Giuseppe Zicari

Chemical agriculture and pollinators: signs of a Planet in danger

Biomonitoring with bees

Environmental indicators of food safety and health: climate, biodiversity, energy, fertility, pesticides and genetic engineering

Back Cover

Bees, these extraordinary creatures that have inhabited the Planet for over 100 million years, are the common thread that tells the story of various ecological challenges such as the reduction of biodiversity, climate change, soil degradation, and energy transition. When the most presumptuous species on the Planet interferes with the course of nature, it causes serious damage, altering the possibility of survival of non-humans, such as the pollinators, without understanding that this is actually a self-destructive ecocide. Paradoxically, agriculture, which is one of the activities most closely dependent on a healthy biosphere, is one of the major causes of irreversible and, therefore, unsustainable changes such as global warming and the extinction of pollinators from which it derives its benefits and wealth. The massive use of fossil fuels, the distribution of poisons such as pesticides (which are persistent, toxic, and bioaccumulative), the loss of fertility in monocultures of plants selected to satisfy economic needs (e.g. genetically modified organisms), are some of the main causes of an ecologically unsustainable food production system. There is no more time, we cannot afford to waste economic resources such as those dedicated to the production of agrofuels (maize cultivated to obtain methane, biogas) and genetically modified plants (e.g. those made resistant to herbicides); we must take a step backwards in the way we manage natural resources. One species can only thrive if all the others are healthy, we must embrace this principle. This book tries to show a different vision of the World we are building, a story full of backstories and full of underestimated dangers.

Giuseppe Zicari is an Italian agrotechnician and biologist (University of Padua), he specialized in Applied Genetics (University of Bologna), he obtained a PhD in Environmental Sciences, inland waters and agro-ecosystems (University of Eastern Piedmont). He worked as a biologist collaborating with companies in the food sector, some Universities and the National Health Service. He teaches natural sciences (*part-time*) in secondary school; he won the National Award for University Publishing (Associazione Italiana del Libro) with the book *ENERGIE RINNOVABILI DA BIOMASSE: RISCHI E OPPORTUNITÀ. COLTIVAZIONI, ALLEVAMENTI, COMPOST, BIOGAS E AGRO-CARBURANTI: ANALISI DEGLI IMPATTI AMBIENTALI, DELLE RICADUTE SULLA SALUTE E DELLA SOSTENIBILITÀ (2016 - Renewable energies from biomass: risks and opportunities. Crops, livestock farming, compost, biogas and agro-fuels: analysis of environmental impacts, effects on health and sustainability).*
He has published more than 90 works including the following books: *L'IGIENE DEGLI ALIMENTI (2001 - Food hygiene); GESTIONE DELLA SICUREZZA ALIMENTARE (2003 - Food safety management); LA TUTELA AMBIENTALE: ADEMPIMENTI OBBLIGATORI E STRATEGIE VOLONTARIE (2008 - Environmental protection: mandatory obligations and voluntary strategies); OLTRE I LIMITI ECOLOGICI: AMBIENTE, SALUTE E CULTURA NON RINNOVABILI (2015 - Beyond ecological limits: non-renewable environment, health and culture);* most of the publications can be downloaded for free from the website: *https://sites.google.com/site/zicari73/*.

Giuseppe Zicari

Chemical agriculture and pollinators: signs of a Planet in danger

Biomonitoring with bees

Environmental indicators of food safety and health: climate, biodiversity, energy, fertility, pesticides and genetic engineering

How to cite this publication: Giuseppe Zicari. *Chemical agriculture and pollinators: signs of a Planet in danger. Biomonitoring with bees. Environmental indicators of food security and health: climate, biodiversity, energy, fertility, pesticides and genetic engineering. 2022, https://sites.google.com/site/zicari73/*

ISBN 979-12-210-0216-4

This is the incomplete version and it is OPEN ACCESS, downloadable from the Internet for free; the original book (in Italian) is: "*Giuseppe Zicari. Agricoltura chimica e impollinatori: segnali di un Pianeta in pericolo. Il biomonitoraggio con le api. Indicatori ambientali della sicurezza alimentare e della salute: clima, biodiversità, energia, fertilità, pesticidi e ingegneria genetica*. Youcanprint, edited in 2022.", (ISBN 979-12-20377-90-4). The first edition of the Italian original book was printed in January of the year 2022; this translation was completed in September 2022. (© Copyright 2022). The translation into the English language was edited by Peter Mazzoglio.

This publication is the result of more than three years of writing and many years of study and activity in the field, and has not received any kind of financial help or indirect sponsorship. Independence is the value that has allowed this publication to be completed without constraints or conditioning of any kind, but with considerable sacrifice. With the hope that a better knowledge of the issues discussed may help to raise awareness of the changes needed, I decided to make available for free the digital version of this work in order to facilitate the widest possible dissemination. The PDF file of incomplete version of this book, in Italian, in English and in French, can be downloaded for free from the website https://sites.google.com/site/zicari73/.
The paper book can be purchased in Italian, English and French languages at the following site: https://www.youcanprint.it/.

On the cover: photo by Marco Basso.

To my parents and Anastasia who have helped me and
supported me especially in the most difficult times;
to my nephews, with the hope of a better future
than common sense would allow for

INDEX

PART TWO

Chemical agriculture and pollinators: signs of a Planet in danger (by *Giuseppe Zicari*)

INTRODUCTION

Foreword

We have difficulty in orienting ourselves in front of the incessant news and forecasts on the state of health of the Planet, oscillating between incredulity and alarmism. We are aware that we have passed from an era controlled by nature to one called *Anthropocene*, that is dominated by the human species, whose hegemonic role has increased with growing speed for over 8,000 years. The theory of competitive exclusion - i.e. the ability of a species to dominate the others when resources are limited - in the case of mankind has reached worrying levels as rapid expansion is saturating all the available space. Mathematics applied to biology together with the observation of nature teach us that in order to avoid succumbing, in the absence of any possibility of escape since we do not have a second Planet available, it is possible to modify habits by exploiting behavioural multiplicity. There is no time to wait for the natural selection of less predatory and parasitic genetic characteristics towards the biosphere. Knowledge can promote awareness of the necessary changes, and with this book we hope to make a small contribution to spreading environmental culture. The synthesis in this work is intended to be a cry of alarm and an exhortation to participate in breaking the silence. A diagnosis of many current trends is offered with the aim of highlighting their unsustainability.

We are witnessing the systematic and constant reduction of a multitude of animals such as insects, with the general idea that this galloping extinction is inevitable. To attribute this mass extinction to fatalism or to a multitude of unstoppable and uncontrollable causes is an incorrect simplification: a true ecocide generated by a single species.

With this study, which has bees and food safety as a double thread, I hope to disseminate important knowledge to build up an independent awareness. More widespread information is probably an essential lever to counteract selfish and short-sighted behaviour spurred on by the advantage of a small fraction of the society. It is necessary to build a new ecological culture that must place the health of other living beings at the top of the political agenda and economic priorities, because it is from their health that that of our species derives. In too many contexts the environmental illiteracy that reigns make the strength sustained by the values of scientific knowledge weaker and corrodes it to the point of offending intelligence.

I wrote this book primarily to participate in change. The effort it required allowed me to transform some of the pessimism and worry into a peaceful form of struggle against the absurd system we have created. The planning and compilation of this bibliographic synthesis, of irrefutable evidence and useful reflections for the formation of an ecological culture, has allowed me to channel pessimism into something that I believe can help plan a way out. It is my contribution to civic participation and collaboration in the planning of a future different from the one we are favouring. I feel powerless in the face of the fury of the changes taking place, and this book is a personal form of advocacy on behalf of the necessary ecological transition.

Even if with pain, we can consider ourselves lucky to be the cause of the environmental disaster that is now more and more evident and worrying, because we would have much less chances to defend ourselves from the forces of nature and its catastrophic manifestations that in the eyes of humanity are ruthless and inhuman. In some centres of power, the environment is treated as if it were a luxury that the market and finance can afford only after satisfying human rules based on ignorance of the basic principles of biology, chemistry, physics and supported by the presumption of omnipotence. In fact, contemporary economic theories, behind a mathematics that is incomprehensible to most people, hide indifference to the fundamental laws of ecology that regulate our health. The exaggerated confidence in the ability to arbitrarily control and manipulate the environment implies the erroneous assumption of the inexhaustibility of resources. The small fraction of society that manages most of the wealth of the Planet and, consequently, the destiny of us all is particularly affected by a psychic disorder: the delusion of omnipotence. It is considered possible by assumption to be able to intervene on the modification of the Planet by overriding the laws of nature, which take a back seat, considering them, erroneously, at the service of economic interests. The manifestation of omnipotence was once reserved for divine, supernatural power, but today, thanks to technological innovation and the concentration of power, it has become a dangerous manifestation of the imperfection of the human society. This almost divine power actually shows its fragility if we observe that it is exercised against something whose capacity for defence is not unlimited: the result will be a collapse that will overwhelm even the creators. In this context, the manifestation of power against nature reveals stupidity and the inability to limit the capacity for self-destruction. The megalomania manifested through economic rules justifies actions that generate irreversible and self-destructive changes, beyond the possibilities of regeneration offered by the biosphere. The ostentation of this fragile grandeur generates a waste of limited resources and produces devastating and irreversible changes. Unfortunately, we are not omnipotent and we do not have infinite powers over nature.

One of the paradoxes of our times is that today the knowledge that each of us can access is endless, but this opportunity does not foster the necessary inhibiting brakes. A child with a smartphone connected to the internet potentially has access to more information than the head of a major power could access a few decades ago. Knowledge is therefore in our possession and it is easy to find information, but this opportunity does not get translated into self-protection mechanisms. For the first time, humanity must have the ability to refrain from doing something that is within its capabilities.

In many social contexts there is a widespread awareness that we need to change our attitude towards the environment. It is increasingly easy to meet people in favour of a better democracy, a cleaner environment and safer food, but it is difficult for them to be actively engaged in change. At present it seems difficult to think of drastic change unless a mass movement is involved. Comprehensive action is needed that will be difficult to organize from the top of the social hierarchy (politicians and businessmen), as the ruling class is largely subservient to financial and economic rules that produce significant monetary benefits, mainly to itself, while managing the environment in a relationship of parasitism and cannibalism. Selfish behaviour prevails to the detriment of the community, also because the human species has never excelled in the protection of common goods and this is why more information can favour the passage from individual sensitivity to collective behaviour. Systems based on participation and sharing are generally more democratic and flexible, but they can be feared by those who hold privileges and power.

The evident repeated failure to avoid the easily foreseeable environmental and social disasters constitutes an unprecedented moral, political and evolutionary defeat for humankind. In the future, the demand for food, both of plant and animal origin, is expected to increase, as well as the need for energy, but this growth has become unsustainable due to the limited availability of natural resources (fossil fuels, soil, clean water) and the damage generated to the Earth's ecosystem (irreversible reduction of biodiversity, climate change). The blindness with which we are witnessing the environmental disaster and ecological destabilization constitute an intergenerational crime, not addressed with due determination. The dissemination of information could perhaps encourage the formation of an environmental culture, stimulate greater awareness and a more coherent sharing with natural balances. The ruthlessness of the rules dictated by nature becomes very evident when the materialism of men clashes with the predominance of natural forces, which remind us of our smallness with uncontrollable manifestations.

The term *Anthropocene* indicates an era in which the World is dominated by humankind: the age of man. The human capacity to move and modify matter, to consume energy and to destroy biodiversity has surpassed that of many forces of nature in intensity and rapidity. Man constitutes a new force acting on a planetary level and is capable of degrading entire continents, of making most ecosystems and species extinct, of altering the water, nitrogen and carbon cycle and of producing in the atmosphere the sharpest and most marked increase in the quantity of greenhouse gases recorded in hundreds of thousands of years. If the planetary population growth, the plundering of resources, pollution and destruction of natural ecosystems are not stopped, economic, social and demographic collapse will inevitably follow very soon. At the current level of exploitation of the Planet's resources, the ability of ecosystems to sustain future generations of human beings can no longer be taken for granted. Negative aspects include: the loss of biodiversity, soil exploitation, the decrease in resources such as water, the alteration of biogeochemical cycles such as nitrogen and phosphorus, ocean acidification, pollution and climate change. For each of these critical issues, many expert committees have already suggested thresholds that should not be exceeded to ensure a future for the next generations. Unfortunately, many of these thresholds, estimated according to very selfish and biased human criteria, have already been exceeded and continue to get worse and worse, such as: the loss of biodiversity, the atmospheric concentration of carbon dioxide, the alteration of the nitrogen cycle, the degradation of soils. Moreover, both chemical and biological pollution are greatly underestimated.

THE COMMON THREAD: POLLINATORS AND FOOD SAFETY

Through the narration of the signs of suffering recorded by the World that revolves around pollinators, in this book, I explore some important issues such as the impact generated by the indiscriminate distribution of substances with biocidal action such as insecticides and herbicides. We need to reflect on the fragility of essential services provided by nature such as fertile soil, uncontaminated air and water, and the effects of pesticides on human health. Pollinating insects - and in particular those bred by man primarily to meet economic needs - have long been sending out alarm signals to which we unfortunately do not pay the necessary attention. These alarms concern food production, which is unsustainable and paradoxically one of the main causes of negative changes. In this synthesis I have tried to put together many different pieces that do not provide an adequate perception of the seriousness of the situation when examined individually. The overall and unitary vision, on the other hand, is much more worrying and should help us accept the limits of the biosphere in which we can operate, above all to safeguard future generations.
To counteract the impact of man on nature, which is now creating frightening damage to the biosphere, many measures have been hypothesized, but they have proved bland and ineffective. The pollinating insects are registering a drastic reduction all over the Planet and, in general, they can be considered an effective manifest of bankrupt nature.

PERSONAL CONSIDERATIONS

One of the reasons I decided to tackle the difficulties associated with writing is the desire to contribute to change through information. The feeling of urgency generated by clear negative omens can become a reason to react. There are two main types of scholars in the scientific field: the first deals with science and popularization in order to earn a living and the second does the opposite, that is, finding a way to earn a living in order to continue studying and dealing freely with science. In any case it is biophilia, i.e. love for the process that generates and sustains life, that probably drives billions of neurons to write books that will be read by other brains. Probably, however, it is not only biophilia that has supported me in this long and at times, seemingly endless and tiring journey. The feeling of helplessness and frustration of being surrounded by people who completely ignore such important environmental issues have been my lifeblood. Continuously witnessing manifestations of irreversible and growing degradation, too great to be opposed by individuals or small collectives, generates a feeling of disbelief and at the same time dismay. How can we let this happen? A tool at my disposal at this time to try to actively participate in the change is to publish for free the synthesis of a laborious bibliographic research. Some passages in this publication will highlight a major failure of the scientific world, such as that of predicting the benefits and disadvantages of using pesticides, because it has greatly underestimated the dangers. Science has also failed because it has not been able to adequately support uncomfortable truths such as those about the reduction of biodiversity, soil degradation, the negative effects of pesticides or climate change. The failure of society to defend itself has brought humanity and the biosphere into environmental and ecological conditions unknown in the history of human evolution. We are irreversibly destabilizing the entire ecosystem: this is a truth of which we must necessarily and finally become aware. Perhaps only in this way a widespread and capillary pressure addressed to politicians and entrepreneurs will be able to encourage the drastic and urgent change necessary for our survival. Precisely because I belong to the category of scholars who, in order to continue to be freely interested in science, must do another job, I have not been able to take advantage of that network of contacts, professional relationships and tools that probably could have improved the publication. However, through sacrifices, including material ones, I decided to make this work, the result of several years of study and research, freely available (the PDF file of this book is available for free on the internet). I hope that this small contribution can encourage a greater sensitivity and awareness necessary to save the future. The activity of free thinker through writing is, in my case, constrained by a uni-directional communication, as I could not establish a concrete and constructive exchange with the Authors of the works I summarize and comment, with whom I would have had the pleasure to exchange views.

In this book several environmental issues are discussed and countless data are presented, supported by hundreds of bibliographic references. Possible inaccuracies in reporting the many data extrapolated from the technical-scientific literature and from the professional experiences can be reported to me (gzicari73@gmail.com): I will be grateful and will provide for the updating in the following editions. In the meantime, I apologize in advance for any omission, error or inaccuracy, warning that in some parts data or estimates are presented that do not perfectly agree with each other, as they are the result of different research and investigation methods which, for scientific correctness, are however reported in order to facilitate more objective comparisons and reflections.

In summary, the purpose of this work - the result of the study of hundreds of publications and some professional experience - is therefore to share information on distressing topics such as the reduction of biodiversity, climate change and the use of pesticides. Moreover, the following pages constitute an attempt to transmit the enthusiasm generated by the knowledge on fundamental topics, together with the sense of urgency for the horror they entail.

PART ONE

BEES BIOINDICATORS OF A WORLD IN DANGER

BEES AND BEEKEEPING

THE BEEKEEPING SECTOR IN THE WORLD AND IN ITALY

According to data from the *Food and Agriculture Organization of the United Nations,* the World production of honey in 2018 was about 1.86 million tons. Honey production is mainly concentrated in three Continents: Asia, which alone weighs 49% (with the dominant role of China), followed by Europe with 21% and the American Continent with 18%. The first 6 producing countries guarantee more than half of the World production; the Chinese primacy stands out with a 29% share of the World production, followed by Turkey. [341, 834] Global production is constantly growing: in 10 years the increase has been 23%.
Between 1961 and 2007 honey production in the World increased by 58% to 1.07 million tons per year (from over 72 million colonies registered in 2007). In the same period, the number of colonies bred in Europe decreased by 26% and in the USA by 49.5%. In the United States of America, at least 5.9 million bee colonies were registered in 1947, which had fallen to about 2.3 million by 2008. Most of the increase recorded at planetary level comes from Asia (426% increase in the same period), Africa (130%) and South America (86%). [484]
The World's largest honey producing Continents were, in 2017: [201, 202]

1. Asia 49.8%;
2. Europe 20.8%;
3. America 17.9%;
4. Africa 10.7%;
5. Oceania 1.5%.

The largest honey producing countries in the World recorded in 2017 were (in descending order): China (with more than 500,000 t), Turkey (with just over 100,000 t), Argentina (with less than 100,000 t), Iran, USA, Ukraine, Russia, India, Mexico and Ethiopia. Globally, almost 400 million euros worth of honey is exported every quarter. According to some surveys, honey is the third most counterfeited product in the World. [196]
Europe is the second largest producer in the World with a total of about 17.5 million hives and over 650 thousand beekeepers. A sector with a limited economic value but of inestimable importance for agriculture, as it is responsible for 80% of the pollination of agricultural products. [834, 821] According to data collected by the European Commission, each European beekeeper has 21 hives, the result is the average of very different data: in Greece and Spain each beekeeper has more than 100 hives and in England and Germany only an average of 6 or 7. Italy, together with France, has an average of 27 hives per beekeeper. However, the average yield of each hive shows substantial differences: while in Germany each hive can yield on average 35 kg/year of honey, in Greece it yields on average only 9 kg/year. In France, the average yield in 2017 was 21.1 kg (there were 53,953 beekeepers who registered 1,344,185 hives), while in 2016 it was 16.5 kg. [243] Italy in this context is close to the European average with a yield of 25 kg/year of honey. Italian honey production is guaranteed by more than 1.47 million hives (78% are managed by professional operators), of which about 390,000 are sedentary and 556,000 nomadic, the rest are instead hives for self-consumption (more than 173,000 hives produce organic honey). Italy is the fourth country in the European Union for the number of hives, after Spain (2.9 million hives), Romania and Poland (1.8 and 1.6 million hives, respectively). The number of hives registered in Italy in 2018 increased by 7% compared to 2017. At least 70,000 beekeepers were registered in Italy (Commission Implementing EU

Regulation No. 768/2013 of 8th August 2013), 90% of which were classified as non-professional. [834]

The annual productivity of honey in Italy has been estimated on the basis of the total number of hives surveyed to be about 22,000 - 23,000 tons. [228] Honey production in Italy steadily decreased: from 2010 to 2017, production decreased by 39% (Eurostat). The annual national production of honey covers about 50% of needs, the rest is ensured by import, while that which is exported is about 3,000 tons. National production of royal jelly amounts to about 4,000 kg and swarm production is estimated at 350,000. [221]

Data from the Apistica Database (Banca Dati Apistica) for the November-December 2018 census, updated as of March 1, 2019, show that there are 55,877 beekeepers in Italy, 36,206 of which produce for self-consumption (65%) and 19,671 are beekeepers with VAT registration who produce for the market (35%). Italian beekeepers hold a total of 1,273,663 hives of which 78% (984,422) are managed by commercial beekeepers who raise bees professionally. [745] In 2018, honey production in Italy was estimated at between 21,000 and 23,000 tons. The region with the greatest number of hives is Piedmont (northwestern Italy). Honey yields are between 5 and 35 kg per hive and productions can fluctuate considerably in different territories, during different seasons, and the way hives are managed changes. Nomadic beekeepers generally get higher yields. Average production data by region in 2018 showed an average yield per hive, for professional farms practicing nomadism, of:

- 33 kg/hive for the North West and North East regions;
- 35 kg/hive for the regions of the Centre;
- 22 kg/hive for the regions of the South and the Islands.

These data show a national average yield of about 30 kg per hive.

Italy imports far more honey from abroad than what it exports. The price of exports per unit is higher than that of imports. The average price of imported honey is about 3 euros per kilogram while the value of exported honey ranges between 4 and just over 5 euros per kilogram. In 2017, Italy spent more than 70 million euros to import honey, while it exported a value of more than 30 million euros. Italy in 2018 results to be as 9th country in the World for quantity of honey imports, and is in 6th place for value of honey imports (150,000 tons of honey were imported in the World in 2018). [745] Imports in 2018 (January-September) were mainly from Hungary, Romania and China, while exports to Germany, France and the United Kingdom.

Most of the honey produced in Italy is sold to large-scale retail trade. Honey sale prices range from 6 euros per kilogram in discount stores to over 10 euros per kilogram in large-scale retail trade. Honey selling prices in about 12 years (2006-2018) have increased more than twice (e.g. citrus, chestnut, wildflower honey). The national sales value of honey for 2018 can be estimated at 141.3 million euros. There are also other types of markets associated with beekeeping that are important although smaller than honey sales. To give examples: bee colonies were sold at prices between 75 euros and 140 euros per swarm (the price varies with the season), queen bees between 13 and 15 euros each, the pollination service with orphan bee nuclei (without queen bee and therefore destined to die after a few weeks) between 24 and 55 euros (for greenhouse vegetables such as strawberries, melon, watermelon, cabbage), the pollination service with hives was rented at prices between 20 and 40 euros per hive (cherry trees, sunflowers). [745] The activity of selling honey is much more profitable than that of pollination. [245] In France, only 2.3% of the beekeepers' income is generated by payment for the pollination service. The situation is different in other parts of the World such as North America where this service can be very profitable (e.g. in almond orchards).

Currently, the consumption of products advertised as "greener", such as those derived from organic farming or that ensure greater respect for the principles on which sustainable production is based, is on the rise. Bees have been used as a brand to promote businesses that self-certify as "green", and therefore environmentally friendly, for at least 30 years. Through the promotion of brands and advertising strategies it is possible to encourage environmental education and better ecological awareness.

THE BEES GUARDIANS OF OUR FUTURE

Bees and pollinators are considered the guardians of our future because they are endangered by rapid and extensive human modification. Bees are ambassadors of the threats to other insects, but also to plants and other animals. The role played by canaries in the mines, which, when they stopped singing, communicated the danger of lethal gases and the miners had to leave as soon as possible, can now be transferred to bees for the agricultural World and beyond. In a few decades man has managed to undermine irreversibly a contract that nature has established over millions of years between plants and pollinators such as insects, in fact at least 40% of pollinating invertebrates are at risk of extinction. [230]

About 120 million years ago plants with flowers (called angiosperms) appeared on Earth, bringing new colours to conifers and ferns. [243] One of the oldest fossils remains of insects has been found in amber and has been dated at 80-100 million years; the first wasp species were probably vegetarian and without sting. True bees appeared between 22 and 25 million years ago. [688]*Apis mellifera* and *Apis cerana* are thought to have been two distinct species as early as 2 million years ago. They were geographically separated at least until 12,000 years ago, when humans put them (tragically) close together.

The respiratory system of insects consists of a network of tracheae communicating with the outside through openings called stigmas. This system is suitable for small animals, which is why there can be no insects larger than 20 cm. Larger insects have existed when there was probably more oxygen in the atmosphere and it was warmer.

Some plants like cereals such as wheat, corn, rice or rye rely on the wind for pollination (they are called anemophilous) and constitute more or less 20% of the plants (rarely a few aquatic plants rely on water to transport pollen), while many plants with flowers rely on pollinators such as insects, birds and mammals for reproduction. Besides cereals, other plants that do not need bees are potatoes, sugar beet and spinach. In summary, the most important plants for energy supply to human nutrition such as cereals do not need bees and pollinating insects for reproduction. [869] We also know that between 60% and 90% of wild plants need varying degrees of pollination by an animal such as insects. [585] The majority of plants that produce flowers rely on pollinators for reproduction. It is estimated that 75% of cultivated plants (87/115) depend to varying degrees on pollinators and that the service provided by pollinators contributes to generating 35% of the wealth of World agricultural production. [230, 867] Another estimate reports that 90% of the 107 most important crops benefit to varying degrees from bee pollination. [481] In the absence of good entomophilous pollination, fruits are absent or less numerous and smaller, as is the case of apples. [232, 233]

Nectar is the sugary substance produced by plants to reciprocate the action of pollinators. Plants such as kiwi do not produce nectar, but only pollen, so they may be less attractive. For other plants, pollination by insects such as bees is necessary to obtain seeds rather than fruits, such as carrots, rape, onions, parsley, turnips and cabbage.

Some crops are completely dependent on the work done by beekeepers. North America has one of the largest artificial migrations on the Planet: at least 1.6 million hives are moved by truck to California's almond orchards that otherwise would not be productive. In just a few weeks, most bees meet in this region to carry out a useful and indispensable service.

In order to introduce a critical aspect that will be extensively discussed later on, it must be pointed out that between the months of March and October apples - like those grown in the

Province of Trento in Italy - can receive more than 30 treatments, each with a mixture of poisonous substances such as: [477]

- fungicides (boscalid, captan, fluazinon, iprodione, penconazole);
- herbicides (glyphosate);
- acaricides (bromopropylate);
- insecticides (chlorpyrifos-ethyl, pyrethrins, methoxyfenozoid).

The distribution of these pesticides is concentrated in the months of May and June, precisely during the period of greatest activity of the bees on which they depend for fruit production.
There are already places on the Planet where there are apocalyptic situations of degradation in which pollination (e.g. cherry trees in China) must be done manually by brushing pollen grains collected in areas where flowering began earlier. In China there are at least six million bee colonies (*Apis mellifera* and *Apis cerana*) managed by over 200,000 beekeepers (in China there are some native species such as *Apis cerana*, *Apis dorsata*, *Apis laboriosa*, *Apis florea* and *Apis andreniformis).* [585, 974]

This book devotes a lot of space to domestic bees (*Apis mellifera*) for which there are alarming increases in mortality. The domestic bee provides a pollination service that is much more important than honey production. Also for this reason it is probably one of the most studied insects: in the *PubMed* database of scientific articles it is possible to find more than 4,500 publications with the name of the species *Apis mellifera* in the title or in the abstract. The domestic honeybee plays an indispensable role in food production but also in the maintenance of biodiversity.

A DISARMING GAP GENERATES THE SYNDROME OF CONSCIOUS BLINDNESS TO THE PLANNED DISASTER

Science that analyses the health status of populations and the quality of the environment in which they live is better at suggesting predictions than economic indicators such as gross domestic product (GDP). Some epidemiological indicators have been pointing to an alarming deterioration for some years: in many European countries women have had a lower life expectancy and worse health than in 2010. On average, Europeans had a healthy life expectancy of 62.2 years in 2008, but this dropped to 62 years in 2009. This reduction in quality of life has been recorded in recent decades for the first time, and is an alarming indicator. Studying the health of bees is also a good indicator of the health of the environment. [209]
If man is the main indicator of everything, since 2000 the number of deaths from non-communicable diseases (cancers, diabetes, cardio-circulatory diseases) has exceeded those from communicable diseases (infectious diseases). Unfortunately, the supporters of immobilism (*business as usual*) reject the existence of environmental limits and biological and natural laws, and underestimate that they are independent of financial rules. Science continually produces new information at a frightening speed and enables the application of new techniques. Knowledge moves forward and discovers the risks consequent to the application of new techniques. Therefore science, besides generating new technological applications, should suggest rules to limit the damage generated by the technologies born from this new knowledge. Unfortunately, this does not happen and very important aspects are completely ignored or confused. In some cases, alarmism is generated that draws attention away from the real causes and the best solutions. The World of beekeeping represents quite well some of the contradictions of our society: clear signals and simple solutions, but ignorance and conflict of interest dominate the scene to the detriment of the community and other living beings. This work of synthesis seeks to counter uncertainty, doubt and confusion, which are often

systematically and diabolically constructed and sown by groups of technicians and experts at the service of great short-term economic interests.

For several years now, there has been a steady decline in biodiversity at a rate of between 1,000 and 10,000 times faster than it would have been measured in the absence of human activities. This massive and rapid mass extinction includes insects, animals that feed on them such as birds, and plants that have been symbiotically linked to them for very long periods of time. Yet this rapid disappearance, like that of honeybees or domestic bees, is often presented as a mystery, an enigma whose unknown causes need to be investigated, researched and deepened. To identify this disappearance presented as mysterious, special expressions have been coined such as *sudden death of bees* or *colony collapse disorder*. In this book we will try to unravel this mystery, which in reality has no secrets except those protected by patents and other industrial interests, which are well shielded by economic and legal rules, even when they damage the collective interest (e.g. health and sustainability). This is the case of the research and studies carried out by chemical companies to test the acute toxicity of pesticides on insects such as bees. It is incredible that the information needed to protect the public is only produced by those with an obvious conflict of interest (the chemical industries) and is not available to the public. Risk analysis documentation can only be viewed by the bodies that have the authority to decide on the placing on the market. In fact, some information may also be withheld from control and authorization bodies.

It rarely happens that scientific documentation succeeds in demonstrating in an unequivocal way that a substance is very dangerous for man (e.g. it is definitely carcinogenic) and even in these cases, however, economic interests often succeed in getting the better of it. With the intention of highlighting the enormous gap between those who have an interest in marketing thousands of chemicals before having reliable toxicological information and those who have the task of protecting collective health and the environment, it is useful to highlight the result of 50 years of work of a prestigious and well-known Agency. The IARC (International Agency for Research on Cancer) believes that a substance can be evaluated in the laboratory as carcinogenic if taken at a specific dose or at a higher one, and not be so at the one at which people come into contact with it in everyday life. As reported on this Agency's website: [304]

> "*If there* is *sufficient evidence of carcinogenicity in humans, the substance is classified as Group 1; if there is limited evidence of carcinogenicity in humans, but sufficient evidence in laboratory animals, the substance is classified as Group 2A; if there is limited evidence of carcinogenicity in both humans and animals, the substance is classified as Group 2B; if the evidence is insufficient, the substance is classified as Group 3; and finally, if the evidence in humans and other animals indicates an absence of carcinogenic activity, the substance is classified as Group 4.*
>
> *Group 1 contains the certain human carcinogens and includes* (at the time of writing) *120 agents; group 2A includes probable human carcinogens and contains 82 agents; group 2B groups possible carcinogens* (for a total of 302 substances)*; group 3 includes substances that cannot be classified as carcinogens* (at the time of writing there are 501)*; finally, group 4 groups substances that are probably not carcinogenic to humans* (in this category there is only one substance, caprolactam, a precursor of nylon). *The IARC lists, compiled from 1971 onwards on the basis of the studies available in the scientific literature, include only those agents studied because there was a suspicion of them.*". [304]

It is decidedly disarming to note the scarcity of information available: from 1971 to date only 1,013 substances have been evaluated for their carcinogenicity compared to millions of molecules produced artificially by man and distributed massively in the environment (there are at least 100,000 molecules registered and produced in large quantities).

This Agency does not have access to the studies carried out by the pesticide companies, so it can only assess data from published research. A search for the word herbicide among the substances (group 2B) generates the result of 2 substances: 2,4-D (2,4-dichlorophenoxyacetic acid) and chlorophenoxy herbicides. This example highlights the imbalance of resources between those who aim to make money at any cost and those who should protect the community. In practice, the burden of proof of dangerousness is left to a slow, ineffective system that does not have access to all the necessary information. [280, 336] In the meantime, industrialists gain time, because substances can only be evaluated after independent scientific research has managed to publish a certain amount of consistent evidence. Under this system, entrepreneurs can rest easy for decades. Another proof of this is the following: over time some of the substances classified in the less dangerous groups are moved to the higher ones, following the publication of new studies. For example, dioxin was moved from group 2 to group 1 in 1994. This means that a substance is only classified in group 1 after it has already caused many cancer patients and deaths. This is proof that prevention through toxicological studies in the laboratory does not work, especially when they are managed almost exclusively to protect private interests. Returning to the death of the bees presented as mysterious and defined by some with the ugly acronym CCD (*Colony Collapse Disorder*) it would be sincerer to define it as the syndrome of *Conscious Blindness* to *Planned Disaster*. The symptoms manifest a very serious disease: the community continues to repeat errors and self-harming behaviours.

THE BIOLOGY OF BEES (*Apis mellifera*): an example of a female society of tireless, organized and altruistic workers

Insects were among the first animals to colonize the land around 400 million years ago. Insects are the living organisms on our Planet with the highest number of species: over one million have been classified. Reasoning in terms of biomass, that is, the weight of living organisms, it is estimated that 97% is represented by plants (and fungi) while 3% by animals, two thirds of which are insects.

The estimated number of pollinator species is about 40,000, of which at least 25,000 are classified bee species and about 2,000 live in Europe (most are solitary bees for which little information is available). [193, 230] At least 547 species of wild bees have been identified in Germany. [869] Insects that are able to pollinate are called pollinators. Plants have developed various stratagems to keep some pollinating insects, thus ensuring the phenomenon of floral constancy. The evolution of bees with plants capable of making flowers (angiosperms) probably began over 100 million years ago. We can affirm that bees are indeed the daughters of flowers.

Among the thousands of species of bees described in the World, about 50 are managed by man and 12 are commonly used for pollination [*Apis mellifera, Apis cerana, Osmia lignaria*, *Osmia bicornis* (solitary bees very widespread in Europe), *Megachile rotundata* (solitary bee pollinator of alfalfa, legumes and carrots), *Bombus terrestris* and *Nomia melanderi*]. [364, 365, 481]

The success of honey bees is due to several factors such as the ability to regulate the temperature of the colony and therefore to resist the cold. Moreover, honey bees are not always the most efficient pollinators, but they do live in colonies made up of thousands of individuals that move for kilometres. Another characteristic that distinguishes foraging bees is floral constancy, that is the tendency to be faithful to the same type of flower, unlike other insects such as bumblebees. [254] Usually the honey bee tends to visit the same types of flowers if it is possible, while only a small fraction explores new resources (5-7% of forager bees explore new flowers). [974]

Our ancestors had already learned to collect honey at least 7,000 to 8,000 years ago. As a reminder of the ancient relationship between man and bees, there is evidence of engravings found in caves on the northern coast of Spain dating from between 6000 and 9000 b.C (before Christ). [35, 81, 972] Egyptians and Assyrians in the past used beeswax and honey for embalming. Some of the oldest evidence of actual beekeeping dating back to 2400 b.C. has been found in Egypt. [243, 254] A papyrus dating back to 256 b.C. refers to a beekeeper with 5,000 colonies. [974] Honey was also an ingredient in over 500 medicines used in ancient Egypt. Traces of the practice of beekeeping have been identified among the Greeks (dating back to 650 b.C.) and among the Romans (dating back to 150 b.C.). [484] One of the alcoholic beverages originated from the fermentation of honey is mead. Vinegar was also discovered as a result of the transformation of mead or wine from honey.

One of the first magazines dedicated to beekeeping probably dates back to 1861 in the USA: *American Bee Journal*; while one of the first books written in English on beekeeping dates back to 1568. [974]

Bee products such as honey are also used by animals: bears (they cover their snouts with mud to protect themselves from stings), badgers, skunks, raccoons, rodents, foxes, martens, monkeys and insects such as beetles, hornets, ants, wasps (e.g. the adult hornet or *Vespa crabro* feeds mainly on fruit while the larvae are carnivorous), dragonflies, praying mantises and moths (nocturnal butterflies). Lizards, hedgehogs and dormice can also feed on bees and mice can sneak into the colony in winter to warm up and feed on honey, wax, pollen and bees that are immobile in this season. [2, 35] Several birds feed on bees such as titmice, bee-eaters, redstarts and swallows. The bee-eater catches them in flight and is able to remove the sting and swallow them. It can place itself near the hive and comfortably feed on them. Woodpeckers (e.g. green woodpeckers) and the great tit can beat the walls of the hives with their beaks to get more of them to come out and then feed on them. We report a very interesting symbiosis recorded in Africa where a bird (*Indicator indicator*) guides the honey badger (*Mellivora capensis*) towards the bee colonies. After the badger has taken its share of the spoils the bird that guided it can feed in turn. This behaviour of the African bird, which guides animals towards food, i.e. the beehive, has also been exploited by human beings. Even today in many places on the Planet there are male honey hunters who prey on wild beehives in the forests, braving various dangers (e.g. in Asia, Africa and Latin America). [196] The male brood is considered a food delicacy in countries such as Mexico, Thailand or Australia. [972]

Bees are insects belonging to the order of Hymenoptera like wasps and ants. This category of insects is united by anatomical characteristics such as having three pairs of legs and two pairs of wings. Most bees are solitary, that is, they do not form colonies (there are at least 14,000 classified species). [13]

In the subfamily of Apinae there is the genus *Apis,* which includes eight species comprising: *florea*, *dorsata, cerana* and *mellifera*. [17] Those used in beekeeping are *Apis cerana* (of Asian origin) and above all *Apis mellifera* (of European origin). [2] *Apis mellifera* includes several subspecies: at least 26 have been classified. According to some studies there are 31 subspecies, of which 15 live in Europe and the Caucasus area; the most important in Europe are: *Apis mellifera mellifera*, *Apis mellifera ligustica*, *Apis mellifera carnica* and *Apis mellifera caucasica*. [972]In Europe, the wild *Apis mellifera* probably no longer exists and the colonies found may not be self-sufficient as they are derived from bred ones.

A hive of *Apis mellifera* is formed by 10,000 to 100,000 bees divided into castes: the queen bee, a few hundred drones (present only during the reproductive period) and worker bees originating from fertilized eggs (10,000 bees weigh about 1 kg). [35] At the end of 1600 the colony of bees was described as an example of monarchy in harmony with the "King"; in 1800 it was understood that instead the colony was governed by a queen. [991] The males (the drones) are completely dependent on their sisters as they are fed by the worker bees (by trophallaxis) or they can take nectar from the uncovered cells (those not closed by a wax plug).

The average size of a worker bee is 12-13 mm in length and 130 mg in weight. The drones within a colony can be about 200-300 individuals, all derived from unfertilized eggs (parthenogenesis). These males are present only in spring and summer; on the whole, a colony can breed between 2,000 and 6,000 drones a year. However, there are exceptions to this rule. In a colony with a queen bee, usually less than one worker bee per 10,000 has active ovaries, while in orphan colonies (without a queen) 10% of the workers are able to produce eggs (the hormones produced by the queen bee play a fundamental role in this case). Therefore, two types of parthenogenesis are possible within the colony: that of the queen bee who generates only males (from unfertilized eggs) and that of a small fraction of worker bees (usually less than 1%). Female bees have 16 pairs of chromosomes while drones have half (female bees will have approximately 30,000 genes in 32 chromosomes while males have half).

The queen bee has the ability to regulate the fertilization of eggs and, therefore, together with the worker bees can change the number of males present in the colony. Drones and worker bees live between 30 and 50 days. [2, 17, 35] Worker bees born in autumn can live up to 180 days, summer drones can live up to 90 days, while the queen bee can live between 2 and 7 years. In Italy, in the spring-summer period, a hive of 50,000 bees has a daily mortality rate of about 1%, that is, equal to about 500 individuals. It follows that in four months all the bees in a colony are no longer the same (except for the queen). It is assumed that forager bees die after flying a maximum of 800 km. [974]

The reproductive system of the bees foresees that different queens will always be fecundated by different males. Without a queen bee the family is doomed to die but with the generation of males, who will fly in search of queens, a way is opened to spread the genetic heritage.

From the second year onwards, the ability of queen bees to lay eggs may be reduced. For this reason, beekeepers tend to replace them before old age. A queen bee can mate in flight with several males, usually only once in her lifetime, and can store 5-7 million sperms for several years. In the colony, males usually appear 3-4 weeks before the generation of new queen bees. [1179]

Virgin queen bees have the need and the possibility to mate only for a short period of a few days during which they will be able to perform 1-3 mating flights during which they will receive all the necessary spermatozoa for their entire reproductive career of 3-5 years (from copulation with 6 and up to 24 males). If the male's copulatory organ gets stuck in the queen bee's vagina, it will have to return to the hive to be cleaned by the workers. From the copulation with different males the queen bee receives many more spermatozoa than necessary, only a part (5-7 million between 80-170 million, coming from different males) will be stored for years in the spermatheca. [974] Therefore in the colony there will be worker bees coming from different fathers, called super-sisters (each group of super-sisters will have the same father, there are as many as the number of fathers).

Following mating, the queen bee can produce up to 1,500-2,000 eggs per day, which is more than one egg per minute. [13] If the queen bee lays more than one million eggs in her lifetime, or about 200,000 eggs per year, that means generating 15 kg of larvae per year. Each egg weighs between 0.03 and 0.1 mg, and after several moults will lead to the formation of a 120 mg worker bee or a 277-290 mg male. [764] The queen bee begins laying in the 2-5 days following mating and lays about 1,000 eggs in January and February. She then goes on to lay 30,000-40,000 eggs in May and June, and continues to decrease in laying intensity during the summer through autumn. During her lifetime, the queen bee can produce over two million bees. The 30,000-40,000 1.5 mm larvae that originate from the egg-laying in May and June will weigh over 2.5 kg.

During the first 3 days the larvae are fed with royal jelly (3 mg/day) and then they are fed with a mixture consisting of 50% honey and 50% pollen (12 mg/day). [13] Royal jelly is produced by special glands of nurse bees and is used to feed the queen bee and larvae in the first few days and is usually not stored. It is estimated that 0.55 kg of royal jelly is consumed per year, 10 g for the queen bee and the remainder for the larvae. Royal jelly contains hormones produced by worker bees that activate particular genes that will allow the transformation of the larvae into queen bees. [254]

Fertilized eggs will give rise to worker (sterile) or queen (fertile) females depending on the diet of the juvenile stages. Worker bees, therefore, with the differentiated diet can regulate the development of a larva into a queen bee. All larvae are nourished with a special secretion called royal jelly for the first 3 days and, later on, only the larvae destined to become queens will continue to be nourished with the jelly, while the other larvae will be fed with a mixture of honey and pollen. For feeding a worker larva about 125 mg of honey and about 70-150 mg of pollen are necessary. [35] The eggs will undergo 5 moults (in the case of workers the first at 12 hours after hatching, the second at one and a half days, the third at two and a half days, and the fourth on the third day). The larvae will weave a silk cocoon in which to pupate and after 5 days they will weigh a thousand times more than when the egg hatched.

Pollen is a very important food for larvae. Fresh pollen is composed of 10-30% water, 10-35% proteins, 15-40% sugars, 1-10% lipids; pollen is an important source of amino acids, vitamins and fats (sterols). [45, 107] A study conducted in Poland reveals that pollen may contain between 0.8 and 5.9% fat. [184] Bees do not eat fresh pollen but after processing and storing it: bees mix the collected pollen with honey and/or nectar, digestive juices and microorganisms (e.g. lactic acidifying bacteria), transforming it into what is called bee bread (fresh, unprocessed pollen develops moulds, so it does not keep). The typical pollen reserve during the summer is 2 kg and in a year a colony can consume 30 kg of pollen. Pollen is consumed by the juvenile stages (about 9.6 mg per day) but not by foragers and is used to make royal jelly. [184]

Therefore, the queen bee has the possibility to generate both fertilized and unfertilized eggs (more rarely). It is the royal jelly that establishes the destiny of the juvenile stages, causing to them become queens: royal jelly is formed by honey (34%) and the secretion of the mandibular glands of the workers. It is composed of 57-70% water, 6.8-20% sugars, 6.4-17% proteins, 1.3-1.7% lipophilic substances (phospholipids, sterols, phenols, fatty acids, glycerides and wax) and 0.75-1.1% minerals and other molecules (e.g. vitamin B5). [13]

Several larvae destined to become queens may be reared in the colony at the same time, but they will fight until only one remains. Cells destined to develop queen bees are usually constructed within 48 hours of the death of the queen bee, due to the absence of the mandibular pheromone. Each larva destined to originate a queen bee receives a total of 1,200 to 1,600 feeds, for a total of 17 hours of work.

Unfertilized eggs will give rise to drones that do not have a sting and are not equipped to collect pollen or produce wax. In the hive, drones can take part in thermoregulation, exchange of food and its transport inside the hive. Usually the number of drones does not exceed 10% of the number of foragers (the bees that go out to forage, that is, to collect the food, the forage). Between 16 days (i.e. queen) and 24 days (i.e. drones) are necessary from the laying of eggs to the formation of an adult: the speed is also influenced by nutritional and environmental conditions. Drones, every sunny afternoon, fly off in search of a new young queen. If they find her they launch themselves at her trying to beat their rivals and mate in flight at 10 or 20 m height. When the drone fails to make contact, it returns home and tries again the next day, and after mating it dies.

The sting is present in the queen and in the worker bees and when they sting a man they are unable to extract it from the skin, so as they move away it tears off and remains stuck in place. The bee with the sting is deprived of a part of the nervous system and parts essential for survival and therefore dies. Bees do not lose their sting when they sting each other, so in this case they do not die. When they sting, in addition to releasing a venom, they secrete an alarm hormone that signals danger to other bees. To avoid stings, beekeepers wear protective suits, preferably light-coloured, probably also so as not to look like a fearsome enemy of the colony: the bear. There is a commercial production of bee venom: from 10,000 bees it is possible to obtain more than a gram of poison (one of the first methods developed to extract the venom is to use electric shock). [974]

Bees perceive ultraviolet radiation, blue and green and do not see the red colour. A bee that forages on artificial flowers, for the same reward, prefers blue flowers over white or yellow ones. Bees are able to produce and perceive sounds and vibrations, and generate various pheromones (it remains largely unknown what kind of communication takes place through vibrations). The queen bee can emit sounds and when the virgin bee emits it the workers around it become immobile (the receptor organ for sounds is located in the antennae). [972, 20, 254]

Worker bees produce different types of hormones to signal alarm; as a result of this chemical signal, the number of bees that come out in flight increases and, therefore, aggression increases. The chemical signal placed on a target which is chased and stung by several bees.

The hormones secreted by the queen bee from at least six different glands are also very important. Some of these hormones are:

- those deposited on the wax to inhibit the construction of cells for other queen bees;
- those that appease the aggressiveness of the workers towards the queen bee and attract them to her;
- the ones that keep the ovaries of the workers atrophied.

Among the hormones produced by the bees there are those produced by the *Nasonov*'s gland which are used to mark the foraging places, to facilitate the finding of the hive and to favour the aggregation of the swarm. The mandibular glands of the worker bees are able to mark the visited flowers generating a repulsion from her sisters, as they have been emptied of their precious forage. Visited flowers can be repulsive for over 40 minutes even to other species such as bumblebees. During this time the flower produces more nectar. The bees also visit flowers that do not produce nectar if they are able to supply pollen, as happens with roses, poppies and helianthus. We can affirm that nectar is the form of reward invented by plants to pay for the pollination service and therefore make it more convenient. Plants can produce different quantities of nectar per flower per day (mg of sugar/flower/day): [869]

- raspberry 3.8;
- apple 1.37;
- cherry between 0.5 and 1.27;
- rape 0.79;
- red clover 0.19;
- sunflower 0.12;
- pear 0.09.

The number of worker bees varies over time, going from about 10,000 in winter to 100,000 in June and then decreasing again. If the number of bees during the summer drops below 4,000 individuals, the colony collapses. An important role in the immunity of the colony is assured by an enzyme secreted by the hypopharyngeal glands and present in honey and royal jelly (glucose oxidase). In the presence of water and oxygen, this enzymatic protein produces substances with antiseptic properties (glucose generates glucuronic acid and hydrogen peroxide). [35]

Worker bees perform different functions depending on their age (they live 40-45 days in summer and those that are born in autumn and spend the winter inside the hive can live up to 6 months): [13, 35, 56]

- Worker bees less than 2 weeks old do not leave the hive, they clean the cells, feed the larvae (also thanks to special secretions), take care of the queen.

- The worker bees, at the age of 12-18 days, take care of the storage of pollen and nectar, produce the wax with four special glands they have in the lower part of the abdomen, and eliminate the dead individuals; in a hive there may be 550 insects, or many more if necessary, with the task of removing the dead bees or those infected by viruses.
- Bees older than 20 days become explorer-gatherers: they are the so-called *foragers* able to fly at 20 km/h and up to 12 km from the hive; about 20% of forager bees spend one or two days guarding the entrance of the hive: in this way they have time to learn their bearings. Most honey comes from nectar collected in a few weeks of the year.
- Other functions carried out after the fifteenth or twentieth day of life of the worker bees, which are by now adult, are those of moving the wings for ventilating and cooling, moving the flight muscles without moving their wings to increase the temperature which, also in winter, is maintained around the 34-35,5°C. To lower the temperature they can also evaporate water taken specifically for this purpose. In winter the bees can remain inside the hive keeping the temperature constant even for months. If they have water at their disposal and experimentally exposed to 70°C, bees can maintain a temperature of about 35°C. However, a bee exposed to 46°C usually dies in about one hour. [764] A fraction of bees (1 or 2%) are responsible for regulating the temperature inside the hive and may decide to do so to counteract the spread of a disease. [230] Small changes in temperature can harm bees. For example, drones exposed for a few minutes to temperatures of 40°C show a sperm mortality of over 40% (in juveniles, during pupal formation, 32°C generates abnormalities in the male reproductive system). [991]

  Bees process honey, pollen, propolis and wax.

Worker bees in the last period of their short lives visit thousands of sites daily collecting pollen, nectar, honeydew (secreted by aphids, especially in coniferous forests) and water. [58] They also collect the resins produced by plants as they serve to produce propolis which is used as a building material or glue, to make an area watertight or to embalm intruders. For these reasons bees are an excellent sampler of the area. The average amount of material collected by a beehive in a year is 240 kg of nectar, 30 kg of pollen, 10 kg of water and 0.1 kg of resins. [13] Only a small proportion of foragers (about 5%) collect both pollen and nectar at the same time. Bees shed their feces in flight outside the hive.

Some products of the hive are permanent, such as wax and propolis even though, for example, the wax in the hive is continually being consumed and rebuilt. Others are consumed continuously. The amount of materials produced daily in a colony are (we consider a production in 8 months per year): [13]

- 0.238 dm^3 of honey corresponding to a production of 80 kg per year;
- 0.178 dm^3 of larval feed corresponding to a consumption of 25 kg per year of honey and 25 kg per year of pollen;
- 0.00231 dm^3 of royal jelly corresponding to a consumption of 0.61 kg per year;
- 0.000648 dm^3 of cap wax corresponding to a production of 0.15 kg per year (mature honey is contained in wax-sealed combs).

The intensity of flights and the number of bees collecting pollen and nectar is also regulated by the stocks accumulated in the hive. For example, if more than 10% of the cells are filled with pollen, the number of bees that come out in search of nourishment decreases. Bees tend to accumulate pollen stocks to last at least 3 days. The number of bees returning to the hive to deliver nectar to other bees may be about 30 bees per minute and within the hive over 1,800 bees may be engaged in receiving nectar and distributing it to the cells every hour. [13]

THE POLLINATION SERVICE

About 73% of crop plants are pollinated by some of the 25,000 species of bees classified. [193] At least a third of food production (by volume) depends on pollination by insects. [45] 70 of the more than 100 major plant species we eat are pollinated by bees: apples, almonds, pears, strawberries, tomatoes, carrots, broccoli, olives, onions, peanuts, avocados, cherries, blueberries, alfalfa, sunflowers. [2, 17, 45] The sunflower (*Heliantus annus*) is cultivated for the production of oil and in the World it is pollinated mainly by honeybees, although the most efficient pollinators are wild bees. [476] Bees also play a very important role in the pollination of wild species. In 2009, the benefits generated by pollinators for the economy were already estimated a considerable figure, which however is not sufficient to highlight their importance: 153 thousand million euros, of which 80% is attributed to honey bees. [55, 75]

The pollination service also depends on other insects and other animals such as bats or some birds (e.g. hummingbirds). To give an example, of the 160 million hectares under cultivation in India, about 55 million depend on pollination. The pollination service is so necessary for some vegetable productions that farmers are willing to pay, during the 20 or so days of flowering, between 30 and 60 euros per hive. [196] In Italy, the beekeepers' main source of income comes from the sale of honey. In other countries, the main source of income for beekeepers is precisely the provision of the pollination service paid for by farmers (e.g. in the USA). For example, almond growers in California, who need at least 5-6 hives for each of their 380,000 hectares, have paid between $50 and $200 (e.g. 2009) per hive over the years. [243] This service is often provided by disposable swarms, i.e. groups of about 20,000 bees in a cardboard hive that are left to die, as they are sold with the sole purpose of performing the pollination service for the 2 or 3 weeks of flowering; it is useful to remember that a colony in the spring months can reach over 60,000 individuals, that worker bees live between 40 and 60 days, and that a queenless colony is destined to die in a short time. Among the paradoxes of growing California almonds is the need for irrigation water in an area that has long suffered from severe drought. It takes about four litres of water to make an almond, much more than it takes to make a strawberry or a grape. Yet the area of California where almonds are grown has been suffering from a severe drought in recent years. To compensate for the lack of rainfall, production has been maintained by consuming groundwater. This strategy is unsustainable and will cause serious problems.

Colonies of bumblebees can be sold for this purpose too: for example to pollinate tomatoes in greenhouses. As an alternative to payment, the farmer who allows the placement of hives in exchange can receive a part of the beekeeping products (e.g. honey).

Worker bees are very loyal during their short lives to the food source, as they are not easily distracted by other blooms: forager bees that choose to look elsewhere for flowers when the primary source is still present are between 0.8% (*Apis mellifera mellifera*) and 14% (*Apis mellifera ligustica*). [35, 192]

Bees easily sample the area up to 2-3 km from the hive: usually if food is available they do not move further than 1.5 km. Exceptionally they fly up to 10-12 km away; with the aim of reducing energy consumption, as is logical to expect, they look for food and water as close as possible to the hive. [17] Every day the bees of a hive make tens of thousands of trips to look for food. About 50% of the bees in a hive are forager bees, i.e. insects that go out in search of food or water: even more than 20,000 bees, at least 10% of which are busy collecting water. A colony consumes several tens of litres of water per year, up to 40 L/year: between 40 g per day per bee to about 300 g per day per bee in summer. [35] In very hot countries a colony can consume from 0.5 to 5 L of water per day. [69]

When temperatures are between 20°C and 25°C, forager bees make 12-15 flights a day. Approximately 5 million flowers must be visited to produce half a litre of honey. Each forager bee can visit over 1,000 flowers a day and carry tens of thousands of pollen grains. To produce one litre of honey, 2,500 bees have to work for about three weeks. By the end of the summer they may have accumulated several dozen kilos of honey in the hive, having to make between 100,000 and 150,000 foraging flights per kilo of honey. [71] On the whole, if a bee travels, on average, distances of three kilometres, to make one kilo of honey they will have flown for at least 170,000 km. [254]

HONEY

Honey production per hive per year is between 20 and 40 kg, but in exceptional conditions even more. Honey is produced from the nectar collected from the flowers. The nectar is a sugary solution and is also produced by other parts of the plant such as the leaves, the trunk, the petioles (in these cases we speak of extra-floral nectars, such as those produced by cotton plants and blueberries). [974] Nectar may consist of many different types of sugar such as sucrose, fructose, glucose, maltose, and trehalose. Some types of nectar may contain sugars that reduce the life expectancy of bees such as the monosaccharides arabinose, mannose, xylose, galactose, and galactose-containing oligosaccharides (e.g. lactose). [107]

Many properties have been attributed to honey - but these need to be verified and contextualized because it is basically water and sugar - such as anti-oxidant, anti-inflammatory, cardio-protective and anti-tumour. [477] Nectar has a sugar concentration of 20-40%) and is composed mainly of simple sugars (70-80% glucose and fructose), the remainder of water (less than 18%) and in small quantities of other substances such as pollen (honeydew honey does not contain pollen) and polyphenols with antioxidant properties. [477] Honey has a density of about 1.39-1.44 kg/dm^3 (at 20°C), meaning that one litre weighs between 1,390 and 1,440 grams. [3]

The minimum content of sugars in honey should be 60% glucose and fructose, it should not contain more than 5% sucrose, while it should contain less than 20% water (a honey with more than 19% water risks fermenting if it has not been pasteurized; the content of sugars can be measured with special instruments including the refractometer). Honey also contains minerals and acids: the pH is usually between 3.5 and 6.0 (for example, the pH of acacia honey is acidic, about 3.9).

Over 300 different natural chemicals have been identified in honey, including at least 20 different types of sugars and 30 different polyphenols; excluded from these are man-made or man-used substances such as pesticides and different types of pollen. [35, 477] There can be between 100 and 100,000 pollen grains in every gram of honey: honey contains pollen from several different plants.

Fourteen elements can be found in honey: calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), phosphorus (P), cobalt (Co), manganese (Mn), iron (Fe), chromium (Cr), nickel (Ni), zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb). In light-coloured honeys, minerals can usually be present at a concentration of 0.04%, but in dark honeys they can be found at a concentration of 0.2%. [754]

Honey also contains some water-soluble vitamins, mostly present in pollen, as well as several enzymes (proteins with a catalytic action) that are altered by heat treatment. The concentration of proteins is usually less than 0.5%. [754] Measuring the presence of proteins and their functionality allows us to know if the honey has been subjected to high temperatures: heat treatments are forbidden (e.g. more than 60°C). Another indicator used to measure the freshness of honey is the concentration of hydroxymethylfurfural. This molecule is practically absent in freshly extracted honey and forms over time as a result of the breakdown of sugars, particularly fructose in an acid environment. This index allows us to evaluate the freshness of honey because the concentration increases during storage, but the process occurs much more quickly if the honey is subjected to heat treatment. The maximum limit set by the regulations is 40 mg/kg, although fresh honey should contain less than 10 mg/kg. [225] Another protein present in honey, the enzyme diastase (its function is to hydrolyze sugars), is degraded by exposure to heat. Therefore, the presence of this protein can also give information about the freshness and heat treatment of honey.

Finally, it should be pointed out that microorganisms such as yeasts (from 1 to 100,000 cells per gram) can be found in honey too. [35]

Honey can be stored for more than 18 months and also for this reason the national legislation allows the indication of the date within which it is advisable to consume it with the month and the year (the Italian legislation provides for a storage period of 24 months). [226]

A colony of bees, which in the best period of the year reaches 40,000 individuals, will in total host more than 150,000 insects. This colony, in order to survive the following year, will need at least 20 kg of pollen and 60 kg of honey; considering that nectar on average has a sugar concentration at most equal to half that of honey, it means having to take and store at least 120 kg of nectar to form the necessary stocks, but it is more realistic to consider a greater quantity.

The nectar produced by the flowers is sucked into the forager bees' honey sac, i.e. an enlarged esophagus with a capacity of 50-60 mL (about 40-70 mg of nectar). Water is also transported in this sort of bag, that can be considered a kind of social stomach where bees store nectar for their sisters. A bee needs to visit between 100 and 1500 flowers to fill this bag. [972] An apple blossom can produce 2 mg of nectar and a cherry blossom up to 40 mg. These are the total quantities produced during flowering. Usually the foraging bee finds only a few milligrams or tenths of milligrams of nectar in a flower. So, if a bee makes between 3 and 10 flights a day and visits a total of 3,000 flowers in a day, it can only bring a few hundred milligrams of nectar to the colony, of which less than 50% is sugar. In practice, to obtain half a kilo of honey, the bees must have visited between 20 and 30 million flowers. A worker forager bee needs at least 11 mg of sugar per day, corresponding to about 22 mg of honey per day (at 50% sugar). A colony with a maximum population of 50,000 bees can consume over 120 kg of honey in a year, just for the survival of the adult bees. Overall, as already mentioned, the entire colony can consume over 200 kg of nectar and between 10 and 30 kg of pollen per year.

When the bee returns to the hive it regurgitates nectar which is collected by other bees (trophallaxis). It can be estimated that 25,000 trips are necessary to transport one litre of nectar. [35] Nectar is made up of water (40-80%), sugars (20-60%, such as sucrose, glucose and fructose) and, to a lesser extent, other substances such as nitrogen compounds, vitamins, pigments, essential oils and mineral salts. Plants may secrete nectar all day long or only during certain hours. When a flower is visited, the bee may release a repellent substance that other bees are able to sense in flight. In addition to nectar, bees also collect other sugary solutions such as those from fruits like grapes or other ripe fruits.

Inside the hive, trophallaxis takes place, i.e. the sharing and exchange of food between insects; nectar is exchanged and enriched with an enzyme (*invertase*) produced by the salivary glands in order to break down sugars: sucrose is broken down into glucose and fructose. The digestive tract of the bees enriches the nectar with enzymes already during flight, immediately after sucking it from the flower, such as amylase and glucose oxidase (the latter produces hydrogen peroxide starting from the oxidation of glucose). Honey therefore derives from nectar that has been exchanged, digested, and from which water has been removed through the ventilation produced by wing movement. By using radioactive isotopes of sugar it was possible to detect that after 24 hours from the ingestion by foraging bees, thanks to trophallaxis, 55% of the bees in the colony had become radioactive. [974] Thus, if the nectar is contaminated with pollutants, these are very quickly distributed in the hive. [865]

Honey is used by bees as nourishment especially in winter and to produce wax through glands located in the abdomen. Bees are able to keep the temperature of the hive constant at around

35°C even in winter, consuming a lot of energy, namely the accumulated honey. During the winter, a fraction of the bees move their flight muscles without flapping their wings to produce heat. Consuming about 20 kg of honey collected mostly in 60 days, the bees survive the winter without hibernating. The typical honey reserve of a hive in summer is 15 kg, but in a year bees can produce up to 80 kg of honey and consume 60 kg of it. The beekeeper subtracts honey from the colony, so he will have to provide a substitute nutrient: feed based on water and sugar. [13]

It is estimated that to produce 1 kg of wax, bees need to consume between 6 and 10 kg of honey, so it is easy to understand why beekeepers recycle it: they usually supply the colony with ready-made artificial combs. [258] This is why bees prefer to occupy hives with ready-made combs. The wax cells are hexagonal in shape and, during the summer, of the approximately 100,000 cells, two to three fifths will be occupied by the brood, two fifths by honey and less than one fifth by pollen. [13] The average amount of wax contained in a colony is 1.5 kg, and can be stored for years. The bees continuously remove part of the wax and rebuild it (0.15 kg per year). The wax cells in a colony can form an area of more than 36 m^2 (100,000 cells). Bees need at least 7.5 kg of honey to build a nest containing 100,000 cells weighing about 1.25 kg. [35]

The largest *per capita* consumers of honey include the Central African Republic (3.5 kg per year), New Zealand (2 kg per year), Slovenia (1.6 kg per year), Greece (1.5 kg per year) and Switzerland (1.4 kg per year). [227] In addition, annual *per capita* consumption of honey is about 1.5 kg for Germany, 800 g for England, 600 g for France and less than 500 g for Italy (in 2018). [229] Honey is also used to prepare soaps, creams and other products not used for human feeding.

THE MELLIFEROUS POTENTIAL

The melliferous potential of a nectariferous species is the quantity of honey that bees can potentially produce under ideal conditions from a hectare (10,000 m^2) in bloom. Hives can be placed near the crop when 20-25% of the flowers are already open, in order to perform the very useful pollination service. It is interesting, to get an idea of the melliferous potential: [35]

- up to 25 kg/ha for pear and almond trees: in the case of almond trees, at least 2 or 3 hives per hectare are needed to ensure pollination during flowering;
- between 25 and 50 kg/ha for sunflower, cherry, apple, and red clover;
- between 50 and 100 kg/ha for raspberry, cornflower, and vetch;
- between 100 and 200 kg/ha for dandelion, lavender, willow, white clover, rosemary, alfalfa, sycamore, horse chestnut, wild mint, and origan;
- between 200 and 500 kg/ha for chestnut, ivy, buckwheat, coriander, rape and sage;
- more than 500 kg/ha for acacia, thyme, field maple and linden tree.

Experimental tests have shown that the shorter the distance between the hive and the crop to be pollinated, the greater the production obtained (e.g. in rape, sunflower or buckwheat seeds). For example, hives placed between 1 and 216 m from an apple tree recorded the production of 29.37 kg per apple tree, if the distance increases between 430 and 600 m the production drops to 14.94 kg. If the distance between apple tree and beehive is between 1,500 m and 2,000 m the production is reduced to 9.5 kilos per tree, thus the production is decreased by 68%.

It can be estimated that each bee weighs about 0.06 g (between 60 and 110 mg; *Apis mellifera*). [75] Forager bees come into contact with 1 g of pollen per day and eat 1.1 mg of pollen per day; they also consume up to 80.2 mg of honey per day. The larvae feed exclusively on pollen for 10 days, consuming about 6,5 mg per day and up to 9,5 mg/bee/day. [32, 45] Overall, 130 mg of pollen are consumed to allow development to the adult bee stage: a colony consumes 20-26 kg of pollen in a year. [13]

To fill the pollen baskets each bee must visit between 80 and 150 flowers. The sacs are located in the hind legs and can carry the weight of 7.5 mg each; it can be estimated that a bee can transport, making a maximum of twenty trips a day, up to 300 mg of pollen to the hive. This quantity depends on the flowers, since some of them produce a lot of pollen, so that one visit is sufficient to complete a load. Over the course of a year, it is estimated that a medium-sized colony collects between 20 and 50 kg of pollen. [184]

Each bee can visit up to 3,000 flowers per day by beating its wings at a rate of 200 to 300 beats per second. The frequency of wing beats can be used to distinguish them from similar species. They can fly at a maximum speed of about 20-22 km/h carrying a load of nectar and pollen equal to the 50% of their body weight which, as already said, is between 100 and 120 mg (the maximum speed recorded in flight was of 34 km/h).

In the animal kingdom also hummingbirds (small birds that can play the role of pollinators) are capable of similar feats: they beat their wings 200 times per second and can take hundreds of breaths per minute (even 500). [848] Because of this intense metabolism, hummingbirds have to eat a daily amount of nectar (visiting up to 1,500 flowers a day) and insects equal to their body weight.

In flight, forager bees consume about 6.8 mg of sugar per hour and can carry 40 to 60 mg of nectar and 10 to 20 mg of pollen. [13] Therefore, the further they have to move away from the hive to collect honey, the less convenient the energy return may be. An average consumption per bee of 10 mg/day of honey and 1 mg/day of pollen is estimated. Beekeepers can artificially feed bees with a 50% sugar syrup or at different concentrations. In experimental (laboratory) tests, worker bees (*Apis mellifera* of the Carniola race) can consume 250 mm^3 of sugar-water solution (at a concentration of 2 mol/dm^3) in 24 hours. [485]

Pollen is the main source of proteins and vitamins; proteins are also important for the immune system and extend the life expectancy of bees. [764] Between 6 and 28% of the weight of pollen is made up of proteins that contain at least 10 amino acids essential for bees; pollen usually contains less than 0.2% of sugars. In addition to proteins, pollen also contains lipids, sterols, vitamins and minerals. It has been demonstrated that bees with a low availability of pollen have a reduced life expectancy (from 21 to 56%), tend to become foragers at an early age and have other problems such as a reduced ability to communicate. [35] The current reduction in floral biodiversity implying food monotony in monocultures easily generates nutritional deficiencies to the colony.

THE MAIN CROPS POLLINATED BY BEES

Honey bees are believed to be able to pollinate at least 4,500 species of flowers. Some plants have hermaphrodite flowers (e.g. apples and tomatoes) in which the male and female parts are found in the same flower. Other plants have male and female flowers in the same individual but separate one from the other (e.g. hazelnuts and courgettes). Others have only female flowers or only male flowers, so they are either male or female plants. Plants of greatest commercial interest to humans include the following. [35, 174]

	Plants for which the pollination service is essential	**Plants for which the pollination service is important**
Fruit plants	Apricot (some cultivars), watermelon, chestnut, sweet cherry, almond, mango, apple (almost all cultivars), melon, Australian walnut, pear (many cultivars), peach (many cultivars)	Apricot, cherry, black cherry, kaki, kiwi, raspberry, blueberry, mango, apple, blackberry, medlar, pear, peach, plum, strawberry, Italian clover
Forage crops for seed	Lucerne, field beans, birdsfoot trefoil, sainfoin, alexandrine clover, hybrid clover, ladino clover, red clover, vetch	Buckwheat
Vegetable crops for seed	Garlic, asparagus, chard, broccoli, carrot, Brussels sprouts, cabbage, Chinese cabbage, cauliflower, Savoy cabbage, watermelon, coriander, melon, leek, parsley, radish, celery, mustard, pumpkin	Onion, eggplant, pepper, chilli, tomato, fennel, bean
Horticultural crops	Cucumber, watermelon, melon, pumpkin, courgette	Turnips, fennel
Oleaginous plants	--	Rapeseed, safflower

Other plants that can be pollinated by bees, although this service is not essential, are soybean, sunflower, cactus, pomegranate, currant, citrus fruit, clover and dog rose. Among the plants that can be visited by bees but which also produce fruit in the absence of this insect are the vine, the strawberry tree and the papaya. The vine generates flowers pollinated mainly by the wind, but bees can still collect pollen or visit the vineyard to collect sugar juice from damaged fruit. Bees help produce 30% of rapeseed intended for oil production and 95% of the seeds of plants intended to produce other plants. [243]

Among the arboreal plants of interest from the point of view of beekeeping in urban areas there are: maple, laurel, hibiscus, horse chestnut, laburnum and magnolia. While among the shrubs, holly, ivy, lavender, hawthorn, American vine, lilac and peony.

The importance of the pollination service provided by bees varies considerably from plant to plant and in different contexts. We report the case of alfalfa (*Medicago sativa*) which is a perennial leguminous plant, with trifoliate leaves, flowers with violet, bluish or yellow corolla in racemes and a legume forming a spiral. It is a very important forage crop in Italy, but also in other countries such as the USA. Alfalfa represents an important nectariferous source only in the absence of competitive flora. This is due to the mechanism that takes place in the flower to favour the dispersion of pollen: when the bee rests on the flower, the stem tube snaps upwards hitting the insect and trapping it against the upper petal. For this reason, bees abandon alfalfa as soon as alternative blooms appear. Honey production from alfalfa is only possible if the plant is allowed to flower fully for seed production and, therefore, is not mowed for use as fodder.

Without the service performed by bees, probably in the World, 23% of fruits, 16% of vegetables and 22% of nuts and seeds would be lost or produced much less. [196] Thanks to bee pollination, many plants produce bigger and better fruit (e.g. sweeter watermelons) and have an increase in the number of seeds and their germination capacity (e.g. rape, sunflower, buckwheat, red clover).

In order to compensate for the lack of pollinators and to increase independence from them, researchers select new varieties of plants that have less or no need for pollinators. One can take the example of rape and kiwi. A mechanism that has survived natural selection over millions of years is replaced by others, dependent on human ingenuity, which are neither sustainable nor durable. Another side effect of artificial plant selection is that it does not take into account the production of nectar and pollen. This is, for example, the case of sunflower selection with the aim of obtaining plants with better oil production, greater stem strength or other characteristics that do not take pollinators into account. This results in plants that are less useful to insects and less attractive.

In Italy, in 2017, on average, each beekeeper (of the 48,889 surveyed by the national bee registry) owned 28 hives and each apiary consisted of an average of 14 hives. It is therefore possible to find dozens of hives located close to each other. The indicative number of hives needed to ensure a good production per hectare in different crops is given below.

Category	**Crop**	**Number of hives per hectare** (depending on cultivar) [35]
Orchards	Apricot	5-8
	Cherry	4-10
	Kiwi	8-10
	Apple	6-8
	Pear	6-10
	Plum tree	7-10
Small fruits	Strawberry (open field), raspberry, blueberry	2-4
Plants for seeds	Alfalfa	6-8
	Red clover	4-6
	Other leguminous plants (field beans, sainfoin, vetch)	2-4
	Cucurbits (melon, watermelon, courgette)	4-6
	Cruciferous vegetables (cabbage, radish, rocket)	3-4
	Composites (chicory, radicchio)	3-4
	Sunflower	8-10

SOME TYPES OF FRAUD INVOLVING HONEY

Some types of fraud that beekeepers can engage in are summarized below: [850]

- The indication of a different floral and/or geographical origin (e.g. Chinese honey passed off as European Community honey). [987] This fraud may be accompanied by the addition of pollen to honey.
- The dilution of honey with sugar syrups, for example from corn, sugar cane, sugar beet, rice or wheat.
- Artificial dehydration (e.g. under vacuum) of honey collected when it was still immature (bees transform nectar into honey through various modifications such as one that reduces the water content).
- The removal of pollen to reduce the possibility of tracing the origin of the honey.
- The purification of ions (by ion exchange) present in honey with the intention of lightening it or changing its colour.
- The artificial feeding of bees with feed and chemicals.

According to some surveys, honey is the third most adulterated food product in the US (2018), after milk and olive oil. [850] Pollen testing is used to test the authenticity of honey and specifically its floral origin. To avoid this type of testing, it is possible to remove pollen from honey and add other pollen.

Another possibility of traceability of origin is that offered by examining the profile of other substances contained in honey, using expensive analytical techniques such as liquid chromatography. [856] With appropriate analytical investigations, it is possible to examine the concentrations of molecules such as flavonoids, which can make it possible to discriminate between honeys of different floral origin and therefore of different geographical origin.

SYMBIOSIS BETWEEN HYMENOPTERA AND FIG PLANTS

There are some plants require pollinating insects other than bees, such as the cocoa plant, the vanilla plant and the fig plant. Bees have a tongue that is about six millimetres long so that flowers in which nectar is contained in deeper districts cannot be used for harvesting. In some cases the symbiosis between plants and insects is indispensable so that only one or a few species can pollinate a particular flower, as in the case of the plant that produces figs. At least sixty-five species of figs (*Ficus* spp.) have been recorded. This group of plants, along 65 million years, has had time to establish a close and indispensable relationship with the wasps that pollinate them. [704] Each species of fig depends on one species of pollinator. This co-evolution has resulted in fig plants that produce neutral flowers, i.e. incapable of producing seeds and serving as a larval chamber for pollinators. If wasps lay their eggs in a female flower, the insect egg does not develop and the seed is not affected. Therefore, it is the fig tree that exercises control over the pollination resources by establishing the number of neutral flowers. The different species of fig plants can therefore produce three types of flowers: male, female and neutral. Depending on the species of fig plant, a tree is able to produce a receptacle that contains three types of flowers at the same time, two types of receptacles or apical parts of the flower (one will contain the neutral and male flowers) or three or four types of receptacles in which various combinations of sexed and neutral flowers are observed. The most curious part of this extravagant invention of nature is that the males are born from the egg inside the flower and are without wings, and they mate and die inside the fig plant. The females, on the other hand, emerge from the receptacle through a tiny pore, collecting pollen along the way. When the females fly out in search of new flowers where they can lay their eggs, they pollinate the female flowers inside. As the plant gets rid of the receptacles that have not been pollinated, any female wasp that has not returned the hospitality with pollination loses its offspring. These wasps (hymenoptera), that live two or three days, are a couple of millimetres long and are able to fly up to ten kilometres far. Wild populations of the common edible fig (*Ficus carica*) native to the Mediterranean area, at the end of the season, produce receptacles that contain only neutral flowers. During the winter, fig wasps are fed inside the receptacles and then released to provide pollination by visiting the receptacles produced in the spring. Domesticated varieties produce very sweet fruit without fertilization.

HONEYDEW HONEY

Honeydew is a sugary secretion emitted by phytophagous insects (Rhynchoptera Homoptera) such as aphids and scale insects that feed on plant sap. Plant sap is rich in sugars but poor in amino acids. For this reason, some insects that feed on sap must ingest a great quantity of sap in order to have the right amount of amino acids. Some insects therefore filter the lymph, expelling the sugars and retaining the proteins. This liquid, secreted by those insects such as aphids, is a sap deprived of amino acids and is called honeydew. If the honeydew remains on the leaves of the plants it will favour the growth of some saprophytic fungi (moulds) which feed on it and form a blackish layer, called sooty mould, because it looks like a deposit of soot but is formed by hyphae. Mold hinders chlorophyll photosynthesis, so its removal, by ants or bees, is a welcome thing for plants.

Honeydew is nothing more than a type of excrement of the animals such as aphids. Honeydew is made up of fructose, glucose, sucrose, fructo-maltose, melicitose (the last two are trisaccharides typical of honeydew and of the resulting honey), enzymes, mineral salts, and organic acids. [35] Honeydew can be produced by insects present in all plants including firs, larches, oaks, lime trees and herbaceous plants. It represents a fundamental food substrate for the nutrition of a large number of insects and some saprophytic fungi and, in some populations, for man himself. It is produced in the woods above all by parasites of conifers and is used by bees for the production of honey, which does not contain pollen.
The collection of honeydew by humans is described in the Old Testament and is known among Australian Aborigines. [710] Honeydew honey is derived from insect excrement taken in and processed by other insects: bees. Interesting symbioses have evolved between the ants that breed and protect the aphids and also benefit other animals such as bees. The aphids are defended by the ants against the attack of wasps, flies and other parasites that could inject their eggs into the aphid’s body or eat them. Then the honeydew is collected and accumulated by ants, bees, other insects and humans.
The ants, in addition to defending the insects that produce honeydew, move them to the best shoots of the same plant and in some cases raise the eggs in the anthills and take them with them when they migrate. Thus this symbiosis can have very elaborate and protective mechanisms, turning the ants into true honeydew-producing breeders. Similar symbioses have also been recorded between ants and butterfly caterpillars, which also produce a nutritious secretion for the ants (they may have a special gland for this purpose). Some butterflies need the help of ants to survive and vice versa. Butterfly adults can feed on honeydew and so are also related to phytophagous insects; caterpillars too are known to feed on both ants and aphids.
The interactions that can be recorded in nature are amazing and incredibly complex. The balances are delicate and depend on multiple factors that we are largely unaware of but that we destroy every time we simplify and intervene in an ecosystem (e.g. ploughing, spreading herbicides and insecticides). Even the nomadism of thousands of bee colonies creates havoc in natural balances. Think of the honeydew which, with the arrival of bees travelling by road, is suddenly competed for by ants, butterflies and millions of bees cared for by man and disconnected from these natural balances. This cannot happen in industrial monocultures where the delicate trophic balances have long since been destroyed.
Hives that are placed near honeydew-producing forests may suffer from a lack of pollen, which results in a reduction in the number of juveniles in the colony: by the end of the summer there will be almost exclusively adult bees. This unnatural negative condition is favoured by the presence of artificial forests made up of few species, but is compensated by nomadism and the use of pollen replacement feed.

BEESWAX IN THE FOOD AND PHARMACEUTICAL SECTORS

Wax is produced by four pairs of abdominal glands in worker bees of two or three weeks of age (the glands degenerate in foragers) and is made up of 80% hydrocarbons and lipids (wax is made up of over 300 different molecules including esters of fatty acids and alcohols, paraffins and free fatty acids). A colony of 50,000 bees can produce 200-300 g in a day (it is estimated that up to 1 kg of wax is produced in the colony for every 40-50 kg of honey). [974]

The beeswax, represents a versatile zootechnical product used in the realization of various products of common use. In fact, beeswax in addition to being recycled by beekeepers is used in different categories of products: [57, 974]

- pharmaceuticals (ointments, pill coatings);

- cosmetics (face creams, lipsticks, soaps); one of the earliest recorded applications of wax as a cosmetic (mixed with olive oil, water and perfumes) dates back at least 2000 years;
- foodstuffs (in chewing gum, as a sealing material in food wrappers, in icings and confectionery);
- other products such as polishing agents (e.g. floor polishes), varnishes (the Egyptians between 60 and 230 a.C. certainly used wax to produce a kind of varnish), candles etc.

The wax that finds applications in the food sector is used as a coating agent in biscuits containing ice cream, as a glazing agent in chocolate or cocoa products (this plant also depends on entomophilous pollination), in candies and chewing gum, flour-based confectionery, for the surface treatment of fruit such as citrus fruits, pears, apples, peaches, mangoes and avocados.

Beeswax is the best matrix among beekeeping products to have information on chemical treatments carried out in the field and by beekeepers, even months in advance. Measuring pesticides in beeswax provides information on any illegal use carried out by both beekeepers and farmers. [55] Pollen is the best matrix for obtaining information on treatments carried out by farmers.

Wax can remain in the hive for several years and for this reason it is often the most contaminated matrix in the hive. [54] Wax is recycled by beekeepers and unfortunately can easily accumulate volatile, fat-soluble and persistent substances. The persistent and heat-resistant molecules that can be found in wax (e.g. lipophilic pesticides) pose a significant risk. Compared to honey, live bees and pollen, wax can record the highest concentrations of acaricides used by beekeepers such as coumaphos, chlorfenvinphos, fluvalinate and acrinathrin, found in 75% of 133 samples. [55] There is no official monitoring of the risks generated by the ingestion or cosmetic use of wax contaminated by pesticides or other molecules (e.g. pollen for allergy sufferers). In Europe, there are no quality standards for beeswax products, although in some countries there are internal rules.

As regards the food sector, beeswax is identified with the acronym E901 (*food additive based on shellac and beeswax, natural purified secretion, formulated with emulsifiers and soluble agents*) and is authorized as a food additive, with multiple technological purposes, especially in the confectionery industry and in bakery products. However, beeswax is not considered in the *food* category and is therefore not subject to any kind of quality control (EC Regulation 178/2002). The same applies to non-food applications such as cosmetics or pharmacology: although it is a fundamental element in the production of soaps, creams, ointments and other cosmetics, especially in the organic and natural product categories, no homogeneous quality criteria have been established for beeswax, consistent with the commercial sector in which it is used. [70] In Italy, a Technical Regulation (no. 16 of Sincert, update 05/11/2018) provides guidelines for the control of raw wax. [77] However, maximum residual limits (MRLs) are only established for five acaricides used in beekeeping and apply only to wax intended for the production of wax sheets used in organic apiaries.

PROPOLIS

Propolis is a resinous, brown substance produced by tree buds (resin, gum, exudate) that bees process and use to close holes and crevices in the hive and to resist attacks by bacteria and fungi. Propolis is collected by bees from the buds and bark of various plants such as poplar, birch, alder, spruce, fir, pine, plum, cherry, elm, oak, horse chestnut and ash. Propolis is obtained from plant secretions to which salivary and enzymatic secretions are added by bees (it consists of 70% resin, 25% wax and 5% volatile oils). [13] More than 200 different substances have been identified in propolis. Its composition differs according to geographical origin and

therefore depends on the plant species from which it is extracted. Propolis can contain 5% pollen and is obtained from the resin of plants mixed with salivary secretions and with wax produced by the abdominal glands of bees (it can contain sterols, essential oils, terpenes, amino acids, flavonoids, phenolic acids, aromatic esters, alcoholic phenols, keto-phenols, coumarins, lipids, polysaccharides, waxes, vitamins, and proteins). [62] According to many Authors, propolis is the bee matrix with the highest concentration of metals. [74]
The necessary resin is collected in baskets in which pollen is also carried. Propolis was used by the priests of ancient Egypt for mummification and in the 18th century to make a varnish suitable for wood and used by the lute-makers of Cremona (Italy). The Greeks used it to accelerate the healing of wounds and today propolis is used in pharmaceutical and cosmetic preparations, mostly as a component of creams but in low proportion, because it can give rise to cases of sensitization (propolis dermatitis). [1] A 6500 year old human jawbone found in Istria has a cavity filled with wax and propolis. [972] Propolis is collected by scraping the hive where it has been deposited and a few dozen grams per hive (23-88 g) can be collected. [35]

BEE BEHAVIOUR

Bees are fascinating insects because of their social behaviour which is generated by a brain consisting of less than a million nerve cells (they fit in a pinhead). [254] For comparison, the human brain contains about 90-100 billion neurons and at least 10 times as many support cells (called glial cells). Compared to bees, a fruit fly (*Drosophila melanogaster*) has five times fewer and a cuttlefish 40 times more. Nerve stimuli cease to function when the temperature drops below 5°C and in the laboratory it is possible to frustrate short-term memory with cold temperatures. One of the interesting aspects of bees is their eyesight, which is provided by about seven thousand punctiform eyes (ommatidia) that can also see ultraviolet and polarized light. Bees are capable of perceiving tactile, odorous and visual signals, but they also have taste and perceive electrostatic fields thanks to their very small nervous system. They are also capable of amazing behaviours. Bees can memorize and remember the time when food is available. Under experimental conditions, bees rewarded at a particular time on one feeder and at a different time on another, learn to fly at the right time to the proper place. [254]

At least 24 types of viruses have been identified in honey bees that may differ in the type of impact they generate. [230] The major cause of virus spread is the mite (*Varroa*). [13] This mite can be considered a bee vampire. Voluntary removal without return of sick bees (altruistic suicide or social immunity) has been recorded.
Bees can defend themselves against enemies by stinging but in the case of small parasites like mites or wax moth this system is useless. Among the social defense behaviours against mites that are settled on the body of bees, it has been observed that they can bite the parasite with their mandibles. At least two genes have been identified that give bees a natural ability to defend themselves against *Varroa*. [974]

The sense of smell of bees is amazing in that they can distinguish substances that differ by even one carbon atom (different alcohols). Odour receptors located on the antennae allow them to perceive odours spatially (with the trill of the antennae; over 170 genes for odor receptors have been identified). [974] The bee uses this capacity to orient itself in the search for flowers and inside the hive (it is able to perceive olfactory stimuli emitted at a distance of six milliseconds, and thanks to this speed it is able to discriminate the presence of odours coming from sources in different positions). By examining the odour, the bees can perceive whether their similars come from the same queen or from the same father, as the queen bee mates with different males. When bees are sleeping, they have their antennae down.
Regarding taste, receptors are present on the tongue and on the whole body (e.g. on hairs).

As far as their ability to communicate is concerned, they can inform their sisters about the position of flowers, water and resin in the darkness of the hive using dances, vibrations and electrostatic fields. A colony of bees forms what we can call a super-organism as there is a complex social organization. Bees perform different functions depending on their sex and age and are able to orient themselves and communicate information in a fairly sophisticated way. They mainly use chemical signals to communicate but can also use vibrations, sounds and movement. [231] Bees are able to determine the quality of nectar (e.g. sugar concentration) and communicate the location of the food source with two types of dance. When the site is less than 50-100 m from the colony, bees perform a circular dance alternating left and righthand rotations. The circular dance is performed after having delivered most of the forage and consists of describing tight circles while continuously changing direction. All this takes place in the dark on the combs inside the hive. The bees that have followed the dance go out in search of their forage and when they return they repeat the dance. Therefore, if the flowers are rich in nectar and pollen, the number of bees enlisted in the search increases rapidly.

Another dance is that of the abdomen (the waggle dance), performed to communicate interesting places further away such as food sources or the place where to build a new hive (up to 10 km away). [254] In this case the bee on its return makes a movement in the shape of the number eight. During the straight stretch it vibrates its abdomen with a pendular movement (12-15 vibrations per second). It makes circles in which the longer the movement phase of the abdomen, that is the rectilinear one, the greater the distance from the source of pollen or nectar. During the dance a buzzing sound is also produced. We can consider the rectilinear run a ritualized version of the flight that the other workers will have to do to go from the hive to the target. The angle with respect to the Sun of the rectilinear stretch provides the direction to be communicated; the bees perceive the position of the Sun even in the presence of clouds, through the polarized light that filters through. The duration of the wagging walk (performed in the dark and in perpendicular honeycombs) is proportional to the duration of the flight outside. A duration of one second of the oscillating movement of the abdomen combined with the buzzing represents an average of one thousand metres of flight. [258] The angle made by the walk, in relation to the vertical line of the honeycomb represents the angle of the outward journey in relation to the direction of the Sun. So, if a forager dances straight upwards it indicates that the flowers are in the same direction as the Sun. Whereas if the wagging walk is tilted 50 degrees to the left it means to fly in the direction 50 degrees to the left of the vertical. A bee that communicates the presence of a site full of flowers rich in forage will be able to perform a dance consisting of over 100 laps completed in more than 200 seconds, while another bee that communicates a less interesting site will be able to perform only 15 laps in 30 seconds. So there is a relationship between intensity and duration of the dance and richness of the site. The more attractive the site, the shorter the return walk from the straight wagging dance. This will make the dance appear more lively.

Bees also produce different types of buzzes (180-800 Hz) that communicate different messages and can be measured and used to predict behaviours such as impending swarming or queen bee death. Video and audio recording systems can be used to monitor and analyze these behaviours. One barbaric application involves clipping the wings of the queen bee to prevent swarming. [764]

Cannibalism is known to occur among bees, for example among larvae. [13] This behaviour may be a response to pollen deficiency. Bees reared in pollen deficiency tend to become foragers at an early age, can easily die during the first day of flight, and if they return to the hive they rarely dance. [35]

A colony that has lost its queen is recognised by neighbouring colonies and can be pillaged. In the presence of the queen bee, one worker out of every 10,000 may have functioning ovaries and begin to lay eggs, but these are recognized and eliminated. The queen marks her eggs with a pheromone, so that other eggs laid by the workers are easily recognised by the workers and destroyed. In addition, the worker bees recognise those that have started to lay eggs and eliminate them.

One of the interesting behaviours of bees is swarming, i.e. the phenomenon during which a part of the worker bees remains in the hive and raises a new queen, while the others (at least 10,000 bees) fly away together with the old queen to create a new colony (by swarming, between 30% and 70% of the bees leave the hive with the old queen). Before swarming, which takes place between April and July, the bees raise ten or more queens, all daughters of the queen mother (in Piedmont, swarming usually takes place between April and May). The swarming bees initially gather in a cluster less than thirty metres from the hive, where they will remain for a few days at most. From this swarm hanging from a branch, several hundred bees will leave in search of a new home, exploring up to 70 km^2. The explorers, older worker bees, will identify at least a dozen different sites chosen using certain criteria. At the end of a democratic process the colony will have to choose only one. The fascinating mechanism of this process is the democratic selection of the best site for all. A collective real estate evaluation of the site (e.g. a tree cavity) is carried out, so to speak, and the site must have the minimum characteristics to convince the majority of the colony. Through the manifestation of a collective intelligence, a crucial choice must be made by about a kilo and a half of bees. The swarm has only a few days to make the choice and occupy the new nest. The time at their disposal is governed by the stock of honey they have taken from the old home before leaving (about 50% of the body weight of swarming bees is made up of ingested honey). The decision mechanism compares different options presented by different scout worker bees. Each one tries to convince the other bees and the option that first passes a consensus threshold will be chosen. The bees involved in the exploration and dance can be between 2.8% and 5.4% of the total number, i.e. between 300 and 500 if the swarm is composed of 10,000 insects; this is the number of bees that actively promotes the debate. [258] In a few hours or a few days, a group decision is made on which the survival of all depends. Initially, each explorer bee will perform the dance indicating the direction of the potential nest discovered; however, if you observe all the dancing bees (or most of them) just before swarming, you will notice that they are indicating the same direction. Some interesting observations have revealed that the number of bees that must be in favour of the site is at least 75 worker bees. That is, it is sufficient that at least 75 scouts have visited the site and deemed it suitable by promoting it with dance for the swarm to move in that direction (plus or minus 1% of the swarm). It is a kind of *quorum*[I]. Before departure the bees warm up their muscles for the flight and make a characteristic noise. The choice of the best site has been made and they prepare for the collective flight that will take them to the new home. In this dimension the bees can be seen as a single collective intelligence of a few pounds. One possible lesson from this behaviour is that promoting open and fair competition among different factions can be a good solution to the problem of having to choose from a scattered set of information available among a group of individuals. The dancers convince other foragers to go and visit the site, and when they return, if they have evaluated the new site positively, they will in turn dance to promote the same proposal. The liveliness of the dance will be proportional to the quality of the site. Over time, the number of female explorers dancing to promote a site increases, as new female explorers are recruited if the likely dwelling has the best characteristics: volume of about 40 L, entrance of about 15 cm^2, exposure of the entrance and absence of ants. Poor sites generate weak dances and tend not to attract bees that will replace them (reports of poor sites becoming extinct). Initially one or a few scout bees have discovered an interesting new nest and by the end of the decision process most or all of the hundreds of scouts will dance promoting

[I] The *quorum* defines the minimum number required for the legal validity of the deliberation or vote of an assembly, a council, in elections or *referendums*.

the same information. Intermediate behaviours may be recorded, although rarely. For example, two groups in the swarm may split because they do not agree on the decision, but as there is only one queen, they move a short distance apart and the two swarms reunite. The decision-making process through the search for consensus is also operated among men, but it is more common, in our case, to delegate the choices to selected groups of people (from the secretariats of the parties winning the elections or with a direct election). Once decided to leave, the swarm takes off with the queen bee. She emits a hormone (called mandibular hormone which is a fatty acid with 10 carbon atoms) which, as long as the bees in flight perceive it, they continue to fly to the new nesting site. If the queen stops to rest and so they cannot pick up the scent, they stop moving forward and search for her until they reach her and wrap themselves around her. The bees that know the direction in which to move are less than 500 out of 10,000-11,000 (at most 5%) and they guide the whole group to the destination that has been marked with a special hormone (a product of *Nasonov*'s gland). The swarm flies between 5 and 12 km/h, even if the bees individually can fly faster.
Interestingly, the group is organized in such a way that the deliberations of individuals in direct confrontation result in a widely shared collective reasoning so as to reduce the probability of making wrong choices. This method has went through a selection over more than 30 million years, i.e. over the estimated time of existence of bees. Bees choose a nest in a democratic way, without a leader among the explorers, a sort of search committee composed of a few hundred bees. So bees make important decisions for their survival without needing to have a leading exponent.

The study of the behaviour of bees, regulated mainly by chemical signals, has made it possible to achieve goals that are both astonishing and worrying. Today it is possible to buy hormones that are released into the hive in a controlled manner and remain active for up to a month. In this way it is possible to induce specific behaviours artificially. As an example, it is possible to buy mixtures of hormones produced by the queen bee for different purposes: [224]

- Increase pollen collection and reduce drone production.
- Keeping bees quiet in the absence of queen bees as happens with "bee packs" i.e. groups of worker bees (about 10,000 individuals or less) used for pollination during the short period of a flowering (2 weeks) and abandoned to their fate, i.e. death.
- Prevent looting that bees do against other colonies of the same species.
- Stop swarming, which can also be artificially inhibited by the beekeeper by clipping the queen bee's wings so that it cannot fly.
- Encourage the production of wax and, therefore, honeycombs.
- Attracting bees within a queenless hive (these calls mimic the attractant pheromone that explorer bees release from their glands to mark an attractive location).

To sum up, it is possible to chemically induce specific behaviours useful to the beekeeper and farmer by purchasing specific chemicals (hormones).

Smoking is used with the intention of taming the bees which, when they smell the smoke, move to the combs and greedily eat the honey. This behaviour is probably a consequence of the danger of fire and therefore the need to leave the hive to save themselves. The use of smoke to calm the bees and facilitate inspection by the beekeeper, if poorly managed, can lead the insects to abandon the hive. The first portable smokers date back to 1873. [974]

BUMBLEBEES

Bumblebees are wild bees living in small colonies that appeared on Earth about 30-40 million years ago. [688] Bumblebees are probably vegetarian descendants of wasps. There are at least 250 species, 48 of which are found in Europe; for comparison, there are about 25,000 known species of bees. They live in matriarchal colonies headed by a queen, the only fertile female in the family. [244] The queens who survive the winter, already fecundated, found a new colony. Therefore, the queen is the only one to survive the winter in a sort of hibernation.

The queen bumblebee usually mates with only one male; during mating the male deposits a sticky glue on the female so that another male cannot mate. The first born are all females, the workers, who take care of community work: they produce the wax for the enlargement of the nest founded by the queen, they collect and store the nectar and pollen to raise new offspring and to feed the queen, who now only takes care of laying eggs. At the end of summer some females are bred to become queens. The females, as for the honey bee, are equipped with a sting, but unlike these they can sting a human being several times by injecting the poison without dying and can also bite (the poison contains various substances such as histamines). The workers of the bumblebees, contrary to what happens among the workers of honey bees, can be very different in size. This variability is also seen in some ants where different workers have different bodies as they have adapted to do different jobs. In bumblebees, the smaller workers usually remain to carry out work in the nest, while the bigger ones are the explorers capable of carrying in flight a quantity of food equal to their body weight (about 150 mg of nectar in the digestive tract and pollen on the legs). The explorers have a life expectancy of about two or three weeks.

Bumblebees can be very cold-hardy: *Bombus polaris* lives within the confines of the Arctic Circle. Many species of bumblebees like to nest underground, but since they are bad diggers, they explore existing holes such as mouse holes. Some species of bumblebees (*Bombus pratorum*) nest in old bird nests and can drive the owner away, as is the case with *Bombus niveatus* which is able to drive away the redstart despite being much smaller than this migratory bird. [688]Other bumblebees can nest in holes in tree trunks (*Bombus hypnorum*).

Knowledge of the ecological habits of insects such as bumblebees makes it possible to open a parenthesis on the importance of no tillage, the presence of trees and birds and the presence of flowers. All these factors are indispensable for bumblebees and thousands of species of insects useful to farmers. The ploughing of the soil, the absence of semi-natural or natural areas, the absence of trees, the extinction of birds and the use of herbicides prevent the survival of these useful pollinating insects and others.

The bumblebees produce wax in special glands which is used to build spaces to contain honey and pollen. The queen bumblebee can hatch her eggs as birds do in order to keep them around 30°C (they warm up by vibrating their muscles). The female, to keep the eggs warm, can consume a quantity of sugar equal to her own weight. It means that every day, in order to incubate the eggs, it may need to visit thousands of flowers. During the flight it beats the wings at the incredible speed of 200 beats per second and can move at 25 km/h. For this reason, if the flowers are few and far from the nest, the eggs will cool down and the bee risks not having the necessary energy to establish the colony formed by up to a few hundred worker daughters (all originating from fertilized eggs). Worker bumblebees can perform several functions such as searching for pollen and nectar and cooling the nest. At some point, males or other queen bumblebees appear in the colony. As with the honey bee, males are derived from unfertilized eggs generated in the summer, following specific hormonal signals. Queen bumblebees mate only once and then hibernate.

Among the curious behaviours observed among bumblebees it is interesting to remember some of them. If the terrestrial bumblebee's tongue is too short to reach the nectar of a flower, it can make a hole to take it away: in this case it does not act as a pollinator.
When the bumblebee settles on a flower it leaves a chemical trace that warns the next likely visitor not to waste time because it has just been emptied (with its legs it leaves volatile molecules). Different flowers have different filling times (in the order of minutes or hours) but bumblebees seem to recognise in every type of flower if it is a waste of time or not: perhaps they correlate the odorous trace of the flower with the intensity of the chemical trace left by the previous visitor.
The tomato plant needs bumblebees in order to implement pollination, as they are able with their movement to extract pollen grains and transport them. In this regard, it should be remembered that Italy is the third greatest producer of tomatoes in the World (2015), the first in Europe and produces 48% of the transformed product in the European Union. [452]

HEREDITY AND SOCIALITY

In humans, each individual has 50% of the genetic characteristics of one's parents and 50% of one's offspring and siblings. In addition, there are two X chromosomes in women and one X and one Y in men (these are the sex chromosomes). In bees such as *Apis mellifera* and bumblebees things are different. First, sex is determined by a single gene. Female bees have two copies of each chromosome as they are derived from mating between male and queen bee and are diploid. So, if the insect has two copies of the gene responsible for sex, it is destined to become female. If on the other hand there is only one or two identical copies of the sex gene it is destined to become a male. Therefore, males do not have a father (they are haploid) and all sperms contain the same set of genes (they do not do meiosis; in males, recessive characteristics will occur more frequently than in females because there will not be a second allele in the homologous chromosome).
A drone can ejaculate between 0.9 and 1.7 microlitres of seminal fluid containing between 3 and 12 million spermatozoa, all genetically identical. This lineage system generates degrees of relatedness different from those of the human species. The daughters are 50% related to the mother and 50% to the father, as they derive from the fecundation of the egg with the sperm. However, they are 75% related to each other as they all receive the same genetic heritage from the father and on average share 50% of the genes with the mother. At the same time the father has 100% of the genes passed on to his daughters, but none in the males. So a worker bee is 50% related to its mother and 75% related to its sisters (if they have the same father). The sisters are more related to each other than to the mother. The mother shares 50% of the genes with her male offspring (the female *Varroa* mite, a feared parasite by beekeepers, generates haploid eggs which will give rise to males and diploid eggs, i.e. fertilized, which will give rise to females, as bees do). One result of this particular transmission of hereditary characters is that worker bees, if they decide to mate, will produce offspring that have 50% of their genes, while if they leave reproduction to the queen bee they will see an increase in the number of individuals that have 75% of their genetic characteristics. Therefore, the worker bees are better off leaving reproduction to the queen bee and helping her sisters to increase the copies of their genes within the colony. This invention of evolution has favoured the highly social behaviour of bees (honey bee and bumblebee) and that of ants. It is more advantageous to help the mother and sisters produce other sisters than to reproduce independently. It is not advantageous for sisters to breed males as they share only 25% of the genes with them. The queen bee could equally benefit from breeding both males and females with whom she shares 50% of the genes, while the sisters should only breed other females (who will be sisters). But worker bees from

unfertilized eggs might produce male offspring with whom they would have 50% of the genes in common instead of the 25% they have with their male siblings. So there is a conflict between the interests of the queen bee and those of the worker bees. The queen bee has no interest in having grandchildren with whom she would share only 25% of the genetic characteristics. Bees have developed many mechanisms to reduce this conflict such as cannibalism: in bumblebees the queen bee eats the eggs of the worker bees. In some species of bumblebees up to 10% of males may originate from the workers instead of the queen. Sometimes the workers may even kill the queen bee. However, most males usually derive from the queen bee and in the case of honey bees, after the breeding season if they return to the hive they are killed.

One interesting behaviour is that of the cuckoo bumblebee. The cuckoo is a bird famous for its particular reproductive behaviour: it lays its eggs in the nests of other bird species and the chick pushes the other chicks out of the nest, killing them. Other birds also adopt this behaviour such as moorhens and some ducks. The advantage of this behaviour is that more offspring can be had. Some studies indicate that within the colony of bumblebees more than 95% of the males derive from the queen and the remainder from the daughters of the queen, but a small percentage from workers who come from other colonies. In the colony it is not in the interest of the worker bee - daughter of the queen - to lay eggs as she is more related to her sisters than to her sons. If a worker bee manages to enter another nest and is therefore not related to the queen bee, she has an advantage in laying eggs because she increases the probability of passing on her genes through care in a different colony. This behaviour has been observed in at least two species of bumblebees (Bombus *terrestris* and *Bombus deuteronymus*).

A behaviour observed in some bumblebee species is the intrusion of a queen bee into a colony of a different bumblebee species. The intruder kills the queen of the colony and thus the early stages of colony care are avoided. The workers who will not be related to the usurper become slaves. This type of cuckoo bumblebee has a bigger and stronger queen bee so that it can easily defeat the rival queen. At the same time, it does not have the structures used to collect pollen on its legs because it does not need them. The invading queen bee will lay her eggs and the slave workers will take care of them until she dies. Then over time the slave workers will be replaced by the daughters of the invader and the cycle continues. At least 30 species of cuckoo bumblebees are known, each specializing in colonizing another species (*Bombus vestalis* targets *Bombus terrestris*). To reduce the aggressiveness of enslaved colonies, cuckoo bumblebees often mimic both the colours and chemical signals of their target species.

ANTS: DIVISION OF LABOUR AND COOPERATION

The animal kingdom has no need of our imagination to reveal itself as extraordinary as are the biologies of insects such as bees, ants or bumblebees. Ants form a very fascinating superorganism. To overwhelm the enemy, they can use multiple strategies such as chemical deception, specialized surveillance and mass assault. In extreme cases, some ants may throw pebbles at their opponents, while others carry out slave raids to increase their workforce. Ants have probably existed for 100 million years, so they originated at the time of the dinosaurs. The species of ants counted in Italy are about 270, more than 500 in Europe and there are probably more than 15,000 in the World. [825] Also in Italy ants are very useful gardeners as they contribute to the distribution of seeds.

Ants, like social bees, live in large communities with a very complex organization that provides numerous advantages in cases where resources are abundant. In general, solitary insects are better pioneers and fare better when resources are scarce. Ant societies probably originated from aggregations of solitary wasps.

Ants are interesting in several ways that, in part, link them to bees: [710]

- Like bees, some species of ants participate in pollination and feed on nectar (e.g. some species have been found to contribute to the pollination of orchids).
- Like bees they form a female society in which the queen has the exclusive task of reproduction. In some ants the development of larvae is influenced by temperature: those reared at higher temperatures tend to develop queens. [974]
- The queen ant produces pheromones that inhibit egg production by the workers and virgin queens (in this case if the queen is removed the workers will begin to lay eggs, if the corpse remains inside the nest the pheromones will continue to inhibit fertility).
- The ants are all sisters as they are daughters of the same queen who will produce males only from unfertilized eggs; males are present during the short periods of the year of reproduction and usually live a few weeks.
- Even in ants, feeding can determine whether they will become workers or queens. In some species it has been found that, instead, it is the temperature that determines the fate of the eggs.
- They can have a sting comparable to that of bees.
- They have a complex organization formed by different castes among which that of the worker ants is the most numerous, as in the case of the honey bee; contrary to the latter, the different castes have very different bodies and the colonies can be much more numerous as they can include millions of individuals. Among the ants there can be many more castes, for example, among the worker ants they will also have very different bodies (the soldier ants can be even hundreds of times bigger than their sisters who carry out other tasks).
- They breed insects that produce the sugary honeydew on which bees also feed (aphids and scale insects); the term "honeydew" is used by entomologists to indicate the excrement of aphids that are rich in sugars and nutritional principles useful to ants and other animals such as bees; some ant colonies can receive a large part of their dietary needs from these secretions.
- They are good bio-indicators, e.g. of the state of health of the soil and the forest; in some areas of the World it is possible to find hundreds of different species in a few hectares (e.g. 300 species in 8 hectares of Peruvian rainforest). [825]
- Ants can plunder bees: a large wood ant (*Formica rufa*) can consume many tens of kilos of honey per year. The Argentine ant (*Linepithema humile*) has arrived from South America to Europe and Italy. It is classified as a very dangerous invasive species and is able to damage bee colonies by feeding on eggs, larvae and young bees. [972] The ants, however, can also play an important role in maintaining hygiene within the colony, picking up dead insects and other organic waste. [973]
- Formic acid - which is produced by some ants to defend themselves (natural chemical warfare) - is also used by beekeepers, for example to control the larvae of parasitic beetles (such as those of *Aethina tumida*).
- Males and females of many ant species make nuptial flights, therefore they form flying swarms to mate; the queen ant after the nuptial flight takes off its wings and begins to look for a suitable place to form a nest. In general ants are longer lived than honey bees as some queens exceed 20 years and those of some termites live even longer (up to 50 years). [972]

  As in the case of bees, worker ants renounce having offspring for the benefit of the colony. Sisters are derived from a father who originated from an unfertilized egg, so they share three-quarters of their genes. All sperms produced by a male ant or bee generated to produce their daughters are the same. Sisters are genetically more related to each other what happens in other animals: 75% instead of 50%. This inheritance favours altruism: sisters are more similar to each other than to their potential offspring. It is more convenient to breed sisters than daughters, if the goal remains to transmit the greatest number of similar genetic characteristics. It becomes favourable to care for one's mother who will produce sisters who are genetically more similar than potential daughters. This altruistic mechanism is not so strong with males who will have only a quarter of the genes in common with their sisters. Therefore, it is better to take care of few males and only when it is necessary to inseminate the queens: this is what happens among the colonies of bees and ants.

- Ants engage in fascinating social behaviours such as growing mushrooms and enslaving other ant species. In addition, unlike bees, they can interact between different colonies building a network of very complex interactions. Some species maintain social relationships between contiguous colonies, so they form super colonies that can occupy large areas (more than 25 hectares) and maintain contact with many colonies of conspecifics. In this case communities are formed that can exceed one hundred million individuals (*Atta cephalotes* ants can form colonies of 5 million individuals, the queen can live 15 years and can form nests 7 metres deep). [825, 826] It has been observed that some ants (*Oecophylla smaragdina*) can even maintain barracks nests with elderly workers standing guard at the borders of the territory. Ants migrate, go to war against conspecifics and other species, can reproduce by parthenogenesis, i.e. without males, produce poisons, silk and build constructions that can be very big.

Other amazing aspects of ants include the following:

- Brunei ants have guards that blow their heads off in case of a threat: the result is a sticky quagmire that slows down invaders. [848]
- They are famous for their ability to carry hundreds of times their own weight.
- They feed on a huge amount of insects, so the considered negative aspect of being able to spread phytophagous insects such as aphids and mealybugs can be compensated by the action of defending plants. A colony of wood ants can feed on hundreds of thousands of insects during the summer period, helping the plants. For this function they have been used in biological control: a colony of wood ants can collect tens of thousands of caterpillars in a single day. In the past, ants (*Formica rufa* or fire ants) have been proposed as a means of biological control against processionary moths. [720, 828] This butterfly generates larvae, the caterpillars, which use to move on the ground one behind the other, forming curious rows (the processionary moths). The larvae of the butterfly feed on the needles of conifers and are stinging. It is believed that four ant hills per hectare can be effective in controlling many plant pests.
- They participate in the useful process that makes the soil alive and fertile. They also feed on dead carcasses by participating in the recycling of organic matter.
- They are responsible for the spread of many types of seeds, so they are the gardeners of many plant species. Like squirrels, ants forget where they have hidden seeds and, therefore, participate in the spread of many plants such as herbaceous plants. A colony of some species can help disperse thousands of seeds each year. It is known that some species have learned to damage the seeds they carry in the nest so that they cannot germinate. Other garden animals include jays and nutcrackers, birds that disperse seeds from some pines. A representative example is the evolution of mistletoe, a parasitic plant, which takes up residence on the branches of trees. Mistletoe seeds must be placed on the aerial part of the plants which will provide nourishment. To achieve this, they produce berries that are palatable to many birds. The berries contain a very viscous gelatinous substance that remains intact as it passes through the digestive tract. When a bird feeding on such berries tries to clear its beak on a branch or defecates it distributes

the seeds on tree branches. Some birds seem to be able to choose the most suitable branches for the distribution of these seeds.

- The seeds of over three thousand species of plants contain a substance that is attractive to ants, called elaiosin, which contains fats. The ants find these seeds and bring them into the anthill, underground. Once inside, the seed is deprived of the elaiosin, which forms a fatty appendix, and thrown, still vital, on a heap of wastes. Here the seed can germinate. The seeds picked up by the ants are more likely to escape consumation from predators such as birds. The interactions between species, in some cases, are truly amazing and wonderful. In Australia where seeds with this gift packet for ants are widespread, there is a species of stick insect that produces eggs that mimic the seeds of the plant. The eggs of this insect also contain a gift for the ants which causes them to collect them and take them underground to protect them from pests. Some plants have evolved an even more elaborate mechanism. For example, some Mediterranean shrubs produce fleshy fruits inside of which there are seeds containing the elaiosome. Birds consume the fruit and evacuate the seeds with the elaiosome still attached. Then ants recover the seeds from the faeces and bury them. The plants in this case produce a double gift, one for the birds and one for the ants: in this way they will be able to travel much farther. We must remember that some seeds, as well as being space travellers, are also time travellers. Dormant seeds are able to slow down their metabolism and do not germinate, remaining viable for centuries. The oldest germinated one dates back to about two thousand years ago: it was recovered during some archaeological excavations in Israel. [704] The soil can therefore constitute a very important reserve of seeds. Nature's gardeners have long known that some seeds keep for years, while others must be procured every year. These examples are useful to underline the fact that the spread of a species can generate many unpredictable changes.
- Ants are able to favour certain plants by modifying the composition of the plant species in the surrounding environment: defending them from phytophagous animals, distributing seeds or damaging certain species. [710] The plants in exchange, as well as giving seeds, can offer water, shelter and nutrients such as fruit and nectar (hundreds of plants are known to offer shelter to ants). A symbiosis that has been known for a long time is that between acacias and some ants which, in exchange for hospitality, reduce the presence of competing plants in the close vicinity and defend them from parasitic insects.
- Other species such as wasps and beetles can also live inside the anthill.
- They can communicate not only with chemical signals (the rule is: anyone who does not smell like me is a potential enemy or possible food) but also with visual, tactile and sound signals. For example, ants buried because of heavy downpours can ask for help through sounds so that their sisters will dig them up.
- The castes of ants show sensational specializations such as the honey bucket ants (*Myrmecocystus mimicus*) in which some individuals have an enlarged abdomen where they accumulate nutrients to be used to feed the colony. [827]
- Queen ants of some species during the early stages of colony establishment, after nuptial flight and mating, may form alliances with each other. For example, a virgin queen may assist another fertilized queen in the search for nourishment. [720]
- More than 200 species of ants are able to be farmers, cultivating fungi which they then eat: they feed them with their excrements as well as with vegetables and defend them with bacteria. [848] Ants (e.g. the genus *Atta*) can cultivate fungi on fresh vegetation brought inside their nests. They behave like gardeners inside underground burrows. The largest nests of some ant colonies, consisting of millions of individuals, may consist of a thousand chambers, of which a few hundred are occupied by the mushroom cultivation.

- Ants can raise aphids and mealybugs by protecting them, caring for them and transporting them. Like shepherds, if the night is too cold, they take aphids from plant shoots and transport them in the warmth of the nest underground. In addition, some species feed on the sap or eat the leaves and fruits. This is why ants can be considered a pest by farmers.

Among the interesting behaviours that social insects such as ants exhibit, there is slavery, which is a form of social parasitism. Some ant colonies attack colonies of the same or other ant species. After neutralizing the adult defenders they kidnap the juvenile stages (the pupae). The kidnapped young ants are raised as slaves to increase the worker population. In extreme cases the slave ants are completely dependent on the slave-making ants. The evolution has produced very particular interactions between enslaved ants and slave-making ants so much that the latter do not escape but consider themselves sisters of their captors. [693] Therefore slavery can be optional or obligatory and can be temporary or permanent.

Another interesting feature of ants is their ability to hunt in groups, which allows them to prey on insects weighing thousands of times more than the individual ant. Legionary ants (*Eciton burchelli*) in Central and South America form colonies consisting of 300,000 to 700,000 adult specimens. During the migration phase, the colony is organized into several columns of workers, protected on the sides by specialized soldiers, while the queen is constantly protected by a group of worker specimens that climb on her body. The larvae and pupae which have not yet developed into adult insects are carried between the mandibles by the smaller workers. Columns of workers can exceed 50 metres in length and move 90 metres a day. During the migration period, a colony can kill more than 90.000 arthropods in a single day. [699, 824] More than 300 species have been identified that live in association with these legionary ants such as formicarian birds and some mites.

Like many animals, some ants exhibit the behaviour of feigning death when in danger and may inhabit the same nest with other ant species as roommates in an apartment building. Building an ant nest underground can involve moving over 100 kg of soil. Ant nests provide food for many animals such as other ant species and arthropod pests (spiders, aphids), birds such as black woodpeckers or pheasants, and lizards. The green woodpecker is able to eat hundreds of ants per meal. [848]

BEES AND TECHNOLOGICAL APPLICATIONS

BEES TRACKED BY MICRO-ANTENNAS

One of the technologies used by researchers to follow insects in flight such as bees (*Apis mellifera*) and bumblebees (*Bombus terrestris audax*) is the radar. [108, 109, 110] The harmonic radar[I] emits waves into the environment, which bounce off non-linear devices (e.g. an antenna fixed on the insect) and are then detected again by the radar itself. [111] It follows that the responders positioned on the insect do not need energy (batteries). The harmonic radars have a rotating antenna, a transponder fixed on the insect to track and need a computer with a special management program. With these instruments it is possible to follow the movement up to a maximum distance of 3 km, in the absence of obstacles such as trees or houses. [195] The radar is able to detect the position of the insect with a certain frequency (e.g. every 3 seconds): this is a limiting factor. [114, 1191] If the bees are moving at a speed of 20 km/h and if the antenna makes a turn every 3 seconds, it means being able to have an accuracy of 15 m, while if they are moving at 5 km/h it means having an accuracy of the position of about 5 m. The ability to track bees at a distance of 1.2 km is restricted between 70 cm and 9 m of height from the ground. So when they fly low or between plants they cannot be tracked. Worker bees (*Apis mellifera*) were probably first studied with this technique less than 20 years ago. [109] Thanks to these instruments, it has been possible to trace exploratory flights made to orient themselves outside the colony (these are the first flights made by the youngest bees) and those aimed at obtaining food (nectar, pollen and honeydew) and water. The flights in search of food are made by the most experienced bees, which are the oldest ones. Before starting to search for food, honeybees make exploratory flights to learn their bearings (the median distance of exploratory flights is 100 m from the hive; instead, bumblebees collect pollen even during their first flight). [112] Exploratory flights take time, cost energy (bees do not collect food during these flights) and present risks. These flights are also carried out again by experienced bees when the hive is moved (nomadic beekeeping). An experienced bee can learn to reorient itself in a single flight, whereas a young bee (three weeks old) needs an average of 6 flights (between 1 and 18 flights lasting about 6 minutes each; according to another study, 3 orientation flights are sufficient for an inexperienced bee) when it first leaves the hive. [108, 113] Thanks to the use of the harmonic radar, it was possible to study these different types of flight and the behaviour of the bees. The harmonic radar recognizes the insect in flight thanks to a responder which is a small antenna positioned on the thorax. This small antenna can be between 12 and 16 mm long, have a diameter of 0.3 mm and weigh between 0.8 and 25 mg: the weight includes that of the antenna, that of the label that marks the thorax of the bee on which the antenna is attached and that of the glue. [13] A worker bee weighs about 100 mg and can carry this weight quite easily, while a bumblebee weighs twice as much and can carry greater loads.

The instruments used by the researchers have a range of between 400 m and 1,000 m, with an accuracy of ±7 and up to ±2 m radius (and a height accuracy of ±3.5 m). [108, 109, 110, 113] It should

[I] The definition of harmonic radar is often used to specify electronic circuits or systems that process and handle high frequency electromagnetic signals. In the case of radio frequency signals by air, this refers to electromagnetic waves of frequencies between a few kHz and 300 GHz. Wavelengths range from 100 km (at 3 kHz) to 1 mm (at 300 GHz). Radio frequency is generally used to indicate an electrical signal or a high frequency electromagnetic wave propagating in space or in a coaxial cable. [111]

be pointed out, however, that bees can easily move to search for food for several kilometres, thus out of radar range.
The information one is able to get through the use of a radar is (if they do not go out of range):

- the time spent in the air;
- the speed of flight;
- distance;
- the route taken;
- the size of the area being explored.

This technique has shown that the flights of experienced foraging bees are faster (experienced bees can maintain an average speed of about 20 km/h), longer and more direct. [113]The bees have been shown to be able to return to the hive without any problems, even if artificially moved to an unfamiliar location, 4 km away. [112]
A prototype entomological radar has been developed to detect *Vespa velutina* nests by capturing individuals (e.g. while they prey on bees near hives) and tracking their flight to the nest, so that it can be detected and destroyed in order to control this enemy of the bees. This wasp (it is a hornet) feeds on bees and is a non-native species in Europe. [1297, 1298]

Tracking systems have some limitations: [108]

- The weight and bulk may be such that flight is hindered and behaviour is altered.
- They have the ability to detect signals only a few hundred meters away.
- They are unable to detect insects resting on flowers or on the ground, and those behind obstacles such as houses and trees. Below 70 cm above the ground the signal is lost.
- The need to have to glue the antenna on the bee at the moment it decides to leave and the need to have to be detached when the bee returns, before it tries to enter the hive (so in the time of a few hours or minutes, in the same day) are other limitations.
- Tracking is not very precise because it indicates a position within a radius of at least 2 m, so it is not possible to determine, for example, which flower the bee has landed on.
- Only one insect can be followed at a time.
- Daily between 7% and 20% of the bees do not return to the hive because they probably die (e.g. predation, insecticides). More than 15% of bees with receivers do not return to the hive, so in this case the signal and the transponder are lost.

Another system is an apparatus for identification through *radio frequency identification* (RFID). This system consists of an electronic label that is attached to the back of the insect. The energy needed to operate this device carried by the insect is provided by the reading instrument called scanner: therefore it does not need batteries like the transponder. When the bee passes near the scanner, it recharges the electronic tag by induction, without contact. [195] The electronic tag contains a microprocessor (chip), weighs a few milligrams (2-4 mg) and has a size of 0.8-1.6 mm^3 (a model of RFID-transmitter is the size of 2 x 1.6 x 0.5 mm and weighs 4 mg). [743, 872] This electronic tag can weigh 3% of the weight of the insect (3 mg); keeping in mind that the worker bee weighs about 120 mg and is able to carry 70 mg of nectar in the digestive tract and 10 mg of pollen on its hind legs. The transported label is detected when the insect passes at a distance between 2 and 4 mm from the detector (the scanner) which requires a computer (this distance is a limit). The label must be perpendicular to the scanner and must not be dirty (e.g. pollen), these are other limitations. [13] Therefore, it is necessary to place tunnels with the scanner that must form an obligatory passage for the insects. In this way it is possible to get information about the marked insects. [385]

Bee behaviour can be studied with different techniques and different objectives, such as detecting behavioural changes generated by insecticides. [195] Some advanced techniques are the use of X-rays so to measure non-invasively the strength and numerosity of a colony. [772] Another possibility is offered by high-resolution video cameras whose images can be processed with special computer applications. In this way it is possible to quickly measure surfaces, such as those of combs filled with larvae rather than honey, or areas occupied by empty cells.

DETECTION OF HAZARDOUS SUBSTANCES WITH BEES

Unfortunately, the construction of mines is simple and inexpensive, so their use is widespread. There are at least 50 million unexploded mines in the World (2001) and over 20,000 victims are killed or maimed each year in 90 countries (2003), 30% of whom are children. [115] If, theoretically, these devices were no longer produced today, it would probably take at least 400 years to make the current known minefields safe.

The search for buried mines and unexploded devices is very dangerous. Dogs (*Canis familiaris*), rats (such as the African giant rat *Cricetomys gambianus*) and metal detectors have been used to search for these devices, but these do not work with plastic explosives. The African giant rat which is a nocturnal rodent, widespread in most of Africa, from Senegal to Kenya and from Angola to Mozambique, is among the largest rodents in the World and has been trained to search for mines in Mozambique. [120] Major advantages over the use of dogs include a lower cost, the ability to easily enter sites where dogs cannot and their weight is not sufficient to detonate mines. Marine animals have been used to search for naval mines such as dolphins (employed in 2003 during the invasion of Iraq), sea lions and killer whales. [119]

Researchers have looked into the possibility of using the sense of smell of bees to detect dangerous substances. [116, 117, 118] Bees are able to detect gaseous substances at concentrations of parts per billion[I] or, in some cases, parts per trillion, thus at very low concentrations. Bees detect very low concentrations of explosives (10-100 ppt) such as 2,4-dinitrotoluene (2,4-DNT), trinitro-toluene (often abbreviated to TNT) and cyclotrimethylene-trinitroamine (or RDX, cyclonite or T4; it is also the explosive material of C4). [122, 123, 124, 125] Mines release a small fraction in the form of gas, which can be perceived by the bees' olfactory system. According to some scholars, bees are able to perceive 50 ppt of 2,4-DNT with less than 2% probability of false positives or false negatives. [126] In experimental field trials, it was possible to generate a congregation of bees over mines. [115] Probably the use of bees in real situations to search for mines is not yet possible, as there are many limitations, such as being able to follow bees within a radius of several kilometres (binoculars and a ladder are not sufficient).

Bees are conditioned to actively seek out particular odours, such as those of certain explosives, while receiving a food reward. [120] To increase attention and memory, bees are given rewards such as water and sugar with added caffeine (it is naturally present in the nectar and pollen of some plants in the genera *Citrus* and *Coffea*). [107] A solution of sugar and water with 25 ppm of caffeine has a greater attractive action than sugar alone, but at 150 ppm caffeine generates a repulsive action (caffeine improves the bees' memory). [718] By exploiting the *Pavlovian* reflex, a congregation of bees can be generated where a mine is buried. Bees can be forced into a small container and as soon as they sense a sugary solution they extend their proboscis to feed. Once the bee is conditioned, when given a certain odour, it extends its proboscis even when the known olfactory stimulus is not followed by the reward. Bees could also be conditioned to pollinate only certain plants.

A punishment, such as a mild electric shock, triggers the sting extraction reflex. Bees can learn that one smell is rewarded and another punished. [254] This learning process is rapid. Local colonies can be used to condition the bees, using soil contaminated by the mine and a solution

---

[I] The acronym *ppb* means part per billion (*bilion* is a prefix of the International System equivalent to 10^{12}), that is to say it indicates the presence of a particle in the air for every billion other particles; parts per trillion or *ppt*, a prefix of the International System equivalent to 10^{18}, are also used.

of water and sugar, so theoretically it is very simple and cheap. It is known that an agency of the United States (*Defense Advanced Research Agency*) has financed this type of research and that several university researchers have explored this application, as in the case of mine research in Croatia. [116, 121]

For the search of dangerous and forbidden substances, such as explosives and drugs inside vehicles and suitcases, dogs are used and, as mentioned above, the possibility of using suitably conditioned bees (conditioned reflex or Pavlov's reflex) has been evaluated using prototypes (*Vasor136 or Volatile Analysis* by *Specific Olfactory Recognition*). [3, 130, 131] These instruments exploit the proboscis extension *reflex* of bees conditioned to associate a specific odour with a food reward (water and sugar). A few hours are enough to condition the bees and, thanks to an apparatus that sucks in the air, the reflex obtained from the molecules present in the air sucked into suitcases, cars or other objects to be controlled is measured electronically and automatically. This instrument has a screen that shows the number of extensions recorded by a few dozen bees (36 in one particular model; the worker bees that have this most active reflex are those between the twelfth and eighteenth day of age). [133] A prototype advertised on the internet is the size of a portable vacuum cleaner and inside it houses a few dozen bees, each with an automatic electronic sensor that can detect the extension of the proboscis. The conditioned bees can do this work for a few hours and up to a week. [115] Theoretically, it would be possible to condition a greater number of bees to different substances and thus, with a single sampler, measure the presence of different substances (e.g. explosives and drugs). There are prototypes that condition 78 bees to perceive 12 different substances.

If it were possible to use these insects to search for explosives, instead of dogs, there would be several advantages, among which lower costs. The bees condition themselves in a few hours while the dogs get trained for several months, the weight of the dogs is enough to activate the mines so it is very dangerous, instead the bees return to the hive. Local bees can be used while using and maintaining wards with trained dogs is much more expensive. However, bees do not fly at night, in bad weather (rain and wind) and if temperatures fall below 10°C. During the winter they stay inside the hive and start flying when, for several consecutive days, temperatures exceed 15°C. [129] The bees may also be distracted by flowers. A prototype of an automatic system for conditioning bees in the field runs on solar energy, is remotely controlled, and can dispense particular odours (e.g. explosives) with a food reward (water and sugar). [4, 11, 16, 126, 128] In this way the bees are attracted to a certain area and learn to look for a desired substance or smell or particular molecules such as those in certain flowers. Taking advantage of the Pavlovian reflex can encourage the search for certain flowers instead of others. Automatic and portable systems can be installed for conditioning bees in the field. [3] They can run on solar energy, be remotely controlled and dispense particular odours with a food reward (water and sugar). [4, 11, 16]

The main problem is to be able to follow the bees during their flight in real contexts. There are instruments (LIDAR) that can detect the bees' wing beats and measure the points where their density increases. [16] The use of radar systems (LIDAR or Light Detection and Ranging) that produce laser light is limited to a range of a few hundred metres (maximum 1 km but more likely less than 150 m) and can only work in the absence of obstacles and barriers, in non-flooded terrain and in the absence of smoke or fire. [3, 128] The radiation produced by this instrument is harmful to human eyes.

The bees' antennae are able to detect substances in the air coming from corpses (they can detect molecules such as cadaverine and putrescine), certain types of diseases (e.g. skin and lung cancer or diabetes) and microorganisms in food (*Escherichia coli* and salmonella). [125, 131, 132] So theoretically several applications could be possible.

ANTENNAS AND ELECTRONIC DETECTORS: THE BIOSENSORS

Proteins present in the olfactory system of bees, particularly on the antennae, because of their ability to bind to substances such as drugs and explosives, could potentially be used to build biosensors. Among the substances that could be easily determined even at low concentrations there are cocaine, amphetamines, methadone, ecstasy and some substances found in cannabis, as well as explosives such as trinitrotoluene. [151] In this regard, it is estimated that about 5% of the World's adult population uses banned drugs (2013) and at least 200,000 people a year die mainly from heroin and cocaine. [152]
In bees (*Apis mellifera* and *Apis cerana*) at least 177 genes responsible for the recognition of volatile substances have been identified. These genes encode the expression of proteins (at least 43 have been studied in depth) which have the function of perceiving odours (e.g. pheromones). The first step in odour perception is the link between the gaseous molecule and the proteins located in the antennae of the insect. This bond produces an electrical stimulus which can be measured with electrodes. A possible futuristic application could be to build electronic apparatuses capable of measuring and quantifying the links between molecules such as heroin and cocaine with the receptors of the antennae of insects such as bees (*Apis mellifera*) in instruments called biosensors. Scientific work in Germany has led to prototypes using bee antennae connected to electronic sensors to measure drug concentrations in the air. It was possible to measure with electrodes the electrical stimuli produced by the binding of substances to the receptors of the bees' antennae. With a portable system for carrying out the *electro-antenna-gram,* it is theoretically also possible to measure the stimuli perceived by the antennae of other insects such as the grape moth (*Lobesia botrana* which is a lepidopterous tortricide, known for the serious damage it causes by feeding on grape berries) and the Madagascar whistling cockroach (*Gromphadorhina portentosa* which is a blattoid insect belonging to the family Blaberidae, endemic to Madagascar). [153] These insects have some useful characteristics: they are easy to breed and have a good ability to sense volatile substances. The antennae can be cut off and inserted into special portable electronic devices (chips). The electrical response to odorigenic substances is dose dependent. A study has shown that bee antennae are more sensitive in measuring the presence of heroin and cocaine, the antennae of the grape moth are more sensitive in measuring the presence of cannabis and the antennae of the Madagascar whistling cockroach are more sensitive in measuring the presence of amphetamines and caffeine (it is used to cut some drugs). [152] The different capabilities of insects could be used in multiple biosensors, capable of using signals from antennas of different species simultaneously. One could also use conditioned live insects and measure their reactions, as described previously (*Vasor136*), in special more or less automatic apparatuses. [131] Therefore, it might be possible to construct portable biosensors capable of exploiting the natural gifts of insects to make reliable measurements and monitor the illegal traffic of drugs and also of explosives. [120]

There are also new nanotechnological applications that make it possible to bind the individual proteins of olfactory receptors (not only of insects but theoretically of any animal such as pigs and dogs) with carbon transistor nano-tubes and measure the presence of odorous substances in the air at low concentrations. [154] In this hypothesis of biotechnological application the antennae are not used but only the proteins that are normally located on them. It is possible to bind proteins of the olfactory receptor system (*olfactory receptor proteins*) with carbon nano-tube transistors and build devices capable of measuring the binding between gaseous molecules and proteins of the olfactory system (they are microscopic biological interfaces between membrane receptor proteins and carbon nano-tubes). In practice, it is possible to build a kind of bio-electronic nose capable of exploiting the receptors built by nature. Bio-electronic noses, if properly constructed, can work for several months. As an example, it is reported that the proteins of the olfactory receptor of the mouse were used to test the capacity of measuring the presence of 8 different molecules in the air (e.g. eugenol: it is an oily liquid, almost colourless or light yellow, which is extracted from some essential oils, especially from clove oil and cinnamon; it is used in perfumery). [156] The establishment of the bond between the odour and the protein generates a change in electrical current that can be measured. These proteins bound in the biosensor were able to detect the presence of a substance that does not exist in nature, but is definitely sensed by mice as the explosive 2,4-dinitrotoluene (2,4-DNT).

Bees produce volatile organic substances that are used to communicate messages within the colony. To carry out this function they have 15 exocrine glands which produce various hormones, but also wax, poison, some enzymes (proteins) and the larvae produce silk. The odours emitted within the colony serve as a means of communication of a great deal of information and can be used artificially. [470] Some messages are very important such as that of the queen's pheromone, which in reality is made up of a mixture of 9 different substances, which favour the aggregation and stimulate the care of the queen bee by the worker bees. It is possible to construct special instruments in which sophisticated chemical equipment (gas chromatography with flame ionisation analyser) can be combined with sensors that measure the activity of the bees' antennae (bio-sensors). In this way, volatile substances and electrical activity, which is generated by one or more sensors linked to the insect's antenna, mounted between two electrodes, can be measured simultaneously: a chemical analyser is combined with a biological one. [470] With this technique it is possible to measure the stimuli produced by some chemical substances that could have different functions (e.g. repellents or attractants).

In addition to bee antennae, other parts of the body that have a sensory activity in response to chemicals can also be used. Artificial biological sensors can be constructed to study the responsiveness to specific chemicals that can bind to a single receptor or multiple receptors and stimulate one or more nerve cells. This type of chemical stimuli analyzers can employ the antennae (electro-antenna chromatographic analyzer), the head or the whole body of the insect. These applications raise ethical considerations that are not discussed in detail.

INSECT MACHINES: CYBERNETIC BEES

Cyborgs in scientific literature are living organisms in which some parts are replaced by artificial prostheses prepared with cybernetic (electronic and mechanical) systems. [119] Attempts are being made to develop electronic technologies to be implanted in the nervous system of bees (and other insects) to try to control their behaviour such as flight. [134] Some projects (hybrid insect micro electro mechanical system or HI-MEMS) have been initiated by the Department of Defense of the United States of America. [118, 135, 136, 137] This Department (through the Defense Advanced Research Project Agency or DARPA) invests millions of dollars in projects aimed at generating cyborg insects (bees, moths, butterflies, beetles or grasshoppers). An interesting attempt made to control the flight of the tobacco sphinx, a hawkmoth (*Manduca sexta*), is reported in scientific literature. [138, 139, 140] In 2008 during a conference one of the first examples of cyborg moth (*Manduca sexta*) was probably disclosed, in which the electronic apparatus was implanted in the pupal stage, so as to be successfully integrated into the body during metamorphosis. [119]

In the future, it will be possible to place a microphone, a gas molecule metre and a video camera in these micro electronic devices. Perhaps one day it will be possible to take measurements, search for toxic substances or take samples by guiding insects artificially.

With the bees, electronic instruments have tried to be implanted in the pupa, before the metamorphosis, so that the adults have a tissue that has integrated these components, inside the exoskeleton. [115] With this technique, also some systems of control of the position have tried to be implanted (Global Positioning System). Perhaps it will also be possible to try to distribute with the sting substances voluntarily added to the poison of the bees and to use their eyes instead of micro video cameras (*cybugs*). On this aspect, it is interesting to remember that bees are able to perceive ultraviolet radiation and do not see the red colour.

Some researchers are trying to control the flight and movement of insects by implanting interfaces that can artificially stimulate nerve areas or muscles. This path is preferred to that of flying micro-drones because one of the smallest prototypes built weighs 3 g, is 10 cm long and can fly for less than 3 minutes, before the batteries run out (the batteries make up at least 1/3 of the weight but cannot ensure a useful autonomy). [141, 145] Also, in order to fly, this small robot requires an experienced technician. It can be argued that the best man-made micro-machine cannot compete with the flying capabilities of insects.

There are wireless systems that can try to control insect flight. These systems have several electrodes, are programmable, have a memory and are equipped with a controlled radio system. They are probably only laboratory prototypes, work from less than 30 minutes up to a few hours, and can be used for a distance of 5 to 25 m between the control instrumentation and the insect. One operative principle is to implant electrodes in nerve areas (e.g. optic lobes of *Mecynorhina ugandensis*, a big African cockroach) that regulate the start and end of flight initiation. [141] By stimulating the left or right area differently, one can hope to regulate the direction of flight. The muscular mechanism is complex so we try to act on the nervous areas that initiate flight so that it is controlled naturally. The flight of an insect is regulated by many different muscles (more than 13) that are difficult to synchronize artificially with peripheral electrical stimuli. [142] During flight, in order to hope for a stable buoyancy, the abdomen and legs must be synchronised too.

Another problem is that of energy. In the future, these micro-electronic instruments could exploit the sugars present in the insects' hemolymph to obtain energy, so that they do not need a battery.

Energy must be available to stimulate the muscles artificially, besides having to overcome the obstacle of synchronism. In contrast, if you use the stimulus only to initiate autonomous flight rather than continuously stimulating it, you save energy. In the lab, you can initiate flight and terminate it, but you probably cannot avoid an obstacle in a guided way. For example, in beetle experiments you can manage flight artificially for less than 10 seconds. [142] Electrodes during the flight may detach, generating other problems. To get around this problem, attempts are being made to implant electronic devices in the larvae so that the adults have these structures integrated inside, forming true cyborgs (insect machines or hybrid robots). Currently there are attempts that have shown some success in controlling the flight of moths and cockroaches.

It is also possible to think of using other bigger insects that weigh up to 100 g for a length of 20 cm and can therefore carry greater loads. [143, 144] All applications involving the use of animals raise very important ethical issues: living beings are treated in the same way as machines or objects, and it is easy to imagine negative applications. [145, 146]

It will probably be possible in the future to tell bees which direction to fly in using robots. Small drones capable of flying are also being tested, which could replace bees, but for the moment they are probably confined to laboratories and science fiction dreams. [157]

Another possibility for mine detection could be the combination of drones and bees. The first drone could place a remote-controlled bee-conditioning dispenser at the site where the absence of mines is to be tested. Another drone could be equipped with video cameras and radar systems to follow what the bees are doing within a radius of tens of metres. This drone could carry bees already conditioned and equipped with an antenna. Airships or radio-controlled helicopters could be used to carry bee tracking systems in flight and on the ground. [118] Probably, given the utility, it might be worth a try.

Other future applications start from the study of the functioning of the brains of insects such as bees and ants in order to build artificial (electronic) intelligences able to guide drones and robots. The bee brain is about one cubic millimetre in size and is composed of 960,000 neurons (over 100,000 times smaller than the human brain). [35] Scientific research is engaged in designing artificial eyes and brains that through electronic circuits and computer programs behave like insect brains. [197, 207, 209] Thus these robots could orient themselves autonomously without the aid of GPS (Global Positioning System).

MILITARY APPLICATIONS OF BEEKEEPING

Memories of the application of bees in the military field date back over 2000 years when they were used to defend fortified positions. Probably the use of bees to defend from enemies is an even more ancient practice as there are Egyptian hieroglyphics that provide evidence of this application. [2] In the past, special weapons were built to launch these insects: for example, in Nigeria cannons were built specifically for this purpose and the Maya produced a sort of hand grenade to launch the bees. [2]

In a 400 b.C. treatise on how to survive a siege, it was advised to release bees into the tunnels dug by enemies at the foot of the fortifications. [115] The naval defense of the Greco-Roman empire could also have had bees in the arsenal, as they constituted a defense system against ships, being catapulted from the land.

In the first century b.C. toxic honey was used in Turkey to poison enemy soldiers. [972] Between the third and fourth centuries a.C., the Romans also used bomb hives, thrown against opponents, as a military technique. The Romans catapulted the hives inside the walls of fortifications. In 908 a.C. the English too defended themselves successfully against the siege of the Danes and Norwegians by releasing bees into tunnels. Swarms of bees launched towards enemies, due to stings, they generate fear and havoc. Bees were also used to generate havoc in cavalry (in 939 in Germany). [115] In the 1100 naked prisoners could be sprinkled with honey and subjected to bee stings as a method of torture.

During World War I, the Germans used beehives to delay the advance of British troops. During the Italian occupation of Ethiopia, locals threw bees into tanks (1935-1936). The same technique was used in Vietnam against American tanks.

Plants can produce less than one millionth of a litre and up to thousands of millionths of a litre of nectar per flower, the sugar content can vary between 7% and 70%. [764] Thus there is considerable variation in nectar content in flowers and, being a limited resource, insects compete for it. Nectar from some plants can give rise to toxic and in rare cases fatal honey. [148] This possibility has been exploited for another application recorded in the past: the use of honey produced from poisonous plant species, which is toxic to humans. Plants can produce either pollen or nectar or both that are poisonous to honeybees. In some cases, the harmful effects are manifested in honey.

Graianotoxins are a group of toxins produced by rhododendrons and other plants in the *Ericaceae* family (e.g. mountain laurel or *Kalmia latifolia* found in North America). Honey made from this nectar causes a very rare reaction called graianotoxin poisoning, honey poisoning or rhododendron poisoning. [149] A record of this strategy reveals that it was used to intoxicate 5,000 soldiers in 946 a.C. by a woman who thus avenged the killing of her son. [115] Probably, however, this type of intoxication was recorded as early as 401 BC. Some symptoms are nausea, vomiting, excessive salivation, dizziness up to more serious phenomena such as cardiac complications and heart block (in 42 intoxicated people recorded in Turkey). [148]

Honey from rhododendrons (*Rhododendrum ponticum* and *Rhododendrum luteum*) caused serious intoxication in Greek times. These intoxications are not fatal and have recently been recorded in the Black Sea areas due to plants containing terpenic substances called andromedotoxins or graianotoxins. [35] In some countries (Turkey and the Caucasus) the honey obtained from it, called *mad honey*, is used in small quantities as it is considered useful against abdominal pains and as an aphrodisiac. *Rhododendrum ponticum* has become an invasive species in some nations like the United Kingdom.

Another plant that can produce dangerous honey is the South African ragwort (*Senecio inaequidens* of the Asteraceae family). This plant can produce substances (alkaloids) with a hepatotoxic and carcinogenic action. Plants of the family Daphnaceae (*Daphne mezerum* and *Daphne gnidium*) are also poisonous and can produce dangerous honey. Buttercups are capable of producing another toxic substance (anemonin generated by *Ranunculus ficaria* and the genus *Delphinium*). [35] Also dangerous is the honeydew produced by a phytophagous insect (*Scolypopa australis* Hemiptera Fulgoroidea) that is a pest of a shrub in New Zealand (*Coriaria arborea*). 974

Below are some plants that produce nectar that can give rise to honey that is toxic to humans:

- *Aesculus californica*;
- *Andromeda* contains graianotoxins which are psychoactive and toxic (they can paralyze the limbic system and diaphragm);
- *Belladonna*;
- *Datura* present in Mexico and Hungary;
- *Hyoscyamus niger* (black henbane) which is a poisonous herbaceous plant;
- *Kalmia latifolia* and *Gelsemium sempervirens* present in America;
- *Melicopeternata* and *Coriariaarborea* present in New Zealand;
- *Nerium* oleander (oleanders) in the Mediterranean area;
- *Rhododendron ponticum* (*Azalea pontica*) contains alkaloids (in the flowers) that are poisonous to humans;
- *Serjanialethalis* present in Brazil.

Another possible military application, probably also used recently, is based on the principle of hoping to damage the health of bees in order to reduce the food self-sufficiency of an area. Damaging the pollination service reduces agricultural production. Spreading diseased colonies encourages the spread of parasites that kill bees. The population of Cuba has accused the Americans of having voluntarily introduced diseased colonies for this purpose. [2]

In conclusion, bees have been used in both offensive and defensive strategies for over 2000 years and probably constitute one of the oldest biological weapons used by humans.

INTRODUCTION TO BIOMONITORING

THE BENEFITS OF BIOMONITORING

Chemical indicators, such as pollutant and nutrient concentrations, or biological indicators can be used to verify the ecological characteristics of an environment. Analytical chemistry techniques allow the measurement of pollutants in different environmental matrices but have some drawbacks:

1) Anthropogenic activities distribute tens of thousands of different chemical molecules in the environment every day and it is impossible to think of basing the management, prevention and monitoring system of the state of health of the biosphere on the measurement of concentrations in different matrices such as water, soil, air and living organisms of such a high number of substances. This is an ineffective strategy with unsustainable costs.

2) Chemicals are transformed by water, light, chemical reactions in the soil, with microorganisms or within animal and plant tissues. The choice of the matrix where to look for a molecule has to be made carefully as hazardous substances may be difficult to find in some matrices. The products of transformation, often unknown, can be numerous and more dangerous than the starting ones. Therefore, some very dangerous substances remain in the environment for a short time and become analytically invisible, but this does not mean that we should not be concerned. The metabolites of many pesticides have been found to be more persistent and dangerous than the starting molecules. Being able to identify even the metabolites of hazardous molecules is expensive and materially impossible in most cases, partly because many of these products of biochemical reactions are unknown.

3) Environmental matrices in some cases have no memory. For example, the discharge of pollutants into surface waters, due to the dilution and flow effect, may not be detected by analytical determinations after some time, while the bioaccumulation or lethal effect on some species may be detected long afterwards. Some chemicals distribute, due to their chemical and physical characteristics, preferentially in certain matrices. For example, the herbicide alachlor, used in the cultivation of maize, is very soluble, so it is easy to find in river water (e.g. in the rivers of the Marche region). [870]

4) Analytical determinations do not measure additive, synergistic, sub-lethal, inhibitory or promoter effects. In some cases, molecules (e.g. human or zootechnical medicines) that contaminate water generate, at very low concentrations, hermaphroditism, feminization, reproductive abnormalities in fish and immunosuppression in mussels. Therefore, by measuring these very evident anomalies we can indirectly get information on serious imbalances of anthropogenic origin from molecules that are difficult to determine.

Since the various contaminants present in the environment can act synergistically and bioaccumulate, amplifying the negative effects in living organisms, in order to have a synthetic and more representative picture of the environmental degradation of an area, it is opportune to place the common methods of instrumental investigation side by side with other methods of a biological type, that is based on the use of living organisms able to act as indicators of the level of pollution. Biological indicators are able to give a lot of information simply by detecting the biodiversity and abundance of single species. The presence of organisms at the terminal position in the food chain is a first indicator of the health of the trophic network. Certain organisms, due to their biology, are suitable for measuring alterations in some ecosystems (e.g. rivers instead of seas), while it is known that others are sensitive to some pollutants or can bioaccumulate them in high concentrations. These evaluations, qualitative (biodiversity and complexity of an ecosystem) and quantitative (numerosity: e.g. of the microbial biomass in the soil), are able to provide information with much less onerous costs and equipment. The well-known phenomenon of bioaccumulation of contaminants in the tissues of living beings makes it easier to measure warning indicators that can sometimes be stored in living beings at concentrations even over 1,000 times higher than in water, air or soil. Another methodological approach is also possible in the laboratory: the environmental matrix can be used to measure lethal and sub-lethal effects in living beings chosen as a model. Trouts can be used to assess the effects of contaminated water or polluted soil samples can be used to assess inhibitory or lethal effects on plant germination (this *test* is used to measure the toxicity of compost from municipal wastes). [741]

Biomonitoring by examining the health status of living beings in a given ecosystem (agricultural, rural, urban or wilderness) has some advantages: [43]

a) It is able to provide more meaningful spatial and temporal information.
b) Several organisms can be particularly sensitive to certain pollutants, such as bees to insecticides and some plants to herbicides. For example, bumblebees (*Bombus terrestris*) are 7 times less sensitive to the contact effects of some insecticides than bees (*Apis mellifera*) and, on the contrary, the latter have been found to be 2 or 3 times more resistant to three insecticides (neonicotinoids), also taking into account the difference in weight. [45]
c) Animals and plants can bioaccumulate, thus providing information on contamination that occurred long before: for example, the wax of honeybees accumulates pesticides used in agriculture and by beekeepers. In addition, bioaccumulation means that it is easier to measure the presence of certain substances as they may be present in animal and plant tissues at higher concentrations than can be measured, for example, in air or water.
d) Animals such as insects (e.g. bees) may suffer additive and synergistic effects (e.g. in behaviour and on reproductive capacity) that are not detected by tests such as those applied by manufacturing companies prior to the marketing of substances such as pesticides.
e) Animals such as bees naturally inspect vast areas so they are an economic method of biological sampling.

Some organisms have been widely used as indicators of soil and water contamination such as mites, nematodes, collemboles, isopods, insects and molluscs. Metal contamination has been monitored with fungi, mosses, lichens and in water with fish and molluscs. [64]

Many organisms have been used as bioindicators of PAH contamination such as fish and molluscs for the aquatic environment, mosses and conifers for the terrestrial environment (polycyclic aromatic hydrocarbons are generated by combustion and are very dangerous as they can be carcinogenic). [59] PAHs can be absorbed by conifers from air, soil and water (young pine needles have been used as bio-indicators). It is possible to make a brief non-exhaustive summary of the various biological matrices used in studies and research to detect certain types of contamination: [43, 181, 870]

- Cow's milk for metals and dioxins.

- Sheep and goat hair for metals.
- Adipose tissue and muscle of pigs for the insecticide DDT (or para-dichlorodiphenyltrichloroethane) and other organochlorine insecticides. [248]
- Lichens for some air pollutants (sulfur dioxide or SO_2).
- Tobacco leaves for tropospheric ozone (O_3).
- Pine needles or holm oak leaves for metals and dioxins.
- The viability of pollen such as that of *Pinus nigra* provides an indication of the level of exposure to environmental pollutants such as nitrogen dioxide (NO_2). Air pollution reduces pollen viability and hence pollen functionality.
- Mosses for metals (including radionuclides), dioxins and polycyclic aromatic hydrocarbons.
- Nematodes for measuring soil health.
- Chicken eggs and liver for polychlorinated biphenyls and dioxins.
- Bees killed by insecticides.
- Ants (*Crematogaster scutellaris*) to assess the level of metal contamination (cadmium or Cd, lead or Pb, nickel or Ni, manganese or Mn).
- Mussels (*Mytilus galloprovincialis*) for polycyclic aromatic hydrocarbons and metals in salt water.
- Eels (*Anguilla anguilla*) for metals, polycyclic aromatic hydrocarbons and organochlorine pesticides (these are persistent organic pollutants) in the water.
- Mullet (*Mugil cephalus*) to monitor shorelines and estuaries for the presence of the insecticide DDT and polychlorinated biphenyls. [183] Great marine mammals such as killer whales are among the animals mostly contaminated by polychlorinated biphenyls (or PCBs) on the Planet. [977] These chemicals are found in the blubber and eggs of turtles (*Chelydra serpentina*) and are suspected of causing alterations in sexual differentiation. [978]
- Common trout (*Salmo trutta*) to monitor metals in freshwater.
- Human urine for some herbicides (e.g. glyphosate, which is an herbicide marketed in over 750 different formulations), organophosphate insecticides (chlorpyrifos) and breast milk for some organochlorines. Glyphosate is classified by the International Agency for Research on Cancer as a potentially carcinogenic substance (it is genotoxic) and acts as an endocrine disruptor at very low doses: less than a thousandth of a gram. [196, 507] It should be remembered that most of the feed used in livestock farming consists of corn, soybeans and canola genetically modified to be resistant to glyphosate. This is probably one of the reasons why the global glyphosate market is worth more than 8 billion dollars a year and, in 2012, 718,600 tons of glyphosate were sold, equivalent to more than 100 g for every inhabitant of the Planet. So the plant food chain and then the animal food chain are contaminated by these molecules in a dangerous way.

In many cases, the mere observation of the presence or absence, numerosity, behaviour and health status of certain organisms (e.g. insects) provides information on the consequences of anthropogenic activities at a very low cost. This type of approach can be much cheaper, provide more information, and record consequences that analytical measurements cannot give such as additive, synergistic or inhibitory effects.

BIOMARKERS OF PESTICIDE EXPOSURE IN HUMANS

Pesticides (e.g. insecticides and acaricides) are very harmful to bees but also to humans: some of the effects of pesticides are skin irritation, birth defects, genetic changes, tumours, nerve dysfunction, endocrine disruption, coma and death. Some pesticides such as aldrin, chlordane, DDT, dihedron, endrin, heptachlor, hexachlorobenzene, mirex and toxaphene are considered persistent organic pollutants (POPs or persistent organic pollutants). [41, 181, 183] This category (POPs) can compromise the endocrine, reproductive and immune systems. Chronic exposures to these molecules can lead to cancer, infertility and nerve problems. These substances can bioaccumulate in the food chain and enter the bio-chemical cycles of the biosphere. Unfortunately, we continue to produce and use these molecules in large quantities, even though we are aware of their negative effects.

Many studies show that humans can be used to biomonitor the presence of pesticides in the environment and possible occupational exposure. Some molecules can be found in blood (organochlorines and their metabolites such as DDT), in urine (organophosphates and glyphosate), in hair (organophosphates), in breast milk (organochlorines), in the umbilical cord. [464] Throughout our lives we are exposed to an huge multitudes of dangerous substances. For example, with the consumption of water we ingest plastics, and hundreds of substances such as more than 300 industrial compounds (arsenic, dry-cleaning solvents, benzene, drugs and derivatives), at least a hundred pesticides and fertilizers (results of drinking water analyses conducted between 2004 and 2009 in the USA). [988] For most of the substances that we release into the environment and that we can also consume through water, we do not have sufficient information on their hazardousness and there are no data on tolerable concentrations.

Biomonitoring can be used to assess occupational exposure through urinalysis, blood analysis or by measuring DNA damage and protein variation. Biological monitoring of exposure is also very important to determine the levels of substances that can be tolerated below predetermined thresholds. These studies provide useful indications for the regulation of maximum tolerable concentrations in water, food, and the estimation of acceptable occupational exposure levels.

Several strategies can be used to implement biomonitoring. One can investigate biomarkers of exposure such as concentrations of foreign substances and their metabolites within the body. Concentrations of molecules can be measured in blood, urine, hair, and exhaled air.

The measurement of exposure biomarkers makes it possible to assess the differences between non-exposed people, diffuse environmental exposure and occupational exposure. In some countries, biomonitoring surveillance is mandatory for occupational groups at particular risk (e.g. in Germany organochlorine insecticides are sought in blood serum or, in Japan, solvents are sought in exposed workers). [489]

Farmed animals may also be used. To measure exposure to metals (e.g. cadmium or Cd, lead or Pb) or organochlorine insecticides, measurements of blood concentrations in cattle can be made.

There are several national and international guidelines on maximum concentrations of biomarkers of occupational exposure. [489] Some limitations of this strategy are its cost and the difficulty of examining thousands of potential pollutants. For reference, in 2005, there were 620 pesticide active molecules registered in the USA marketed in more than 20,000 formulations, i.e. mixed with hundreds of other substances. Many studies have been conducted that provide useful indications: unfortunately, some humans, such as those exposed professionally, have been test subjects.

Biomarkers of occupational exposure, but not limited to this, include: [489, 517, 987]

- Lindane, lead, carbon monoxide in the blood.

- Methyl mercury in hair. The increase in the concentration of this metal in hair is a consequence of an increase in the consumption of contaminated marine fish (swordfish can contain 1,212 µg/kg, sea bass, tuna or sea bream over 200 µg/kg).
- Cadmium, metabolites of organophosphorus insecticides, some herbicides (e.g. 2,4-D, atrazines and their metabolites in urine such as deethyl-atrazine, which is a metabolite of atrazines, and 3,5,6-trichloro-2-pyridinol which is a metabolite of organophosphates), some polycyclic aromatic hydrocarbons, some volatile organic compounds (e.g. benzene, n-hexane, toluene, xylene, styrene), formic acid, nickel, and mercury in urine. The concentrations of some volatile organic compounds can be 10 times higher in blood than in urine.
- Some polycyclic aromatic hydrocarbons in feces or urine.
- Organochlorine pesticides and dioxins in breast milk.
- The organochlorine insecticides in the blood.

Some of these markers may give information about chronic exposures to organochlorine insecticides (even those that occurred months earlier).

Living near apple orchards (in Val di Non, Province of Trento, Italy) increases the concentration of pesticide metabolites in urine, with peaks in the period when treatments are carried out, i.e. in May and June (e.g. metabolites of chlorpyrifos ethyl, an organophosphate insecticide). [487] Residents in the vicinity of apple orchards are exposed to pesticides such as insecticides and fungicides. Several pesticides such as bromopropylate (acaricide), iprodione (fungicide), boscalid (fungicide) are found inside the homes in dust; chlorpyrifos ethyl (insecticide), difenocolazole (fungicide), methoxyfenozide (insecticide) are found in air dusts. This exposure has been associated with a reduction in the ability to repair DNA. Children living for long periods in agricultural areas experience more DNA damage, which can promote the onset of diseases such as cancer; even non-carcinogenic molecules can promote cancer indirectly, for example, by damaging mitochondria or altering the ability to defend from DNA damage. Exposure to low doses of pesticides, even if they are not carcinogenic, can promote the alteration of physiological functions that can promote carcinogenesis. These studies show that humans can be useful bioindicators of environmental contamination, for example by detecting certain pesticides and their metabolites in urine (e.g. organophosphate insecticides and herbicides).

A biomonitoring of the presence of organophosphates was carried out using the urine of 195 Italian children aged 6 and 7 years (53% of the sample were girls), and 124 adults (living in Siena, Tuscany). [488] Metabolites were found in all the urine examined: the metabolite dimethyl-phosphate in 96% of the samples and the metabolite dimethylthiophosphate in 94% of the samples. The six metabolites found in urine can be generated by different types of pesticides such as: azinphos-methyl, azinphos-ethyl, dimethoate, chlorpyrifos, chlorpyrifos methyl, fenitrothion, chlorfenvinphos, dichlorvos and others. [488]

Importantly, a metabolite can originate from several pesticides, so some substances may be useful exposure biomarkers. In this Italian study, no families had used the insecticides. In the urine of children, the presence of metabolites was significantly higher than that found in adults. [488] Probably, the higher presence of these molecules in the urine of children can be explained by the higher exposure due to the ingestion of dust and soil (12 times higher than adults) and the higher exposure through food, in relation to body weight. [488] Research conducted in the USA found that children living in agricultural areas where pesticides are used in orchards (such as azinphos-methyl, chloprpyrifos, parathion, phosmet) are exposed to high concentrations in dust. Household dust had higher concentrations than those found in the soil: in household dust the maximum concentration was 14,000 ng/g, while it was 930 ng/g in the soil of the treated orchards. [488] An interesting aspect, highlighted by the authors of this research, is that in previous studies it appears that biomonitoring with humans did not produce positive results

because the detection limits of the concentrations were much higher. In this work, analytical methods capable of detecting concentrations of 0.01 µg/L were used, while in previous work they measured up to 20 µg/L: a concentration 2,000 times higher. If samples with metabolite concentrations of less than 20 µg/L are eliminated in this work, less than a third of them are positive. [488] Thus, the analytical method used can make a big difference in being able to measure small doses that still have physiological effects, and which are very useful indicators of exposure and risk.
In the domestic environment, in the USA, chlorpyrifos is the most widely used insecticide: in children, between 3 and 6 years of age, non-diet-related exposure can be considered to be around 208 µg/kg body weight per day and up to 356 µg/kg body weight per day. [488] Organophosphate metabolites were also found in 80% of urine samples from 1,000 German adults representative of the general population and therefore not occupationally exposed. [489]
So urine can be used to assess exposure to organophosphate insecticides in children, for example. Humans can use themselves to biomonitor the behaviours of other humans and predict health effects. For some molecules, urine monitoring is more sensitive than monitoring of dust, water and food. Advantages of environmental biomonitoring with urine include being able to measure diffuse environmental contamination, being able to measure chronic exposures and bioaccumulation, and being able to more easily detect concentrations that are high and therefore above the analytical detection limit. In addition, since the same metabolites can be produced by different pesticides, it is easier to reach concentrations above the detection limit and we have information on additive effects, i.e. the overall bioaccumulation from the environmental and food contamination to which we are exposed.

Another category of exposure biomarkers are effect biomarkers, i.e. those that allow quantification of physiological, biochemical or behavioural changes produced by the presence of interferents. They are often a warning sign of certain diseases. This strategy measures changes in immune defences, in the ability to detoxify certain substances or to transform them into other more toxic ones. Specific biomarkers have been identified for kidney, liver, central nervous system and other important physiological functions; [489] for example, pyrethroids (insecticides such as permethrin) generate oxidative stress by altering the function of certain enzymes and the detoxification system called cytochrome P450. [527] Unfortunately bees, compared to other insects, are less equipped with detoxification systems. It is also possible to test the mutagenic effects of urine, for example of subjects exposed to polycyclic aromatic hydrocarbons, in experimental models in the laboratory (Ames *test*). Some biomarkers of the effects are: [489, 517]

- Measurement of acetylcholinesterase activity in red blood cells. Following exposure to organophosphate insecticides, the activity of this enzyme is reduced: exposures occurring even 3 or 4 months previously can be assessed.
- Mouth mucosa and cells in urine to measure DNA damage (e.g.: to measure compounds associated with bladder cancer).

These types of alterations show signs of broad-spectrum toxicity.

Biomarkers of susceptibility to certain substances can be measured, e.g. through the use of genetic surveys. Exposure biomarkers are the most widely used while genetic surveys may raise unacceptable ethical issues (e.g. in the case of occupational exposure). Some proteins (enzymes) may have very important roles in detoxifying certain pesticides. Genetic characteristics of mothers may increase the likelihood of recording nerve developmental damage in their children. [510] As an example, the enzyme paraxonase (PON1) is involved in the degradation of organophosphate insecticides. Certain forms of these proteins, which are derived from genetic variables, may increase the toxic effects of these pesticides and affect neurodevelopment in school-aged children. In pregnant women, reduced concentrations of this enzyme (PON1) or the presence of genetic variations that make it less active in detoxifying organophosphate insecticides increase the susceptibility of their offspring to neurotoxicity. [510] In this case, the different genetic characteristics may increase or decrease the susceptibility to damage the nervous system of children, generated by exposure of mothers to organophosphate insecticides. Deficiency in the detoxification system in mothers exposes children to toxic molecules, during pregnancy and during lactation. In addition, this detoxification system in children is not functional until the age of 7 years, so environmental exposure of children may also be more dangerous than that for adults. In school age, due to these genetic characteristics of mothers exposed to organophosphate insecticides, there is an increase in attention disorders and cognitive deficits (reduced IQ or Intelligence Quotient). [510]

THE USE OF BEES AS BIO-INDICATORS

Bees (*Apis mellifera*) are the main pollinator of many plant species used for human consumption. For several years, researchers, scholars and public bodies have been proposing the products of beekeeping for biomonitoring the state of health, especially of agricultural areas. Inside the colony there are more durable matrices such as wax and propolis, and others that are renewed quickly such as honey and pollen. Whether bees are an effective bio-indicator to verify the presence of pollutants into the environment is a question to which it is not possible to give an unequivocal answer, since beekeeping products have many advantages but also several limitations, such as the fact that they do not bioaccumulate some pollutants (e.g. PAHs and metals in honey). [71] Non-bioaccumulation means that measurable concentrations in beekeeping products are lower than those in environmental matrices. As an example, metals are more concentrated in soil, even more than 1,000 times than the concentrations recorded in honey. Therefore, if the objective of monitoring is to get information on the presence of metals in the environment, it is advisable to measure the quantities also in the soil (and in flowers that can bioconcentrate them). If, on the other hand, the objective of monitoring is to estimate the healthiness of honey, it is necessary to measure the concentrations of pollutants in this matrix. It should be considered that bees, by sampling a very wide territory, can still provide useful information on the state of health of the environment, on the emissions produced in a given site and on the causes of lethal and sub-lethal effects on the colony. Among the advantages of using beekeeping products to monitor the state of health of the agricultural environment it is possible to highlight that: [71]

- The behaviour, physiology, ecology and biology of bees are well known.
- Beekeepers are practically everywhere in the World, so it is easy to find bee products.
- Breeding bees costs less than other sampling systems and does not require sophisticated equipment.
- Bees sample a large area of land (several square kilometres) and over a long period of time around the hive.
- Bees can provide rapid warning signals in case of sudden negative events such as illegal pesticide treatments (e.g. insecticides and acaricides distributed during flowering), release of toxic substances into water (e.g. radionuclides) or emissions of hazardous substances into the air (e.g. metals and PAHs from incinerators).

It is important to highlight some characteristics of bee biology to better understand the advantages and limitations of using bees as bioindicators. An *Apis mellifera* hive consists of 20,000 to 100,000 bees, depending on the season and conditions, divided into castes: a queen bee, several hundred drones (about 200-300 individuals present only in spring and summer; overall, a colony breeds between 2,000 and 6,000 drones per year) and worker bees. Drones and worker bees live between 20 and 50 days. [2, 17, 35] Worker bees born in autumn can live up to 180 days, summer drones can live up to 90 days, and the queen bee can live between 2 and 7 years.

It takes 16 days for the queen bee, 21 days for the worker bee and 24 days for the drones to become adult. [764]
Bees are able to sample the area up to a few kilometres from the hive; if food is available they do not move further than 1.5 km. Exceptionally they may move up to 10-12 km; with the aim of reducing energy consumption, as is logical to expect that they look for food and water as close to the hive as possible.
Bees are excellent samplers because every day they visit the soil, flowers, trees (they collect resin to produce propolis), shoots (they collect honeydew produced by aphids), water sources and passively collect dust from the air (thanks to the hairs covering part of their bodies). They make thousands of inspections a day and fly over vast areas around the hive. In a hive at least a quarter of the bees are engaged in the activity of searching for food and water. In many cases up to 50% of the bees in a hive are forager bees, i.e. insects that go out in search of food or water (even more than 20,000 bees, of which at least 10% are engaged in collecting water). If the environmental conditions are favourable, each worker bee makes at least 10 trips a day, easily visiting 1,000 flowers from which it collects pollen and nectar. Other interesting features of bee biology that make these insects fascinating and at the same time efficient samplers of the environment are the following: [35]

- In order to produce one gram of nectar honey, it is necessary to take forage on 5,000-8,000 flowers (each bee can visit up to 3,000 flowers a day); a colony of bees takes about 10 million micro-samples a day in an area that can be between 5 and 100 square kilometres. In this regard, it is useful to remember that honey is a hydrophilic substance as it contains about 18% water and, therefore, can more easily contain water-soluble substances.
- Nectar is sucked into the bursa melaria which consists of a dilation of the oesophagus with a capacity of 50-60 mL (about 40 mg). When the bee returns to the hive it regurgitates the nectar. The transport of one litre of nectar takes 25,000 trips.
- A colony of bees also needs more than 40 L/year of water; between 40 mg of water per day per bee and up to about 300 mg of water per day per bee in summer. They can carry up to 0.5 L of water daily, but in very hot countries or in summer a colony can consume up to 5 L of water per day. [17]
- Each bee must visit between 80 and 150 flowers to fill its pollen sacs. The sacs are located in the hind legs and can carry the weight of 7.5 mg of pollen each; by making a maximum of twenty trips a day, it can be estimated that a bee can carry up to 200-300 mg of pollen to the hive; this depends on the flowers, as some produce a lot of pollen so that one visit is sufficient to complete a load. Over the course of a year, a medium-sized colony can collect between 40 and 50 kg of pollen.

The biology of bees allows them to be a bio-indicator capable of providing information both on ubiquitous and constantly present pollutants - such as those produced by diesel and gasoline combustion - and on those detectable only after certain events (e.g. industrial accidents, phytosanitary treatments in agriculture, discharge of pollutants into water).

The chemical substances (xenobiotics)[I], with which they come into contact during the search for pollen, nectar, resins (to produce propolis), honeydew (produced by aphids) and water generate sub-lethal effects (e.g. behavioural changes, effects on the endocrine system,

[I] The term xenobiotic is defined as a substance of any kind, of natural or synthetic origin, foreign to an organism. The chemicals produced (between 1 and 100 t/year), marketed and registered (by 5,314 companies) in Europe (in 2018) are at least 10,708 (of which 2,049 registered in Italy); the list of registered substances includes a list of candidate substances of very high concern for authorization (at least 200 substances). [23]The *CAS* registry is a registry that has catalogued over 148 million chemical, organic and inorganic substances with sequences of numbers (the CAS number or *Chemical Abstract Service* or CAS registry is a sequence of numbers that uniquely identifies a chemical substance, for example: water or H_2O, CAS:7732-18-5). [22]

metabolism or nervous system such as decreased ability to orient and fly) and lethal effects (e.g. by insecticides). [22, 23]

Between 9% and 55% of nectars produced by flowers contain xenobiotics, and some sugars in nectar may be indigestible. [107] Pollen may also contain potentially toxic xenobiotics, such as some phenolic compounds, pesticides and metals. Bees may also accumulate certain substances in their bodies or products. Bees' bodies are covered with hairs that not collect only pollen, but also dust and air pollutants. So bees can provide information about environmental contaminants through various indicators:

- Behaviour modification and measurement of sub-lethal effects such as reduced honey production and disease occurrence. Indicators used to monitor colony health include brood viability (measured as the percentage of colonies tested that have low viability), percentage of eggs and larvae lost.
- The measurement of lethal effects such as the number of dead bees daily or weekly, the detection of the presence or absence of the queen bee, the presence and number of larvae.
- The measurement of the concentration of pollutants in the different matrices: live bees, dead bees, honey, pollen, propolis and/or wax. Pesticides found in dead or live bees provide information over a period of time that may be a few days, pesticides found in honey provide information over a longer period and a wider area, while those found in wax indicate events that may have occurred up to a year earlier.
  Pollen transported by bees is an unprocessed environmental matrix, unlike honey and wax. The analysis of pollen found on bees makes it possible to know which crops have been visited and provides information on prohibited treatments such as those carried out during flowering.

Some important information to know before employing bees to perform biomonitoring is as follows:

- Bees do not fly at night.
- Bees do not fly at temperatures below +7°C or +10°C and above +38°C. [65]
- During the winter the bees do not leave the hive.
- It is necessary to comply with specific national and regional rules for the movement and positioning of hives; some of the requirements in Italy are listed below. The registration of the declaration of origin and destination of bees in the case of movements of hives for any reason (in Italy, via internet on the *vetinfo.it* website in the beekeeping section).
- Identification of the apiaries (an apiary is made up of several hives) by means of a code number issued by one of the regional agriculture departments.
- When the hives are placed in a new location, the veterinary of the National Sanitary Service responsible for the area must be notified of the location of the hives and the size of the apiary; the health certificate issued by the Regional Sanitary Service of origin on a date not prior to thirty days (attesting to the state of health of the colonies) must be attached. The certificate must refer to the last extra-regional foraging site of the apiary.
- Respect the safety distances from houses, roads and busy places or particularly vulnerable sites (e.g. schools and hospitals). The Piedmontese regional legislation (Piedmont Regional Law n. 20/1998) states that apiaries must be located at least ten metres from public roads and at least five metres from the boundaries of public or private property. The beekeeper is not obliged to respect these distances if there is a difference in height of at least two metres between the apiary and the mentioned places,

or if there are walls, hedges or other shelters in between, without interruption, that do not allow the bees to pass. These shelters must be at least two metres high. As can be seen, these precautionary measures are not sufficient, especially in urban areas. In the case of particularly attractive sites such as sugar refineries or honey processing plants, hives should be placed at a much greater distance, i.e. at least 1 km away.

There are several methods that can be used to measure changes in bee behaviour to investigate certain factors (e.g. insecticides): [195]

- Measurement of the variation of the proboscis extension reflex at various concentrations of different substances, and under different conditions. In this laboratory assay, stimuli are delivered to the bees' antennae and it is tested whether the proboscis extension reflex starts and how often. With this simple test it is possible to measure the physiological state of the bees as the percentage of positive responses to certain concentrations, for example, of sugar. The proboscis extension reflex can be associated with chemical stimuli but also with tactile stimuli (e.g. with sugar after covering the bees' eyes).
- The measurement of phototaxis, i.e. the movement of insects towards the light. In this case, artificial environments are used with different compartments that can be illuminated or darkened at will. Alterations in visual capacity generated by certain substances are thus measured. These tests show that bees of different ages have the same phototaxis behaviour.
- The measurement of the ability to be attracted by different odours. Also in this case, artificial environments are used in which the emission, intensity and direction of odorous emissions can be controlled and the movements of the insects are recorded. With this laboratory equipment it has been possible to demonstrate that bees of different ages respond differently to certain hormones produced by the queen bee (e.g. the mandibular hormone of the queen bee attracts bees of 5 days of age more intensely).
- Reactions to temperature changes can be measured and preferred choices (thermotaxis) can be verified.
- The ability to fly can be assessed with special equipment.

With simple methods applicable in the laboratory, it is possible to evaluate the effects on bee behaviour of xenobiotics, parasites or feeding under different conditions.

EXAMINATION OF THE CHEMICAL COMPOSITION OF THE AIR INSIDE THE HIVE

Bees need oxygen to live, consuming about 30 kg per year, and at the same time their metabolism produces 52 kg/year of carbon dioxide and 34 kg/year of water. [13] The volume of air inside a beehive is about 0.2 m^3, so an air exchange of over 150 m^3 per year is necessary. During flight, bees consume about 5 mL of oxygen per hour, which corresponds to 641 mL of oxygen per kilogram of body weight per minute. For comparison, a human being can consume as much as 94 mL of oxygen per kilogram of body weight per minute during intense sports activity. So the metabolism of bees is much more intense.

Using sensors that can measure the composition of gases and volatile molecules within the hive can provide a wealth of information. Volatile chemicals in the hive such as combustion products, industrial solvents, pesticides and explosives can be monitored. With a pump that sucks the air and a filtration system, it is possible to convey the air present inside the hive into a gas-chromatograph equipped with mass spectrometry as a detector.[3, 17] The air aspiration system in the hive can be calibrated to aspire with different frequency and intensity. This analytical chemistry laboratory instrumentation has the potential to detect even very low concentrations of thousands of different molecules and is therefore able to measure the presence of substances brought by bees into the hive. [62] It has been possible to associate the presence of molecules in the air of the hive, such as perchloroethylene transported by bees into the hive from a landfill, with a mortality rate of queen bees in colonies of 50%.

Using this technique, the researchers measured the presence of hundreds of molecules of anthropogenic origin and potentially harmful to bees and humans. The instrumentation allows very sensitive analytical capabilities but is expensive and requires highly qualified personnel. It should be remembered that the air inside the hive is kept at around 34-35°C, so many volatile organic substances are easily determined with this method.

Chemical analytical methods make it possible to measure differences in concentrations of different substances in hives located in uncontaminated and contaminated environments, or to measure qualitative and quantitative variations over time. It is also possible to measure substances that are naturally present in the hive, such as pheromones (such as the one produced by queen bees to manage and control the workers or those produced in the event of an alarm), substances contained in plant products such as propolis, metabolites of pesticides or those released by their parasites. The substances released by the construction materials used to make the hive are also detected. The analytical system is very sensitive even at very low concentrations. It is therefore necessary to pay attention to the materials with which the hive is built, such as paints and other materials, for example wood, polystyrene, plastic, polycarbonate, polyurethane and metals.

Examples of the substances that can be determined with these techniques include: alkanes, alkenes, alkynes, cyclo-alkanes, aromatic hydrocarbons, terpenes, diphenyls, alcohols, ethers, ketones, aldehydes, acids, esters, amines, and other organic compounds of nitrogen and sulphur, organochlorinated compounds such as insecticides, and solvents. To get an idea of the power of this technique we report the results of a very interesting scientific work in which the following substances were detected in the air of beehives located in an urban area of the University of Montana in the USA: [17]

- over 85 different pheromones produced by bees such as histamine (worker bee defence), geraniol (worker bee guidance), diesters (cell construction by worker bees), hexanoic acid (queen recognition);
- over 45 substances typically released by nectar, honey, wax, pollen or propolis such as palmitic acid, vanillin, eucalyptol and propylene glycol from propolis, sterols from pollen, alkanes from waxes, and organic acids, alcohols and alkaloids from honey;
- more than 10 wood-derived substances used to build the hive (e.g. limonene); wood can be treated with substances (such as copper naphthenate) that can be found in bees at high concentrations (21 ppm); [107]
- more than 15 substances derived from the varnish used on the wood of the hive such as phenols and methylnaphthalene;
- more than 10 substances derived from the plastic parts of the hive (polyethylene and vinyl) such as xylene and propylbenzene;
- more than 10 substances derived from the combustion of plant parts used for the fumigation of hives;
- over 100 substances derived from combustion (diesel, petrol and methane vehicles) such as benzene, ethyl benzene, toluene, xylenes, naphthalene;
- more than 45 substances derived from industrial activities such as solvents, trichloroethene, dibromo-methane-trichloromethane, chlorotoluene, tetrachloroethene, tetrachloromethane;
- over 10 pesticides such as endosulfan, aldrin, dieldrin, carbaryl and DDT.

Many of these substances would probably have been found by sampling the air outside the hive. By way of example, the maximum concentrations of some very hazardous substances measured in the air of the hive are given below:

- naphthalene (also known commercially as naphthalene) 16 ppt or parts per trillion: International System prefix equivalent to 10^{18};
- benzene 170 ppt (carcinogenic); [100]
- 1,1,1-Trichloroethane 826 ppt: this is a chlorinated solvent and an aerosol propellant banned under the Montreal Protocol because it is believed to be responsible for enlarging the ozone hole, and is deadly to insects and species in the aquatic environment); [59]
- cis 1,2-Dichloroethene 839 ppt;
- toluene 1,643 ppt (harmful substance); [101]
- styrene 9,239 ppt (carcinogenic).

It is easy to understand that with this analytical capacity it is possible to know and predict the health of bees but also to have detailed information on industrial, agricultural and urban activities in the area. It is possible to monitor substances released by bees, honey, wax, propolis but also those present in the air due to human activities. Some limitations of this method are the costs, the need for qualified personnel and some dangerous pollutants may be detectable in the air of the hive only for a few hours or a few days.

HYPOTHESIS OF BIOMONITORING WITH BEES

CHARACTERISATION OF THE SITE WHERE BIOMONITORING IS TO BE CARRIED OUT

The implementation of biomonitoring requires the planning of different activities and to save resources it is important to consider the consolidated results from previous surveys. A preliminary step requires the characterization of the site, for example by classifying it as low or high agricultural intensity. The classification must take into account anthropogenic activities present in the vicinity of the hives, within a few kilometres. The characterisation of the site and its classification as urban, agricultural or industrial should be carried out within a minimum radius of 1.5 km and if necessary up to 12 km from the hive. The classification of an area is important because, to give an example, pesticides are found more frequently and in higher quantities in agricultural areas than in urban areas. The area can be classified as agricultural (with intensive agriculture) if more than 50% of the surface is engaged in cultivation. [54] The purpose of the classification is to highlight the sources of possible emissions (e.g. incinerators, crops, highways, etc.). To carry out this activity, subdivisions that have already been implemented can be used.

Therefore, the biomonitoring activity can be accompanied by very useful and often necessary complementary support operations. Several categories of information can be used to perform a classification of the area.

- The census of the activities performed in the territory such as industrial activities, waste deposits and treatment centres, sites to be reclaimed, highways or other roads.
- In some territories, inventories of civil and industrial emissions and dumps are available.
- The census of the existing crops and the compilation of cultivation maps that highlight the prevalent or most important plants present in the site (e.g. those in bloom).
- The collection of information related to other chemical monitoring such as soil, water and air monitoring carried out in the same areas. In Italy the health status of water and air pollutants are monitored by research institutions and National Sanitary Service, and many results are public.
- Monitoring of soil fertility and soil health. Soils are classified by the Regions in different categories according to their level of fertility.
- The examination of results obtained from other biomonitoring such as those with lichens to assess air pollution. The lichen biodiversity index is used to obtain information on the level of atmospheric pollution and is calculated by measuring the number of lichen species present per unit area. Lichen biodiversity drops rapidly in the presence of toxic gaseous substances and, if conditions improve, it recovers in a few years. In general, the lichens taken into consideration are the epiphytic ones, that is, those that grow on the trunk of trees. The lichen biodiversity index is used to assess pollution by metals such as mercury or cadmium. [51]
- Classification of the health status of watercourses near the biomonitoring station. In Italy and most of Europe, surface watercourses and lakes have been classified according to their chemical, physical and biological quality. Biomonitoring carried out in streams and lakes with fish and other organisms can also provide some useful information.

- The collection of information on the pesticides used, for example by consulting the field notebooks, pesticide sales data and the production specifications adopted (traditional, integrated or organic). The active molecules most used are also those most easily found. It may be useful to know that every year the official monitoring of pesticides found in food of plant origin is published by the Health Service and the Ministry of Agriculture.

The final elaboration of the results in addition to taking into account the information just highlighted can make use of all the results published in the technical and scientific literature.

HOW MANY HIVES TO USE AND WHERE TO PLACE THEM

Hives can be placed near the crop when 20-25% of the flowers are already open, so that they begin attending the desired bloom in time to perform pollination and honey production services.

In order to carry out biomonitoring with bees, at least two hives are often positioned a few dozen or at most a few hundred metres away from the site to be monitored. In some biomonitoring works, 2 to 8 hives per apiary are chosen from which to take samples: they will constitute a biological sampling station. Colonies should not be moved for at least three months as nomadism must be avoided. [71] The movement of hives by beekeepers can be an obstacle to the interpretation of data, e.g. on sources of pollutants such as pesticides, metals or polycyclic aromatic hydrocarbons. If information over longer periods of time is desired, it is useful not to move colonies from a site for even a few years. In this way, for example, the concentrations of radionuclides in wax can be compared over time.

With the aim of measuring significant differences and variations in pollutant concentration over space and time, it is advantageous to use several hives placed in areas with a predictably different level of contamination. It could be planned to place the hives in contaminated and non-contaminated areas in order to measure the differences. Furthermore, by arranging the colonies appropriately in the territory and studying the gradients of pollutant concentration in different hives, it is possible to identify the sources of point emissions.

Monitoring could involve the use of 12 permanent colonies: three in an industrial area, three in an agricultural area, three in an urban area and three in an area such as a nature reserve or park. [71] Dedicated hives may be used or agreements may be established with local beekeepers. It must be repeated that all apiaries should not be nomadic. It is preferable to use new colonies (on an annual basis) and of the same age in order to reduce effects that could alter the results.

HIVE EQUIPPED WITH SENSORS TO GET INFORMATION ON THE STATE OF HEALTH AND BEHAVIOUR OF BEES

It is possible to monitor the state of health of the individual colony by equipping the hive with a series of sensors capable of continuously measuring weight, humidity, temperature, sounds, vibrations, respiratory gases, and the number of bees entering and leaving. [11, 15, 82, 85] By putting these parameters together it is possible to assess the state of health of the colony and the presence of factors that in some way damage the insects and alter their behaviour. [15, 20, 87] The objective is to insert a series of sensors in the hive which can be controlled remotely and which do not disturb the colony. [20]

You can monitor the bees entering and leaving the hive or the change in the weight of the hive with special sensors. [15, 216] Periodically it will be necessary to check that the sensors are not altered by the bees, for example by covering them with their products such as wax or propolis. By means of photo-electric systems which must necessarily be crossed by the bees entering or leaving the hive, it is possible to count the insects outside or not returned. [195]

The distribution of insecticides can rapidly reduce the number of returning bees, while sudden cold weather reduces the number of bees flying in search of food, even to the point of stopping them altogether. Temperature, humidity and concentration of respiratory gases are closely related to bee metabolism. All these parameters can be used as indicators of colony health.

An increase in the intensity of the sounds and a reduction in humidity and temperature are considered premonitory signs of swarming (with a humidity of less than 50% the eggs do not hatch). [20, 972] Some instruments capable of detecting the sounds produced by bees were already sold in the 1960s in the United Kingdom. [31, 92] By examining the sounds it is possible to predict, for example, when the bees are about to swarm, that is, when the old queen with some of the workers (about 10,000) will go in search of a new home, leaving the hive to a new generation. There are several models. [3, 89, 90]

The sounds made by the colony are affected by exposure to pesticides. Exposure to neonicotinoid insecticides makes the colony less noisy. [3] By measuring the variation in sounds, it is possible to hope to detect the loss of the queen bee or the presence of parasites such as mites (*Varroa*).

Another tool allows counting the number of bees entering and leaving the hive. Using computer and telephone programs, it is possible to know from a distance how many bees are inside the hive and how many are in flight. [93] The number of non-returning bees, exceeding certain thresholds, is a worrying sign of increased mortality. Every day about 90% of the bees that have flown out return to the hive, and if the daily return rate falls below 80% there may have been an episode of acute toxicity (e.g. distribution of insecticides).

Over time, weight variation of the hive provides important information on the health status and availability of honey. Rapid weight variation has also been associated with the presence of the tracheal mite parasite (*Acarapis woodi*)[I]. [20] Detection of weight change may provide information on nectar and pollen availability and hive abandonment. The lack of weight increase, due to the lack of honey production or to variations in internal temperature, is another alarm bell (bees keep constantly between 34°C and 35.5°C even during winter). [95] Weight and temperature are therefore two important indicators.

It is possible to build technological hives capable of remotely communicating a series of information on the state of health of the insects. [83] The automatic monitoring systems make it possible to save trips and controls, to have information continuously, at a distance and without disturbing the colony with inspections, which among other things require smoking; they also signal any acts of vandalism. [13] With these sensors it is possible to measure both lethal and sub-lethal effects (e.g. behavioural modification) generated by pollutants on the bee colony.

Monitoring sensors could be used to monitor sensitive sites. In Germany some airports implement biomonitoring using bees to get information on the possible presence of hazardous substances. [3] When certain thresholds are exceeded (e.g. mortality, temperature and weight changes), a thorough inspection can be considered to assess the presence of parasites and sampling of dead bees, honey, pollen and wax.

Some limitations to the use of sensors and wireless systems for communication are cost, the need for electrical power at the site where the hives are located, the use of qualified people to interpret the data, and the use of computer programs (some sensors are sold with solar panels and batteries).

[I] The tracheal mite *Acarapis woodi* lives and reproduces inside the bee's respiratory tract, which it only leaves to infest other bees. The mite pierces the bee's tracheal tubes with its mouthparts and sucks the host's hemolymph. Due to the small size of the mite, the parasite can only be identified under a microscope. The symptoms of mites are difficult to identify and overlap with those of other typical diseases affecting beehives. Since the diagnosis and, therefore, the degree of infestation is difficult to identify, the most effective treatment is the destruction of infected families. [21]

INDICATORS FOR SAMPLING IN HIVES

Monitoring should be carried out at the time of the year when bees are most active, i.e. between April and July, but can be extended into autumn (October in Italy). It is useful to remember that bees do not fly at night, if it rains and in winter (they do not fly at temperatures below 10°C), and that blooms are necessary for honey production. It should also be considered that rainfall reduces the amount of pollutants in the air, so weather conditions will be a variable to consider. In order to get better information on polycyclic aromatic hydrocarbons, metals and pollutants in the air and soil, sampling should be avoided when it rains. [66]

A number of indicators can be used to assess colony health that can provide useful information to establish thresholds, above which sampling becomes more useful. Examples of signals to be examined to decide when to sample are given below.

The sudden change in the number of dead bees in the colony is a useful indicator. Threshold values of 250 or 350 dead bees per week have been adopted in some scientific studies. Dead bees are collected from two hives forming a control station; dead bees can be collected near and inside the hive and removed from the colony by means of special traps. [17, 33]

At least one thousand bees die in a hive every day, but they are a fraction of the foragers who do not return to the hive (especially in the months between May and July). It can be estimated that a mortality rate of about 2.5% of the individuals in the colony, about 175-250 bees per 10,000 bees per hive per week, is normal. However, daily or weekly monitoring and recording of abnormal variations will be useful. Indices for assessing increasing severity can be assumed as: up to 200 dead bees per week, between 201 and 400, between 401 and 800 or more than 800 dead bees per week.

The daily percentage of bees not returning to the hive can be determined with the use of electronic bee detectors that automatically record the insects that have entered and exited. In this case, a threshold of 20% of non-returned bees of the bees that have flown out can be considered an alarm threshold. Specific evaluations must be carried out for each context and sudden changes such as: absence of queen bees, lack of larvae and/or excess of drones (more than 300 per colony) must be recorded. Alteration of colony composition can also be assessed by examining the change in comb cell content over time. Measuring empty and occupied cells and distinguishing between different cell contents can provide useful information. Some scientific studies photograph the combs weekly and with special programs that process the images measure in detail the number of cells, their content and the variations of the areas. In this way it is possible to have information on the state of health of the colony but a disturbance is created which can be significant.

The change in hive weight due to bee death or honey consumption can be measured by automated systems and provides a remote indication of health status.

The presence of parasites such as mites, bacteria or viruses can signal a weakening of the colony's ability to defend itself, which can be aided by the presence of chemical contaminants.

Temperature variation within the colony can be measured with special sensors that record it automatically. Bees keep the temperature in the hive constant at around 34-35°C even in winter, so variations may indicate the presence of negative factors.

The monitoring of the above parameters can provide useful indications on when to carry out the sampling of bees (dead or alive), honey or wax. If the hive is equipped with devices capable of monitoring weight, bees entering and leaving, and the internal temperature of the colony, sampling can be scheduled at the first signs of colony distress. Changes in these indicators can be correlated with concentrations of toxic substances. Many scientific works confirm what is logical to expect: mortality and failure to return to the hive increase as the concentration of certain pesticides in beekeeping products, larvae and adult bees increases.

WHICH HIVE MATRIX TO SAMPLE FOR METAL MONITORING

There is probably no conclusive answer as to which hive matrix (live or dead bees, larvae, honey, wax, propolis) is most suitable for biomonitoring. In each context, several variables will have to be examined that will influence the choice of methodology.

Pollen and propolis are the least processed matrices in the hive and therefore better represent environmental pollution (e.g. by pesticides or radioactive isotopes), while wax is produced by glands located on the abdomen of bees and honey comes from flower nectar that has been processed in the digestive tract and exchanged between bees (trophallaxis or social sharing of nectar).

Live bees are a better indicator than honey for measuring metal air pollution. [71] The following metals can be found in honey at very low concentrations: silver (Ag), aluminium (Al), chromium (Cr), nickel (Ni), copper (Cu), lead (Pb), zinc (Zn) and sulphur (S). Bees contain higher concentrations of these than honey although the information provided by honey analysis is more repeatable. The concentration in propolis is similar to that recorded in bees' bodies and is about 10 times higher than that found in honey. [34] Cadmium, chromium and lead concentrations are higher in live bees than in dead bees and honey. [33] Live bees are a possible bioindicator of metal air pollution. 100 insects can be sampled and a monitoring station can consist of 2 hives, so 50 bees per hive can be sampled weekly or monthly. Measuring bi-weekly concentrations in live bees or monthly concentrations in fresh honey can detect peaks during months associated with anthropogenic activities, such as those of industries that release hazardous emissions at certain times of the year (e.g. chromium). [17, 33, 34] Some research shows that bees highly contaminated with metals produce low contaminated honey. [71] Therefore, bees are able to detect an increase in environmental metal concentrations more effectively than honey.

Honey does not bioaccumulate metals and it is possible to record a considerable variability among different samples, influenced by different floral origin, season, etc. According to some researchers, it is not possible to correlate the concentration of metals in soil with that in honey.

In conclusion, for metal monitoring it is preferable to use live bees rather than dead bees (the opposite happens for pesticides). Furthermore, live bees are a better matrix for metal monitoring than honey. [71]

By sampling live bees, it is possible to decide on the age, the size of the sample, when to take the sample, to carry out analysis of the floral origin of the pollen transported, and to monitor where the bees have flown. The determination of metals in pollen can provide information on plant contamination. The different types of plants visited by bees greatly influence metal contamination: for example, evergreen plants generate nectar and therefore honey with higher quantities of metals than deciduous plants; aromatic plants concentrate certain pollutants more easily than herbaceous plants. [71] Some plants are tolerant to high concentrations of metals and can bioconcentrate them in high amounts. Pollen and propolis can be used as indicators of atmospheric contamination by metals such as cadmium (Cd) and lead (Pb). [71]

Wax is probably the best matrix in which to look for metals. A biomonitoring that looked for cadmium, nickel, lead, iron, zinc and magnesium in samples of honey (multifloral), propolis, pollen taken by bees upon return to the colony and wax confirmed that the latter contained the highest amount and honey the lowest. [40] In conclusion, wax is the best matrix for this type of analysis (e.g. lead and cadmium), followed by pollen and propolis, and finally bees. The measurement of metals in honey, since it is a food, may have other purposes such as food safety assessment.

BIOMONITORING RADIONUCLIDES IN BEEHIVES

As far as radionuclides are concerned, some Authors agree in considering both live bees and honey as good matrices, but pollen is probably the best one even if many studies do not use it. [71] Radioactive isotopes (or radionuclides) of caesium and strontium (^{137}Cs and ^{90}Sr) have been sought in dead bees and pollen, and the latter has been found to be a better environmental indicator of radioactivity than bees (dead bee sampling can be weekly or monthly). [17, 34, 38]

The concentration of some radionuclides (^{239}Pu, ^{240}Pu, ^{137}Cs, ^{90}Sr and ^{40}K) in honey is greatly influenced by the plants visited. Honeydew honey (from firs and conifers) and honey from heather (*Calluna vulgaris*) are good indicators of the presence of caesium isotopes into the environment. [17] These are isotopes that do not exist in nature and are therefore produced by nuclear accidents and explosions; they are also used for research purposes, in clinics and in industrial sectors where they could be released illegally as waste or due to industrial accidents.
The measurement of the presence of radionuclides is very sensitive so it probably works well even in less contaminated matrices such as honey. [34]

Bioaccumulation of pollutants in the food chain is a dangerous phenomenon and can also be recorded in hive products. It is useful at this point to introduce an aspect that will be discussed in more detail later. Honeydew honey is a very interesting bio-indicator because it has the capacity to accumulate certain substances more easily (e.g. pesticides, polycyclic aromatic hydrocarbons, radioactive isotopes and metals). In this case, the food chain involves a passage first between plant and aphids and then between aphids and bees. Therefore, bioconcentration factors have the potential to be 10 or 100-fold higher due to the passage through one more trophic levels. Honeydew honey showed, among beekeeping products, the highest levels of radioactivity as is to be expected as the isotopes are bioaccumulated first in plants, then in aphids and subsequently in bees, increasing in the food chain concentration. Honeydew honey is a better indicator of radionuclide (radioactive metal isotope) contamination in the environment as the radioactivity is 10 to 100 times higher than in nectar honeys (e.g. in the case of the caesium isotope: ^{137}Cs). [17] Honeydew honey from hives located near fir and coniferous trees can be used to monitor many metals (caesium, chromium, rubidium, copper, lead and nickel). Therefore, honeydew honey is a better bioindicator than nectar honey and can be used to monitor the variation of radioactive contamination levels over large areas.

For measuring radioactivity, pollen is a better bioindicator than bees and bees are better than honey. [71] Wax and propolis are better matrices than honey and bees for measuring radionuclides. [61] In conclusion, if you want to measure the presence of radioactive metals through beekeeping products, it is preferable to investigate on pollen, wax, propolis or honeydew honey (pollen allows us to have information on the bioaccumulation in plants). Wax is the easiest matrix to find to search for radionuclides in the hive and pollen is useful to get information on radioactive contamination of plants visited by bees. Palynological analysis can be associated with the measurement of stable and unstable metals in order to obtain information on the plants visited and the geographical area.
Different monitoring objectives should take into account this variability. The measurement of contaminants in bee products should be carried out in potentially uncontaminated sites (as a control) and in environmental reference matrices (e.g. soil and plants) with an appropriate choice of molecules to be monitored. To give two examples, caesium is a good marker of accidents and nuclear explosions that occurred even 30 years earlier and hundreds of kilometres away. Potassium (isotope 40) is a good indicator of natural radioactivity.
With the objective of monitoring the variation of the presence of radionuclides over time, 2-4 samples per year could be taken depending on the half-life of the isotopes sought. For monitoring isotopes with a half-life of several years, one sample per year may be sufficient.

If adverse events such as accidental water contamination or sudden releases of toxic substances into the air are expected, weekly sampling can be scheduled. An example is given of an industrial accident that released the isotope 137 of caesium: it happened in 1998 in a foundry in Spain. [17, 34]

DETECTING POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic Aromatic Hydrocarbons (PAHs) can be monitored by examining concentrations in live bees and honey. However, wax is probably the best matrix to detect these hydrophobic molecules, as together with propolis they record the highest amount of them. If information on the presence of PAHs and metals released into the atmosphere is required, sampling can be carried out every 15 or 30 days, trying to obtain information also from air monitoring stations (e.g. in Italy those of ARPA, Regional Agency for Environmental Protection). It could be useful to place an apiary near the stations that measure air pollutants.

MONITORING OF PESTICIDES IN DIFFERENT BEEKEEPING PRODUCTS

Pesticide monitoring can be triggered when mortality thresholds are exceeded in adult bees, which are better bioindicators than live bees for determining the molecule that likely contributed to their death. [71] Pesticide analyses can be conducted on live bees and on dead bees in the colony; the latter are collected with special traps when the threshold of the number of dead bees is exceeded. Between 5 and 50 dead bees per hive are sampled; the greater the sample, the more reliable the information. Bees can be washed with solvents (such as acetone) to extract and measure pesticides on the surface of the body and then homogenized to extract pesticides accumulated inside.

Pesticides can also be searched in honey and pollen, as honey is a foodstuff and pollen provides information on plant contamination. The search for pesticides in pollen allows us to investigate possible illegal uses of pesticides and exclude their use by beekeepers: it is possible to take a sample of pollen directly from the bees as soon as they return to the hive with special traps, which will be placed 2 to 7 days before sampling. [32] Pollen stored in the hive cells can be used, as it may also be contaminated by the products used by beekeepers and may have exchanged some substances with wax. Pollen is the most suitable matrix to monitor phytosanitary treatments in the field and honey can be used to investigate the products used by beekeepers. [39] Wax is the matrix where the highest concentrations of pesticides can be measured, such as the acaricides used by beekeepers to control *Varroa*. Wax has a long record of treatments carried out both in the field and by beekeepers, and honey is more likely to contain hydrophilic molecules. Pesticide concentrations decrease from wax, to pollen and finally to honey. [41]

A study shows that 161 pesticides were found in beehives all over the World, of which 124 in pollen, 95 in wax and 77 in honey or nectar. [45] The highest concentrations of pesticides are found in wax (average concentration of 126 µg/kg), followed by pollen (average concentration of 66 µg/kg). Of the 124 pesticides found in pollen, 70 are found only in this matrix and not in honey. Of the 77 active molecules found in honey, 23 were found only in this matrix and neonicotinoid insecticides are the most easily found. Therefore, it is not easy to generalize and many factors must be taken into account, such as the chemical and physical characteristics of the molecules to be searched for and of the available matrices.

Some studies show that the matrices most contaminated by pesticides are, in decreasing order, honey, pollen and bees: honey, in these cases, is very contaminated by the products used

by beekeepers and residues in wax have not been investigated; while other studies show a different order, with pollen being more contaminated (in decreasing order: pollen, honey and bees). Also in the latter case, residues in wax were not investigated. [39] Wax is an excellent matrix for detecting contaminants used by farmers and beekeepers.
If you decide to investigate wax and honey it will be possible to take samples in late spring, during the summer and possibly also in early autumn.
In order to get information on phytosanitary treatments, it will be possible to schedule samplings every 5-15 days, depending on the duration of the flowering plants visited by bees. In Italy, most phytosanitary treatments are carried out in spring, so in this period it will be possible to record the peaks of environmental concentration of pesticides and the highest number of mortality or suffering events in bees. Pollen is the most suitable matrix for monitoring phytosanitary treatments in the field, if taken before being stored in the hive; honey and wax are suitable for obtaining information on the products used by beekeepers. Wax is the best matrix in this case, as many pollutants are lipophilic and are accumulated for months or years (wax is recycled by beekeepers, increasing its contamination and thus the risk to bees). Apolar substances such as the acaricides used by beekeepers against *Varroa* (coumaphos and tau-fluvalinate) can be found in this matrix long after the treatment. [67] One of the first records of *Varroa* mite colonies resistant to fluvalinate dates back to 1998. [974] Honey will more easily contain hydrophilic substances such as some insecticides (neonicotinoids and lindane[I]) while wax will contain hydrophobic substances. [45, 182]
In order to know the species visited by the bees, palynological analysis can be carried out, i.e. the determination of pollen species. The pollen can be taken from the bees when they return to the hive (with a special mechanical collection system) or in the honey (if the honey is not filtered and if it does not come from honeydew).

SOME INDICATIONS FOR BIOMONITORING

Depending on the information you want to collect you will use different sampling methods taking into account many factors. For example:

- If you want to investigate the causes of bee death, it may be useful to take dead bees removed by the workers outside the hive.
- In order to measure the contaminants present in the pollen, it is advisable to take it from the foraging bees when they return to the hive instead of taking it from the comb cells, as it may have exchanged pollutants with the wax or may have been exposed to treatments carried out by beekeepers (e.g. fumigation that releases PAHs and metals, or acaricides used to treat *Varroa*). Until it is brought into the hive, pollen is a good indicator of the treatments carried out by farmers. Moreover, it should be underlined that it is the main food in the juvenile stages of bees.
- To detect phytosanitary treatments carried out by both farmers and beekeepers, even in the months before or the year before, it is advisable to use wax; for some hydrophilic molecules, it is preferable to use honey (e.g. neonicotinoid insecticides such as

[I] Lindane or γ-HCH is a chlororganic insecticide marketed since 1938. Also known as γ-hexachlorocyclohexane, it is neurotoxic. It was used in agriculture and pharmaceutical production to treat scabies and eliminate lice. Lindane is a neurotoxin that interferes with the neurotransmission functions of GABA (*gamma*-Aminobutyric acid); it interacts with the chloride channel of the GABA receptor. In humans, lindane primarily attacks the nervous system, liver, and kidneys and is also likely to be an endocrine disruptor. Its use is currently banned in more than 50 countries including Italy.[182] It is considered a persistent organic pollutant and is classified as a probable carcinogen: it was banned in Europe in 2006.

imidacloprid). Pollen and dead bees can be used to detect treatments carried out by farmers a few days earlier.

- In order to assess the variation over time (over months and years) of the presence of radionuclides, it is advisable to use wax from sedentary hives where it should not be recycled.
- Wax and propolis should be used to monitor polycyclic aromatic hydrocarbons.
- Propolis, wax and honeydew honey can be used to monitor the presence of metals. If the objective is also to assess food safety and healthiness, it will be necessary to measure the concentrations of contaminants such as metals and pesticides in honey as well.
- If it is useful to have information on the plant species visited by the bees, the surveys can be supported by the identification of the types of pollen transported by the bees. In general, the melisso-palinological analysis will provide indications on the geographical area visited by bees to collect nectar and produce honey, and it will allow us to know the species that have been contaminated. Pollen can be collected by the bees upon return to the hive or from the cells and is contained in honey too.
- In order to know the identity of the honey and its geographical origin, as has just been pointed out, it will be possible to carry out melisso-palinological analyses. If this investigation is not possible (e.g. the honey has been filtered or is from honeydew) by examining the profile of the concentrations of metals or polycyclic aromatic hydrocarbons and comparing them with a sample of control honey, it will be possible to determine the similarity and, therefore, the origin of the honey. [44, 62] In some cases, the examination of the profile of metals or polycyclic aromatic hydrocarbons in honey may be sufficient, even without a reference honey, to verify the area of origin or to make comparisons.

HONEY SAMPLES

Honey is not a good indicator of contamination by metals, polycyclic aromatic hydrocarbons (PAHs), and pesticides. Honey is derived from nectar, which is a sugary substance that has little affinity for PAHs, which are usually hydrophobic and therefore do not bioaccumulate in it. [60] Honey may contain water-soluble pesticides such as some insecticides (neonicotinoids such as imidacloprid which is persistent). [76] Examination of the concentration of hazardous substances in honey is important even if they are not bioconcentrated because it is a food. In addition, honey samples from different agricultural areas contain different pesticides confirming the possibility of using honey to get information on the molecules used by farmers and beekeepers. [58] There will be a choice of taking "young" or older honey. Honey from some plant species is more suitable than others for biomonitoring because of the ability of some plants to accumulate certain molecules more easily. Honey is collected for many days over a wide area and therefore provides a more extensive spatio-temporal indication than, for example, the use of foraging bees.

Honeydew honey is a very interesting bioindicator because it has the capacity to accumulate substances (e.g. pesticides, polycyclic aromatic hydrocarbons, radioactive isotopes and metals) more easily as the food chain involves a passage between plant and aphids before reaching the bees. Bioconcentration factors have the potential to be 10 to 100 times greater due to passage through one more trophic level. It is also important to remember that honeydew honey does not contain pollen.

WAX SAMPLES

Wax consists of hydrocarbons, fatty acids and esters, which are chemically stable substances that are resistant to hydrolysis, oxidation, attack by organic acids and gastric juices. [70] The wax can remain in the hive for years and beekeepers have the habit of recycling it by melting. The raw wax taken from a hive can be processed by various melting systems to produce wax sheets, which are used for beekeeping itself.

Wax samples may be obtained from several colonies, from each of which a piece of 10 cm x 10 cm size or 10-20 g weight may be extracted (2 g will be used for analytical determinations in the laboratory). Wax, in some cases, may also be taken from samples of honey such as that marketed with wax.

Among the products of the hive, beeswax is certainly the matrix in which pollutants and contaminants spread in the environment, especially pesticides used in agriculture, are more concentrated: especially lipophilic molecules. In fact, the active molecules of pesticides are often hydrophobic (they do not like water), they are not very volatile and persistent, they tend to absorb and accumulate in lipidic matrices such as beeswax. Beeswax is the best matrix among beekeeping products to have information about the phytosanitary treatments carried out in the field and by beekeepers, even months before. Both chemicals used by beekeepers and pesticides can remain in the wax for years and therefore allow information on any illegal treatments carried out by both beekeepers and farmers. [55, 107]

Wax can provide different information depending on its age. Virgin wax can have 18 times less pesticide contamination than wax recycled by beekeepers. [54] Coumaphos (an acaricide used by beekeepers against the *Varroa* mite) is found in high concentrations in wax even 6 months after the treatment of the colony (529 µg/kg). [68]

Studies have shown that wax can release some substances it absorbed into the substrates it contains in the hive such as pollen, honey or larvae and vice versa. If the wax is taken after storing pollen in the cells, it may also have absorbed some pollutants contained in the pollen. [54] In wax, coumaphos is found at a concentration more than 1,000 times higher than in honey. [35] Naturally, the higher the concentration of the pesticide in wax, the more easily it can be absorbed by honey or larvae.

Some active molecules such as coumaphos are not reduced by the 2-hour heat treatment at 140°C applied to recycle the wax; moreover, this acaricide has a half-life that can vary between 115 and 346 days. [57] The results of some biomonitoring show that wax is often contaminated mainly by acaricides used by beekeepers and, to a lesser extent, by insecticides and fungicides used in the field. Residues of some active molecules of acaricides widely used by beekeepers in Switzerland (and elsewhere) are found in wax treated at high temperatures (bromopropylate, coumaphos, fluvalinate, flumethrin and thymol). [70] Therefore, the practice of beekeepers to recycle wax poses an increased risk to bees as some molecules remain in the hive and accumulate. [68]

As already mentioned, wax is the matrix most contaminated by pesticides in terms of quantity, whereas pollen contains a greater number of pesticide molecules. The samples of live bees are less contaminated than wax, confirming the better representativeness of the pollution of the latter. In conclusion, wax freshly made by bees provides short-term information, whereas old wax such as that recycled by beekeepers can provide information on contamination received months or years earlier.

BEE SAMPLES

Live bees can be an indicator of airborne molecules taken in by contact and also by ingestion such as metals, pesticides and organofluorides. Many studies detect pollutants at lower concentrations than in pollen and wax. If you choose to use bees it is advisable to take individuals of the same age and caste for each sample. Some contaminants distribute differently in different stages and castes. For example, air pollutants may be measured in foraging bees, whereas larvae may be more sensitive to certain pesticides. As far as bees are concerned, 20 or 50 may be collected per hive. One hundred and fifty specimens may be taken per monitoring station, i.e. from three hives, from which 50 are taken in each. The bees will be washed if it is desired to measure the concentration of pesticides and metals on the outside of the body (substances are extracted from the surface of the body with a solvent to analyse their concentration). Hydrophobic compounds are more likely to be found inside the bees' bodies and hydrophilic compounds outside. [47] The body of the bees will be homogenized to measure the concentration inside the body; contaminants can be selectively extracted to look for only in one part of the body such as the abdomen which contains most of the hemolymph (this does not have the function of transporting oxygen as it does in the blood, but does have the function of transporting nutrients). Bees before being frozen must be cleaned of pollen, in the sacs of the hind legs and on the body. In addition, bees transport nectar in the digestive tract, so by analyzing the bees you will also have information about the nectar. To obtain more information, the different anatomical parts of the bee (e.g. abdomen) can be separated and the external content of the body examined after washing with solvents, and the internal content after cleaning the external part. Freshly collected bees must be stored at -20°C until analysis.

In dead bees it is possible to try to investigate the causes that generated lethal effects such as insecticides-acaricides or other anthropogenic contaminants. Between 2 and 5 g of dead bees can be collected per sample; considering that the weight of each bee is about 90 mg (between 60 and 110 mg), this means collecting between 20 and 55 bees. If the bee has just collected nectar and pollen it may weigh about 150 mg. As already explained, if the bees are not cleaned of the pollen and if they have collected nectar we will get indications of the contamination of all three matrices: pollen, nectar and insect.

POLLEN SAMPLES

Pollen can be collected by foraging bees returning to the hive with special mechanical capture systems placed at the entrance of the hive from three days and up to one week before sample collection. Collection can be carried out for a few days so to collect up to a few tens of grams of pollen. Pollen can also be collected from the cells in the hive. When pollen is collected from bees returning to the hive, information about environmental contamination such as that from pesticides used by farmers is obtained. On the other hand, if pollen is collected after it has been stored for weeks in the cells, the pesticide contamination generated by the beekeepers will also be measured. In addition, when they are in contact in the cells, pollen and wax can exchange molecules, so the pollen may become contaminated with some molecules contained in the wax. If you do not have a lot of resources to perform chemical analysis of pesticides, you can measure them in the pollen stored in the combs. Pollen usually contains more active molecules than wax, although wax has the highest concentrations, especially of chemicals used by beekeepers.

There are many studies that show a dangerous contamination of pollen with pesticides used in agriculture, but there is little information on the contamination of the female part of the flowers.

PALYNOLOGICAL AND CHEMICAL ANALYSIS TO IDENTIFY THE GEOGRAPHICAL ORIGIN OF HONEY

In order to determine both the geographical area and the floral area of the honey, it is possible to catalogue the types of pollen contained in it: this is the melisso-palinological analysis. In this regard it is necessary to remember that between 100 and 100,000 pollen grains can be naturally present in every gram of honey. Recognition of the species of pollen collected by bee bags and those contained in honey makes it possible to determine which plants have been visited by the insects. By examining the composition of the pollen, that is, by identifying the species to which it belongs using an optical microscope and special atlases, it is possible to trace the most visited sites. The examination of the pollen composition also allows us to trace back possible illegal behaviours such as the distribution of pesticides during flowering or the use of forbidden substances (e.g. pesticides not authorized on a particular cultivation). Determining the vegetable origin of pollen allows us to highlight possible commercial frauds: honey wrongly classified as chestnut instead of wildflower (mixed blossom). Honeydew honey does not contain pollen and beekeepers may have filtered the honey. In such cases, it is not possible to trace the floral origin. In addition, blending or artificial addition of pollen may be carried out.

As already mentioned, when pollen is collected by bees as soon as they return to the hive, it represents an indicator of agricultural contamination e.g from pesticides used in the field. If it is collected inside the hive cells, it may also contain molecules used by beekeepers and those released by the wax.

Through the use of special mechanical systems that are installed at the entrance of the hive, it is possible to collect pollen from the returning bees. When the bees return to the hive, with a simple system, the bags located in the hind legs of the worker bees are emptied into a special container. In this way it is possible to collect the pollen that has just been taken and not yet processed by the bees. Pollen can also be collected inside the hive, from the cells where it is stored or from the honey if it has not been filtered. In order to identify the plant species visited by the bees, it is sufficient to examine 300 pollen grains. The analysis of the pollen content is carried out under an optical microscope using special melisso-palinology atlases. [78]

A honey is considered mono-floral when at least 45% of the pollen comes from a single botanical species. There are exceptions such as chestnut honey, which must contain at least 90% of pollen from that species. To be defined mono-floral acacia honey, it is sufficient that it has 15% of pollen from this species.

It is possible, with more difficulty, to trace the geographical origin of honey by examining the profile of metals, polycyclic aromatic hydrocarbons and radionuclides. However, these methods may require reference samples for comparison and are much more expensive.

ENVIRONMENTAL MATRICES FOR COMPARISON

Honey, wax, bees, pollen are not equivalent in providing information on different pollutants such as pesticides or metals. Pollen and wax are the most suitable matrices if it is necessary to collect information on pesticides. Pollen transported by bees represents the contamination of the visited plants while wax also provides information on the products used by beekeepers, even from the year before. Wax is the matrix where it is possible to have more information on polycyclic aromatic hydrocarbons and radionuclides. Propolis can also be used to obtain information on polycyclic aromatic hydrocarbons and radionuclides. Bees and honey provide less information in this respect, as monitoring shows that concentrations and numbers of pesticides and polycyclic aromatic hydrocarbons are lower. An exception is honeydew honey.

It is fair to point out that other matrices may be more representative of the level of environmental contamination than beekeeping products, as they may contain higher concentrations of metals. In order to obtain information on metal contamination, to examine their concentrations in soil and in plants that bioaccumulate them should be considered, while polycyclic aromatic hydrocarbons are well represented in leaves.

Information obtained from beekeeping products can be compared with that obtained from environmental matrices such as concentrations in flowers, soil, water and air. For example, for metal detection, three aliquots of soil (100 g, from the top 10 cm) can be taken within 400-600 m of the hives, which will be mixed to form a single sample. It will be possible to sample:

- plant leaves for information on metals and polycyclic aromatic hydrocarbons;
- flowers, water and soil for metals (and radionuclides) and plant protection products.

When the objective of monitoring is to get information on metal contamination in soil some studies reveal that the concentration measured in acacia and sunflower flowers is a better indicator than honey, as they bioconcentrate them. [44] To get information on radionuclides it is possible to sample conifer leaves (young needles) that can be taken from 25-30 trees. [59]

Beekeeping products may not necessarily provide better information than other environmental matrices. It depends on the objective of biomonitoring, which, in a preliminary phase, should also evaluate alternatives to the use of expensive analytical techniques on beekeeping products.

SUITABILITY OF DIFFERENT BEEKEEPING PRODUCTS FOR BIOMONITORING

Some information on the different capacity of different matrices to provide information on certain environmental pollutants is summarized. These indications, extrapolated from different studies, allow us to state that metals, in general, are not accumulated in beekeeping products: it is better to look for these contaminants directly in the soil or in plant tissues that bioaccumulate them, unless we want to assess a risk for food safety (e.g. metals in honey) or for bee health. [65, 72] It is possible to try to generalise, with due caution, by stating that metals can be found in different matrices in the following decreasing order of concentration (e.g. cadmium, chromium and lead): [176, 72]

soil > flowers (pollen) > bees > water ≥ honey from nectar.

It should be noted that many plants are able to bioaccumulate the metals present in the soil in certain tissues, therefore they can register higher concentrations than in the soil (e.g. acacia flowers bioconcentrate more than 14 times the potassium, 11 times the phosphorus, 8 times the sulphur, 5 times the boron and 3 times the molybdenum, so they can be used to obtain information on these metals present in the soil; sunflower flowers bioconcentrate more than 22 times the boron, 8 times the sulphur, 6 times the potassium, 3 times the phosphorus, and 3 times the molybdenum, compared to concentrations recorded in the soil). [44]

Bees may record higher concentrations of metals released into the atmosphere than honey. It may be useful to have some general indications even if they are not valid for all possible conditions; for example, the ratios between metal concentrations measured in different matrices will be influenced by the type of contamination source such as atmospheric rather than discharges into water or fertilizers that contaminate the soil (e.g. metals in sludge derived from water purification plants or compost derived from municipal solid waste and industrial waste). It is important to consider that elements such as copper, manganese, arsenic, and aluminium are also constituents of pesticides, so this may be another source of contamination. Cadmium and lead can be derived from manure, sewage, industrial effluents and fossil fuels through atmospheric deposition. Under certain conditions concentrations measured in honey may be higher than those measured in water (e.g. metals from air pollution). The search for the presence of metals in water is useful for the evaluation of health risks (e.g. drinking water) or for other types of investigations such as those on the consequences generated by sewage discharges or industrial activities. Therefore, the suggested concentration level and the indications reported in the following table cannot be applied for all metals and in all possible conditions, but the message that we want to emphasize is the following: beekeeping products are not the most suitable matrix for biomonitoring studies of environmental metal contamination (to give an example, the highest concentrations of lead released into the atmosphere are found in soil, if compared with those measured in beekeeping products). [708, 732] The search for metals can have different purposes such as investigating increases in mortality or geographic identification based on relationships between different concentrations. [62]

To take another example, wax is probably one of the best matrices that can be used to obtain information on the use of chemicals by beekeepers. Furthermore, pollen stored in the hive, in addition to recording the contamination caused by the beekeeper, will be able to provide information on pesticides used by farmers (e.g. during flowering).

With regard to polycyclic aromatic hydrocarbons, it is interesting to report a surprising finding. These substances can be found at high concentrations in propolis because bees, instead of taking resin from plants (such as conifers), collect hydrocarbons and gummy substances from asphalt and tar, or from paints. [59, 60, 974] Bees, in the absence of plant resins, such as those produced by pine needles, have learned to use asphalt and tar that contain various polycyclic aromatic hydrocarbons (or PAHs).
Some types of pollen can have as much as 25.4% lipids (e.g. *Brassica napus* plant) and, therefore, PAHs can be easily accumulated there as they are lipophilic.

In the following table different suitability gradations of different matrices for the detection of some categories of substances are suggested. In the planning phase of biomonitoring all variables should be examined to achieve the required objective and it is important to note that the indications given in the table below may not be the most representative of particular conditions. In order to save time and resources, before carrying out biomonitoring test, we should try to predict how pollutants are distributed in the environment so that we can identify the matrices where they are concentrated and, therefore, in which it becomes easier to identify them.

	Pesticides	**IPA**	**Metals**
Live Bees	II[A]	I	II[A]
Dead bees	II[A]	I	II[A]
Nectar honey	I[B]	I	I
Honeydew honey[D]	II	II	II
Pollen [C]	**III**	II	II
Wax[E]	**III** (lipophilic)	**III**	II
Propolis	X	**III**	II
Soil	X	**III**	**III**
Air	--	II	II
Water	II	II	II
Vegetables	**III**	**III** (e.g.: leaves)	**III** (e.g.: flowers)
Lichens	II	II	**III**

Notes:
PAHs = polycyclic aromatic hydrocarbons.

Representativeness of the matrix:

I = suitable only in certain cases (e.g. comparison samples must be available; information on food safety or bioaccumulation capacity may be available).
II = good.
III = matrix very good for the type of investigation. In this type of matrix the highest concentrations can be detected.
X = presumably suitable matrix although no information on this has been reviewed in the scientific literature.

[A] They reveal contamination that occurred only a few days ago. Some persistent and bioaccumulative pesticides in live bees may show higher concentrations than those in soil and flowers.
[B] Nectar consists mainly of water and sugar, so water-soluble pesticides such as some neonicotinoids (imidacloprid) and lindane may be found in it. [45, 182] Honey is not a suitable matrix for the detection of lipophilic pesticides.
[C] Pollen, if collected before bees enter the hive, may provide information on plant contamination. If it is collected after it has been stored in the honeycomb cells, it may also have been contaminated by the products contained in the wax and those used by beekeepers. Iron, zinc and magnesium are essential metals, so it is possible to find them in high concentrations in pollen.
[D] Higher bioconcentration due to aphids.
[E] Virgin wax will be less contaminated than old wax or wax recycled by beekeepers. Therefore, the latter will have a memory of what happened more than a year before.

BIOMONITORING AND CONCENTRATION GRADIENTS OF POLLUTANTS IN THE ENVIRONMENT

By examining the concentration gradients of pollutants (e.g. metals) measured in beekeeping products from hives located in different sites, it is possible to obtain information on the most likely sources of contamination (e.g. motorways, textile industries). [17] The different concentrations recorded in hives located in distant sites and the gradient that can be obtained can suggest where the pollutants come from. By comparing the variation of concentrations over time at different sites, it is possible to identify the area from which the contaminant under study comes (e.g. factory, waste depot, farmer). The presence of some pollutants is often associated with others, and this information can also give useful indications.
In the event of nuclear accidents or explosions (e.g. tests with nuclear devices conducted in the air or in the ground), it is possible to record the variation in space and time of the different radioactive isotopes, even hundreds or thousands of kilometres away.

SAMPLING OF BEEKEEPING PRODUCTS

Samples should be handled appropriately and analysed as soon as possible after collection, preferably within 48 hours. In general, samples should be stored below -20°C until analysis. Honey and wax can be stored at +4°C and in the dark for a few days. [71] It must be pointed out that some pesticides are rapidly transformed into metabolites which, in some cases, are as toxic or more toxic than the starting molecules, much more persistent but difficult to determine.

For sampling it is advisable to take samples from several hives (at least 2 or better 5 hives per apiary or monitoring station) and to follow two possible procedures:

1) Mix the samples and analyze them together. One may use 100 g of honey, or wax, or a few grams of pollen.
2) Take several samples from the same monitoring station, i.e. from 2 and up to 5 hives and analyse the samples separately, without mixing them. The final result could be obtained as an average of 3 or 5 samples. This procedure may be used for less expensive analytical methods, such as measuring the presence of radionuclides (the radioactive isotopes).

In general, all samples such as honey, wax, propolis, and bee samples will be more representative if obtained from several colonies at the same site (apiary). A few grams of honey, which could be obtained from several elementary samples (e.g. 500 g of honey obtained by mixing honey collected from 5 hives), are sufficient for laboratory analysis. In general, the samples obtained by mixing may weigh from a few dozen to a few hundred grams. From the mixture of matrices obtained from several colonies in the same apiary, the sample for the laboratory is extracted, which can be a few dozen grams (most analytical techniques require less than 3 g of sample).

THE PREVENTION OF POSSIBLE INTERFERENCES

Care must be taken to use metal-free hives if these substances are to be monitored and not to use fumigations. The fumigations used by beekeepers, to calm and stun bees during hive inspections, are a source of metals and also polycyclic aromatic hydrocarbons. Operators should be prohibited from smoking during hive inspection and honey should be extracted using slow extraction systems.

It must be considered that paints and plastics can release polycyclic aromatic hydrocarbons and other substances that could alter the analytical results. Hives should not be painted or treated with the sought-after molecules. Sample containers should also not be a source of contamination. Therefore, care should be taken to consider and exclude all possible interfering factors. If it is decided to investigate plant protection products used in the field, stationary apiaries should be chosen and not treated by beekeepers, otherwise molecules distributed by farmers and those used by beekeepers can be recorded simultaneously.

Suction systems and traps can be used to pick up bees, and cold and narcosis (carbon dioxide) can be used to immobilize or calm them. [195]

LABORATORY ANALYTICAL METHODS

Various techniques such as atomic spectrometry, flame atomic absorption spectrometry and flame atomic emission spectrometry can be used to examine metals.

Radionuclides (radioactive isotopes of metals) have energy in their nuclei that makes them unstable. This excess energy must be dissipated to transform unstable nuclei into stable ones, through the release of new particles such as alpha and beta radiation or gamma radiation. Different radionuclides emit gamma rays with different energy levels (between 15 keV and 10MeV). By measuring the emission of gamma rays, it is possible to have both qualitative (the nature of the radionuclide that emits them) and quantitative information (the concentration). The study of the spectrum of the gamma radiation allows us therefore to have information on the radionuclide. For the measurement of radionuclides, it is possible to use the gamma-ray spectrometer which has the advantage of being able to be used directly in untreated samples, for example honey or bees. Thus measuring radionuclides is easier than measuring metals or pesticides as no preparation is required. [71] The analytical measurements may be repeated two to five times for each sample to get a better representation of the concentration (thus the reliability of the method can be assessed). Reference samples should be used and other procedures adopted to check the quality of the result obtained.

Speaking of radiation, it is useful to remember that gamma radiation can also be used to sterilize infected materials including wax and honey. [35]

The choice of the analytical technique will influence the results such as those on residues of active molecules of pesticides that can be searched with the instrument called gas-chromatograph followed by the triple-quadrupole mass spectrometer, or high-pressure liquid chromatography can be used with different types of detectors (e.g. FID or flame ionization detector). [76]

THE USE OF MOLECULAR BIOMARKERS IN BEES

Another possibility, which is briefly mentioned, is the use of biomarkers that can be defined as observable and measurable changes at the molecular, cellular, or physiological level. These biomarkers allow the detection of an organism's exposure to xenobiotics, i.e. foreign substances. [43] Biomarkers may be detected in sentinel species and, for example, may be DNA or enzymatic systems, and allow us to have indications on the exposure to toxic substances for the nervous system such as metals or information on the functioning of the metabolism. Among the enzymatic biomarkers (proteins with a catalytic action) there are those that measure the level of oxidative stress and those that measure alterations in the detoxification system (they have the task of transforming dangerous molecules into other which are less dangerous ones and easier to eliminate). Some of the enzymes whose activity can be measured to detect deleterious effects on bees are:

- Alkaline phosphatase and glutathione-S-transferase as metabolic indicators of detoxification processes. Glutathione-S-transferase is an enzyme whose action is induced by some contaminants and increases in the presence of pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and metals.
- Acetylcholinesterase as a biomarker of the nervous system (e.g. it is altered by organophosphate insecticides, pyrethroids, and carbamates). Acetylcholinesterase can provide information on neurotoxic molecules such as insecticides and also on some metals: in the presence of some of these substances its function is reduced.

- Metallothioneins as biomarkers of adverse effects from metals. Metallothioneins in the nervous system are implicated in the detoxification of certain metals that have neurotoxic effects. [42]

By measuring the reduction or increase in the activity of these proteins, and the variation in their concentration, it is possible to obtain information on the state of health of the bees and on the negative mechanisms of action generated by pollutants, without detecting the identity of the molecules responsible. These effects are sub-lethal (they do not kill in the short term) and can still generate disastrous effects on the colony. Measuring and comparing the activity of these biomarkers makes it possible to hypothesize the category of pollutant to which the insects have been exposed and to discriminate one polluted site from another. A limitation of this methodological approach is that it requires equipped laboratories and highly qualified personnel. Moreover, it does not allow the identification of the pollutants responsible. It is a bit like measuring a fever: you have an indication that alone, in most cases, is not sufficient to identify the cause of the disease.

PESTICIDES: MANIFESTO OF UNSUSTAINABLE FOOD PRODUCTION

SOME MECHANISMS OF ACTION OF PESTICIDES

The first record of the use of insecticides to protect crops probably dates back to 1000 b.C. [193] In Italian they are indicated as plant protection products, pesticides, phytopharmaceuticals or agro-pharmaceuticals. Phytopharmaceuticals (*prodotti fitosanitari* in Italian) is an expression that lightens and makes less visible the purpose of these products: to annihilate insects or kill other living beings such as fungi or plants. [1295] Pesticides are chemicals (sometimes microorganisms or their products such as toxins) used in agriculture to eliminate anything that harms crop plants from the farmers' point of view.

Based on their chemical characteristics, pesticides may be classified into different categories: organochlorines, organophosphates, carbamates, pyrethroids, phenoxy-derivatives, triazines and amides. They are also classified according to their mechanism of action. For example, organochlorine, organophosphorus and pyrethroid insecticides act basically as neurotoxins. Some herbicides (the phenoxiderivatives) act like plant hormones (e.g. auxins), some fungicides (the dithiocarbamates) alter energy processes and generate oxidative stress at the cellular level.

The first pesticides to be used were inorganic ones based on copper sulphate or arsenic. In 1881 the first death of bees was recorded due to the use of an insecticide, based on arsenic, in fields cultivated with plums. [974] In the 1940s, organochlorinated pesticides appeared, including the famous DDT, and subsequently, organophosphates were produced, which proved to be even more dangerous for bees. Following the extensive use of these molecules, resistant insects appeared and the chemical industry put carbamates on the market. These too soon proved to be very toxic to bees. For example, in 1967 carbaryl (a derivative of the carbamic acid) killed 70,000 colonies in North America when used on cotton and 33,000 colonies when used on maize (out of 700,000 colonies). [243, 249] Immediately afterwards pyrethroids appeared on the market, insecticides which are synthetic products inspired by the natural pyrethrins of flowers, and are arthropod endocrine disruptors, which prevent the normal formation of chitin in the exoskeleton of insects (such as diflubenzuron which is an insecticide of the benzoylurea class). In nature after cellulose, chitin is the most abundant biomolecule.

In the 1990s neonicotinoid insecticides and phenylpyrazole (fipronil) arrived. [250] A few grams of neonicotinoids (e.g. imidacloprid) are sufficient to protect one hectare of crop from insects (e.g. elaterid beetles).

Over time, the molecules used in agriculture have proven to be effective at lower doses, and some have also proven to be very persistent in the environment. History repeats itself: insects develop resistance even to newer and more toxic pesticides such as neonicotinoids. Examples include the cotton aphid (*Aphis gossypii*) which has become resistant to imidacloprid and acetamiprid, the potato beetle (*Leptinotarsa decemlineata*) and a rice pest (*Nilaparvata lugens*) which has become resistant to neonicotinoids. [415]

Pesticides, depending on the organism they are used against, can be classified into the following categories: [25]

- Insecticides are used to kill insects considered to be harmful to agricultural crops (such as aphids and scale insects), but also to control insects that are bothersome or disease-carrying to humans or domestic animals.

- Fungicides that combat fungal diseases.
- Herbicides used to destroy weeds (this includes defoliants).
- Nematocides that exterminate worms or nematodes.
- Acaricides that kill mites and other arachnids.
- Plant growth regulators which are plant hormones used to regulate crop growth.

Insecticides are also classified according to the site where they act: [193]

- Carbamates (e.g. propomocarb hydrochloride, oxamyl) and organophosphates (e.g. acephate, chlorpyrifos, diazinon, dimethoate, fenitrohion) are acetylcholinesterase inhibitors.
- Pyrethroids (e.g. cypermethrin, cyfluthrin, deltamethrin) modulate sodium channels.
- Neonicotinoids (e.g. acetamiprid, clothianidin, imidacloprid, thiametoxam) are acetylcholine receptor agonists.

Unfortunately, there are no selective molecules, i.e. able to kill only the target species, the one considered an enemy by the farmer. [1175] All molecules act negatively on non-target organisms and often manifest actions that are completely different from those for which they are used. Thus, for example, herbicides have also been found to act as antibiotics and insecticides as hormones (endocrine disruptors). Moreover, many insecticides do not have any repellent effect on bees, which even prefer to consume a sugar solution enriched with pesticides, such as the herbicide glyphosate or the fungicide chlortalonil. [718] This abnormal behaviour encourages harmful exposure and synergistic effects.

In North America, a four-year study (2009-2012) on beekeeping products from stationary apiaries in six States found 82 pesticides, eight metabolites and one synergistic molecule in 168 pollen and 142 wax samples. [1203] Of the 91 molecules found 38 (42%) are insecticides, 29 (32%) are fungicides and 23 (25%) are herbicides. 78 pesticides were found in pollen (32 were found only in this matrix), 58 in wax (12 were found only in this matrix). Of the 91 molecules found, information on lethal doses by contact and ingestion is available only for 51. The mechanisms of action of some pesticides found in beekeeping products in North America are summarized below. It is essential to emphasize the destructive power of these molecules as they alter mechanisms that are essential to the functioning of all living beings. These are biochemical reactions and physiological functions on which the essence of life is based, such as the synthesis of lipids, proteins, nerve transmission, cell division, photosynthesis or growth regulation. We systematically distribute in the environment tons of chemical molecules capable of damaging, at infinitesimal concentrations, biochemical reactions that are ubiquitous because they are fundamental: they inhibit the synthesis of nucleic acids (DNA or RNA), alter nerve transmission, inhibit cellular reproduction and damage the mechanism of photosynthesis. This is an insane attack as it is suicidal. Persistent and bioaccumulative molecules capable of irreversibly changing the foundations of the essence of life should be banned without delay.

Mechanisms of action of pesticides found in wax and pollen in North America

Fungicides [1203]

- Alteration of nucleic acid synthesis: benalaxyl, metalaxyl, octhilinone.
- Alteration of the cell division process (effects on beta-tubulin protein assembly during mitosis): carbendazim, thiabendazole, thiophanate-methyl.
- Alteration of respiration biochemistry complex II (succinate-dehydrogenase): boscalid; complex III (cytochrome bc1): azoxystrobin, fluoxastrobin, pyraclostrobin, trifloxystrobin; (oxidative phosphorylation uncoupler): fluazinam.
- Alteration of amino acid and protein synthesis (e.g. methionine synthesis); cyprodinil, pyrimethanil.
- Alteration of signal modulators (MAP/Histidine-kinase in osmotic signal transduction): iprodione.
- Alteration of membrane lipid biosynthesis: diphenoconazole, epoxiconazole, fenbuconazole, imazalil, metconazole, myclobutanil, propiconazole-1, tebuconazole (C14-demthylase in sterol biosynthesis); spiroxamine (D14-reductase and D8-D7-isomerase in sterol biosynthesis).
- Alteration of the cell wall synthesis process in plants (cellulose): dimethomorph, mandipropamid.
- Action at many sites: chlorothalonil.
- Unknown mode of action: cymoxanil, diphenylamine.

Herbicides

- Inhibition of the enzyme acetyl CoA carboxylase: sethoxydim.
- Inhibition of the enzyme acetolactate synthase: halosulfuron-methyl, metsulfuron-methyl.
- Inhibition of the enzyme photosynthesis at photosystem II: atrazine, metribuzin, prometon, prometryn, simazine. diruon, fenuron, linuron, siduron, bentazon.
- Inhibition of the enzyme protoporphyrinogen oxidase: sulfentrazone.
- Inhibition of mitosis (inhibition of microtubule formation): dithiopyr, pendimethalin, prodiamine, propyzamide.
- Inhibition of mitosis (inhibition of long chain fatty acid synthesis): acetochlor, alachlor, metolachlor, napropamide.
- Alteration of auxin synthesis: MCPA.

Insecticides

- Action on the nervous system (reversible inhibition of acetylcholinesterase): aminocarb, bendiocarb, carbaryl, carbofuran, methiocarb, methomyl.
- Action on the nervous system (irreversible inhibition of acetylcholinesterase): acephate, azinphos-methyl, chlorpyrifos, coumaphos, diazinon, dichlorvos, dimethoate, malathion, omethoate, phorate, phosmet.
- Action on the nervous system (sodium channel modulator): fluvalinate, resmethrin.
- Action on the nervous system (agonist of the nicotinic acetylcholine receptor): acetamiprid, clothianidin, imidacloprid, thiamethoxam, spinetoram.
- Mitochondrial enzyme (ATP synthetase) inhibitor: propargite.
- Inhibitor of chitin biosynthesis (type 0): diflubenzuron.
- Alteration of growth regulation (ecdysone receptor agonist): methoxyfenozide.
- Action on the nervous system (octopamine receptor agonist): amitraz.
- Alteration of energy metabolism (inhibition of electron transport in mitochondria): fenpyroximate, pyridaben, rotenone.
- Alteration of lipid synthesis and growth regulation (inhibition of acetyl CoA carboxylase): spiriclofen.
- Nerve and muscle impairment [ryanodine (calcium) receptor modulator]: chlorantraniliprole.
- Synergistic action (Cytochrome P450-dependent monooxygenase inhibitor): piperonyl butoxide.

TOXICOLOGICAL CLASSIFICATION

Insecticides are classified according to their estimated toxicity by measuring the dose that kills 50% of the individuals exposed orally or by contact. Very toxic insecticides have a LD_{50} (Lethal Dose) of less than 50 mg/kg body weight, while the less toxic ones have an LD_{50} greater than 5,000 mg/kg body weight. [193] The toxicity assessment does not take into account synergistic, additive, sub-lethal effects and the fact that these molecules may accumulate in the soil and biosphere.

On many websites you can learn more about the physical and chemical properties of pesticides, the results of acute toxicity tests and the effects on certain organisms and humans.

- AGRITOX is a free French data archive on the physical and chemical properties, toxicity, ecotoxicity, environmental impact and regulation of plant protection products. Much of the information comes from the documentation used to obtain marketing authorization filed by French and European industries; bibliographical information is also included. [189]
- PPDB: Pesticide Properties DataBase is a repository of pesticide information. [190]
- ECOTOX Knowledgebase is a USA Environmental Protection Agency (EPA) repository on the toxicity of many chemicals. [191]
- EU Pesticides database is the European pesticides database. [186]

A limitation of the risk assessment system is the fact that the toxicological assessment, which is used to establish the maximum acceptable concentrations, varies considerably from country to country, so that different tolerance limits are adopted, even by orders of magnitude (more than 10 times). [243] The regulations on the use of pesticides vary from country to country, generating heterogeneous behaviours such as bans and very different limits. In Europe, a pesticide database has been created, which catalogues various useful information such as the authorizations for the use of a molecule for certain crops and the reference legislation (EU Pesticides database). [214] The variability between national and sometimes regional regulations is an obstacle to the exchange of foods such as cereals, fruits and vegetables. As an example, the USA exports millions of kilos of pesticides that are banned in the USA or are classified as very toxic to bees.

Some statistics show a drop in the sale of pesticides, for example in Italy, between 2001 and 2014 of 12%. [504] This figure does not consider the fact that over time the use of pesticides that have a toxicity from tens to over a thousand times higher has increased, so the same effect is obtained by using smaller quantities, but the equivalent harmful effect in proportion has increased. This is borne out by monitoring data on the presence of molecules and metabolites in water and the biosphere in general. In the course of more than 50 years, the destructive potential (toxicity) of chemical weapons available to farmers has increased even more than 1000 times, as has happened with insecticides.

A BULLISH SELF-DESTRUCTIVE EXPERIMENT

There are probably more than 1,000 different active molecules on the market in the World, which act by more than 100 different known mechanisms and have countless adverse effects, many of which have yet to be discovered. [605] Over the course of 70 years, the molecules used have been replaced by more toxic ones. [500] The increase in acute toxicity in the different categories of pesticides over time has had the effect of reducing the amounts that can be used to achieve the same result per unit area. As an example, in the USA, the insecticide DDT (organochlorine) in 1945 was used at a dose of about 2 kg/ha, while 45 years later pyrethroids

were used at a dose of 0.1 kg/ha. [538] Synthetic molecules such as pyrethroids, although resembling natural molecules, may have an acute toxicity comparable to that of neonicotinoids. Even considering the differences in acute toxicity levels, which in the case of pyrethroids is at least a few hundred times greater than DDT, the fact to use pyrethroids at the recommended doses is equivalent to using a few dozen kilos of DDT. We must not be fooled by statistics that record a reduction in the use of pesticides, as we must take into account and compare their equivalent toxicity. Over time, the quantities distributed have decreased but the equivalent toxicity released into the environment has increased dozens of times.
One of the strategies proposed several times over the decades to reduce risks has been to propose that preference be given to less dangerous molecules. [517] In fact, this strategy has remained only a proposal, since exactly the opposite has happened, as is shown in the table below.

Insecticides	**Chemical class**	**LD_{50}** (ng/bee)
DDT	Organochlorinated	27,000
Amitraz	Azotorganics	12,000
Coumaphos	Organophosphorous	3,000
Tau-fluvalinate	Pyrethroids	2,000
Methiocarb	Carbamates	230
Carbofuran	Carbamates	160
Lmabda-cyhalotrin	Pyrethroids	38
Deltamethrin	Pyrethroids	10
Thiametoxam	Neonicotinoids	5
Fipronil	Fenilpirazoili	4.2
Clothianidin	Neonicotinoids	4
Imidacloprid	Neonicotinoids	3.7

Notes: they are all insecticides (coumaphos and tau-fluvalinate are also acaricides). LD_{50} = lethal dose capable of killing 50% of the exposed bees in a few hours.

The toxicity of the molecules, over time, has had to increase due to several factors that are intrinsic to monocultures: the simplification of the agricultural ecosystem and the undesirable selection of resistant pests.
At least 2.4 billion kilos of pesticides are distributed worldwide each year (in 2006 and 2007). [13, 280] The USA is the greatest producer and seller in the World, using 22% of the total consumption, followed by Brazil. [280]
In 2003, more than 219,000 tonnes of pesticides were used in Europe and, in 2016, more than 400,000 tonnes were sold. [13, 210, 212] On average, between 500 g and 1 kg of pesticides are used per year per European inhabitant. Among the 25 European states, Spain (more than 76,940 t), France (more than 72,000 t), Italy (more than 60,000 t) and Germany (more than 40,000 t) recorded 79% of sales (Turkey in the same year recorded a sale of more than 49,000 t). [210]
The categories of active molecules sold in the highest quantities, in the 4 main European user countries (Spain, France, Italy and Germany), were: fungicides and bactericides (38,905 t), herbicides (15,224 t), insecticides and acaricides (7,501 t). In Italy, the categories of pesticides sold in the highest quantity, according to this estimate, were fungicides and bactericides (37,000 t). [210] In Italy, in 2014, an average of more than 5 kg of active molecules were used per hectare, in Spain more than 3 kg/ha and in France and Germany more than 2.5 kg/ha. [213] In Italy, in 2015, at least 140 million kilos of pesticides were sold: 20 million kilos in Veneto, over 19 million kilos in Emilia Romagna, over 14 million kilos in Sicily and Apulia, over 10 million kilos in each of the following Regions: Piedmont, Lombardy and Campania. [500] These are certainly underestimates (therefore they are optimistic) as they are based on self-certified data:

unfortunately the current monitoring networks are not able to make cross-checks. Moreover, the average quantities are calculated on the whole agricultural area while the real distribution is not homogeneous. Assuming that active substances are distributed homogeneously over the 12 million hectares of Italian agricultural land, this means distributing an average of more than 11 kg per hectare each year: this fact confirms that Italy is one of the biggest consumers of pesticides in Europe. Some crops, such as vineyards and apple orchards, may receive a much higher number of treatments and quantities: in the Autonomous Province of Trento, 50 kg/ha per year are exceeded, in Bolzano 43 kg/ha per year, in Valle d'Aosta 22 kg/ha per year and in Veneto 14 kg/ha per year. [500] In apple orchards, peaks of 90 kg/ha per year may be recorded. [532] The distribution of pesticides is not homogeneous and, as a result, exposures can vary considerably. In France, an average of 6.7 treatments are made in rapeseed cultivation, 6.6 in wheat and 3.7 in maize. [280] In France, vineyards occupy about 10% of the agricultural area but receive 80% of the fungicides and 46% of the insecticides used in this country. Also for this reason, it has been discovered that wine growers in France have a twice higher risk of developing a brain tumour and three times a higher risk of having a glioma (gliomas are primary tumours that originate in the brain parenchyma; symptoms and diagnosis are similar to those of other brain tumours). [284, 285] The risk of contracting Parkinson's disease or Alzheimer's is also higher in winegrowers: 5.6 and 2.4 times, respectively. [292]

It is estimated that at least 2 million tons of pesticides were used in the World in 2019. [715] The greatest pesticide-using countries were (in descending order): China, USA and Argentina. The most used categories of pesticides (in descending order) were: herbicides (47.5%), insecticides (29.5%), and fungicides (17.5%). Unfortunately, the forecasts are for a possible increase.

In some areas of the Planet the ability to regulate and control the pesticide market is limited, so pesticides are sold by unregistered companies and, to give an example, in Africa 38% of pesticides sold have an incomplete label and at least 6% are not labelled. [715] At least 20% of all pesticides are sold in developing countries, from which it is not possible to obtain sufficient information on their sales and use. The quantities of pesticides used, per hectare and per year, estimated in some countries of the World (in 2014) are the following: [715]

- Mauritius (Africa): 27.2 kg/ha.
- The Netherlands (Europe): 9.9 kg/ha.
- Belgium (Europe): 7.7 kg/ha.
- Portugal (Europe): 6.84 kg/ha.
- Italy (Europe): 6.5 kg/ha.
- France (Europe): 3.9 kg/ha.
- Germany (Europe): 3.8 kg/ha.
- Spain (Europe): 3.4 kg/ha.
- Congo (Africa): 3 kg/ha.

Pesticides reach their target only to a very small extent and most of them move into the environment: only between 0.1% and 0.3% of the molecules distributed in the fields reach the target organism.[280, 295] Therefore, the greatest quantity migrates into the environment, poisoning it and generating many devastating side effects, including human health. The molecules that persist in the biosphere circulate (e.g. in the food chain), thus being able to multiplying the negative effects over time. It can be said that a systematic and constant process of general poisoning of the entire biosphere is being carried out.

Despite the widespread use of these poisons a large percentage of crops are destroyed by pests. For example, 37% of crops in the USA are lost because of weeds, insects and other pests. [715] Between 1945 and 2000, the use of insecticides (organochlorines, organophosphates and carbamates) in the US increased 10-fold and insect damage doubled (from 7% to 13%). 35% of the World agricultural production is destroyed by pests, 13% of which are insects: many pests become resistant to the active molecules used. [280]

It is important to remember that the lethal effects in target organisms, such as insects for insecticides, occur at concentrations in the order of millionths of a gram per insect. Adverse effects on human health can also occur at very low doses, on the order of thousandths of a gram or less. Therefore, the distribution in the fields of quantities of hundreds of grams or kilograms per hectare, in the case of certain categories of pesticides, means contaminating the biosphere with extremely powerful and persistent chemical bombs. The massive use of pesticides has generated several problems, including:

- The generation of metabolites that may be as or more dangerous than the original molecules such as metabolites of DDT, amitraz or omethoate which is an equally toxic metabolite of dimethoate. [181, 183] Other metabolites include aldicarb sulfoxide, endosulfan sulfate, fipronil sulfone, 5-hydroxy-imidacloprid, DDE. [45, 75] Carbendazim, in addition to being a fungicide, is also a metabolite of thiophanate-methyl, another fungicide. [39]

- The persistence of pesticides in soil: for example in Mexico, the organochlorine insecticide DDT is detected in the concentration range of 0.057 ng/g to 360 ng/g.

- The development of herbicide-resistant weeds. For example, in Australia, between 13% and 28% of weeds (depending on geography) have experienced the emergence of resistant plants (an example being plants in the genera *Avena*, *Bromus* and *Hordeum*). [715]

- The finding of several toxic molecules in drinking water at concentrations of micrograms per liter (e.g. 12 of the 43 compounds searched in Italy are measured at the overall concentration of 0.5 µg/L). [715] In Italy, in 2016, 224 pesticides were found in surface and deep waters: sites that contained up to 36 molecules in the water at the same time are recorded. [504] In the drinking water of Lombardy, the most sold herbicide in the World, glyphosate, is found up to concentrations of 4 µg/L. [716]

- It’s difficult find areas in Europe (but not only) that are not contaminated by herbicides and insecticides. To give some examples, already in 2010, in England at least one third of the agricultural area was treated with neonicotinoid insecticides. In the same year, in the USA, at least 33 million hectares of corn, 31 million hectares of soybeans and 21 million hectares of wheat were treated with neonicotinoid insecticides.

- The finding of pesticides within the colonies of farmed bees. The parasites against which they are directed become resistant. In Italy, cases of *Varroa* resistant to amitraz, fluvalinate and coumaphos have been reported. [35, 55]

- Contamination of the food chain. In Europe, in the period 2012-2013, 33 different active molecules were found in plant products used for human consumption, at concentrations in the order of parts per million, such as metalaxyl (0.16 - 0.4 mg/kg), methomyl (0.015 - 0.21 mg/kg), and imidacloprid (0.017 - 0.036 mg/kg). In Italy at least one third of food contains pesticides, often in the form of multi-residue (i.e. the presence of at least two poisons at the same time). [504]

In Europe, the search for pesticides in food shows that 43.4% of 83,000 samples contain at least one molecule. [443] In products of vegetable origin, 154 different substances are found. In Italy, pesticides were found in more than 60% of fruit samples, with frequent multi-residues: 5 in apples, 8 in strawberries, 15 in grapes. [500] In a sample of tea, 21 residues were found at the same time. Among the substances most frequently found in fruit were imidacloprid, dimethoate, boscalid (suspected carcinogen and endocrine disruptor), captan (possible carcinogen and endocrine disruptor, as it inhibits the action of oestrogen), chlorpyrifos (probable mutagen and endocrine disruptor), phosmet, metalaxyl and iprodione. Overall, one fifth of fruit or vegetable samples contain potentially carcinogenic or endocrine disrupting substances. [500] Cereals are also contaminated: 17% contain dangerous residues such as chlormequata, which is the most frequently detected substance (in 50% of the positive samples). This molecule is suspected of being carcinogenic and an endocrine disruptor. Carbosulfan, withdrawn since 2008 and not approved by the European Union, was found in 6.6% of olive oil samples. Wine is also contaminated: 42% contains pesticides and 22.4% has more than one residue. Among the substances searched for in wine, those found most frequently were: boscalid, captan, chlorpyrifos, phosmet, metalaxyl, iprodione, imidacloprid and dimethoate. Among the substances not authorized but found in wine in national monitoring are carbendazim and benomyl in 4.2% of samples, and fensulfothion in 2.4% of samples (in 2014). However, it must be highlighted that carbendazim is also a metabolite formed in the soil from a permitted substance: thiophanate methyl. Out of 37 wines analysed (2017) 24 contain on average 3 residues and up to 8 substances are found at the same time. [500]
As a result, pesticides are found in the umbilical cord, blood, urine and breast milk.

As mentioned above, pesticides can be very persistent and mobile. Pesticides distributed in the US in the 1990s have been found hundreds of miles north, due to natural mobility in the biosphere and decades after their last recorded use: chlorpyrifos (insecticide) was found in Arctic glaciers. [443] Some organochlorine insecticides (DDT, HCH, heptachlor, toxaphene and lindane) can remain in soil and sediments for years, and can be found in remote areas such as the depths of the oceans. Organochlorines (e.g. HCH, DDT and lindane) are still found in European rivers despite being banned for decades. [443]

The use of pesticides has always been aggressively advocated by presenting them as a solution to feeding the World. Reliance on pesticides has generated short-term benefits, but today it is clear that we are undermining the rights to food and health for current and especially future generations. We must also consider that a large part of agricultural production is used to feed farmed animals and at least a third becomes waste. Today it is increasingly clear that the problems of malnutrition and undernourishment cannot be solved with the help of pesticides but by implementing other environmentally sustainable strategies that reduce inequalities.

Pesticides cause a number of health problems for both occupationally exposed workers and the general population. The main symptoms of acute pesticide exposure recorded among farmers include skin and eye irritation, headaches and dizziness. [487] Chronic exposure to pesticides promotes many different diseases such as reduced cognitive ability, diabetes, neurodegenerative diseases such as Parkinson's, Alzheimer's and amyotrophic lateral sclerosis (ALS). [296, 487] In addition, pesticides can promote cancer and be endocrine disruptors. [502]

In conclusion, a fundamental principle to stress is that pesticides do not disappear but spread, that is, they invade the biosphere, persist for decades and can bioaccumulate, reaching very dangerous concentrations.
We should have the collective intelligence sufficient to understand that pesticides and intensive agriculture create dependence on chemicals as they promote or create pests. Pesticides kill beneficial organisms that are the natural enemies of crop pests. At the same time crop pests become resistant to pesticides. Intensive agriculture destroys biodiversity and favours a few,

often artificial, alien or highly resistant species. Thus a diabolical, devastating and self-feeding engine has been generated thanks to stupid rules that favour private economic interests. In addition to generating a massacre among non-target organisms, today there is no segment of the human population that can escape the very serious health effects. We use molecules originally designed for military purposes, which are highly poisonous and only a small part (less than 1%) reaches the target organisms at the dose necessary to generate immediate death, as desired by the economic rules espoused by industrial agricultural production.

THE BEST-SELLING HERBICIDE IN THE WORLD: GLYPHOSATE

Glyphosate is a systemic herbicide and resembles an amino acid. It is absorbed by the leaves and translocated everywhere in the plant, including the roots. Absorption occurs in a few hours, but the complete desiccation of the plant occurs in a few days.

Glyphosate is the most widely sold herbicide in the World (at least 850,000 t in 2017) and is also the most widely used active ingredient: in the USA, between 1974 (the year its marketing began) and 2014, an average of 1 kg/ha per year was distributed; in the same period, in the World, an average of 0.53 kg/ha per year was distributed. [713] In the USA, 19% of the amount distributed in the same years in the World was used. [714, 715]

Glyphosate is probably the most widely and systematically distributed poison on the Planet, partly because genetically modified plants are cultivated to resist its phytotoxicity. Genetically modified plants favour the spread of this poisonous molecule, exactly the opposite of what the promoters of biotechnology claimed.

Glyphosate-tolerant genetically modified (GM) plants were introduced in 1996 and the use of this herbicide has spread accordingly. Today, at least 56% of the herbicide glyphosate is distributed in genetically modified crops that are tolerant to this herbicide: maize, soya, cotton and rape. [714] Before the spread of genetically modified plants, these crops (maize, soybean and cotton) received less than 10% of glyphosate. In some countries most of the crops are genetically modified: 100% of soya in Argentina, 93% in Brazil. The European Union consumes 30 times more soy than it produces, so it has to import most of it. [985] Already in 2015, about 84% of 180 million hectares planted with genetically modified crops were occupied by glyphosate-resistant plants. [1169]

Glyphosate is present in more than 750 different commercial products and can be easily found in food: in 25% (27/109) of bread samples analysed in the UK or in 90% of soya samples in the USA. [507, 552] Furthermore, it is important to underline that it is estimated that up to 44% of the glyphosate distributed on the Planet is not used in agriculture, but for example to remove weeds in urban areas, on railways, and from roadsides.

There are many studies showing that soil fertility and quality are negatively affected by pesticides. A 2017 European Commission report reveals that 45% of European soils analysed contain glyphosate residues: of the 317 soil samples (the top 20 cm), taken in 10 European countries, 21% were found to contain glyphosate, 42% contained one of the most toxic metabolites of glyphosate (AMPA or aminomethylphosphonic acid) and 18% of the samples contained both (at a maximum concentration of 2 mg/kg). [268, 269, 275] The soil was taken from the following crops: wheat, barley, rice, maize, triticale, oats, potatoes, sugar cane, sunflower, rape, citrus, vineyards, olive groves, tomatoes and others. [275] In Italy, 17% of soil samples contain the metabolite AMPA, while in Denmark it reaches 80%. Glyphosate is contained in up to 53% of soil samples in Portugal. These results show that the risk of accumulation and persistence in the environment is widely underestimated. Glyphosate can remain in the soil for up to 387 days and its metabolite (AMPA) for more than 1,000 days, depending on environmental conditions. The half-life of the concentration can be less than three months in water and six months in soil, but this generates hazardous metabolites (e.g. AMPA) that are more persistent: at least 240 days in water. [1165] Both glyphosate and its reported metabolite are toxic to worms, bacteria, fungi and weaken plants by making them more susceptible to diseases. Paradoxically, the reduction of soil fertility and its pollution with pesticides generates weaker plants and thus induces farmers to use more pesticides. A self-destructive mechanism is thus created and amplified.

Unfortunately, glyphosate and the metabolite AMPA are also found in water. In Europe, glyphosate was found in 33% of 75,350 surface water samples (at concentrations of up to 370 µg/L) and the metabolite AMPA in 54% of 57,112 surface water samples (at concentrations of up to 200 µg/L). [270] This herbicide is so widely distributed that it is found in European rainwater: in 10% of samples (at concentrations up to 6.2 µg/L) and AMPA in 13% of samples (at concentrations up to 1.2 µg/L). [275]

In Italy glyphosate is found in surface water (20% of samples), groundwater, drinking water, cotton gauze, sanitary towels, and in many foods such as flour, pasta (up to 0.16 mg/kg), bread, oats, soy sauce, honey and beer. [1165]

Glyphosate is also found in honey: in 45% of 11 samples of organic honey (at concentrations between 26 and 93 ppb) and in 62% of 58 samples of conventional honey (at concentrations between 17 and 163 ppb) from all parts of the World. An alarming finding is that it has also been found in honeys from wildflowers. [1167]

It is important to stress that significant concentrations of glyphosate can be recorded in nectar and pollen: between 2.8 and 31 mg/kg in nectar and between 87 and 629 mg/kg in pollen (concentrations measured a few days after spraying). [1169] The nectar in the hive undergoes a concentration process (it loses water) so the concentrations to which the colony will be exposed may be higher. Consequently, concentrations of glyphosate up to 1 mg/kg can be recorded in honey. [1169]

Sub-lethal effects recorded in honey bees following glyphosate exposure include altered circadian rhythm, impaired olfactory learning, and reduced orientation skills (these effects are measured at very low concentrations). [86]

As one would expect, in countries where plants genetically modified to be resistant to the herbicide glyphosate are not grown or are not widespread, the lowest amounts of glyphosate are recorded in honeys.

More than 173 different pesticides (and/or metabolites) can be found in honey and other beekeeping products, so the synergistic and additive effects, both on insects and humans, are partly unknown but predictably undesirable. [1168] Colonies exposed to these molecules may suffer damage to the foragers' learning abilities, sensory skills, and may experience developmental delay. [1169] For example, memory between the association of a positive stimulus (odour followed by administration of sugar solution) and the subsequent proboscis extension reflex is reduced in the presence of glyphosate. Thus, the short-term memory capacity is altered. In addition, bees exposed to this molecule need more stimuli to be able to associate them with the presence of a reward (nectar). The ability to discriminate between the combination of different odours is also reduced. Overall, exposure to glyphosate generates the impairment of skills important for the survival of the colony and, in the long term, devastating effects can be recorded such as the reduction of the ability to orient themselves and the impairment of the important function of foragers. These effects are sub-lethal, i.e. they can impair the colony's ability to survive at low doses and in the long term. Glyphosate is wrongly considered as not very dangerous but it should be classified among the substances toxic to bees (and other life forms). [1226]

This molecule also alters the composition of the gut flora. Another serious indirect effect of the herbicide is to reduce the floral availability for bees and other pollinating insects, which results in malnutrition and undernutrition.

Pesticides can have several negative effects such as those of insecticides and fungicides on birds, foxes and soil worms. Earthworms in the soil are killed by herbicides such as glyphosate or suffer an altered reproductive capacity. [280, 552] Glyphosate also damages mycorrhizae, microorganisms that live near roots, molluscs, echinoderms, reptiles (e.g. it damages the DNA of caimans), amphibians, birds (e.g. it alters the reproductive system of the mallard, a duck), fish, mammals (e.g. it damages the circulatory system of pigs) and reduces the photosynthetic activity of *Euglena* (a single-celled freshwater alga). [552]

Glyphosate was classified as a probable carcinogen by the International Agency for Cancer, in 2015. Glyphosate is also suspected of damaging certain microorganisms (in our gut and in the soil), promoting autism (in 1975 it was recorded in one out of 5000 children, in 1995 in one out of 500 children, and in 2017 in one out of 68 children), promoting the celiac disease and other diseases of children. [1165, 1166]

Among the undesirable consequences of massive use, there is the discovery of this molecule in the human body. Glyphosate and its metabolites (such as aminomethylphosphonic acid or AMPA) can be found in urine. In the urine of occupationally exposed people, concentrations between 0.26 and 73.5 μg/L may be measured, while in the general population, including pregnant women and children, concentrations between 0.16 and 7.6 μg/L may be measured. [712] Glyphosate is also found in the umbilical cord (range between 0.2 and 949 μg/L), in the blood of pregnant women (in serum between 0.2 and 189 μg/L), and in children from non-occupationally exposed families (in urine between 0.1 and 9.4 μg/L). [712] An aspect that emerges from this publication, which is both interesting and worrying, is that the lowest concentrations that various laboratories are able to measure, with the available analytical methods, vary between 0.05 μg/L and 100 μg/L: looking for glyphosate at very low concentrations from an analytical point of view is not easy, also because it is a soluble molecule, small and similar to many other naturally occurring ones. Not all laboratories are able to detect the expected concentrations in urine because the detection limits are too high. As a result, the concentrations of active molecules, such as those of glyphosate and its metabolites, are so low that they cannot be detected with the most common analytical techniques.

Sixty percent of occupationally exposed individuals record glyphosate in their urine on the day of treatment and 27% three days later. 4% of the wives of farmers who spray the herbicide record glyphosate in their urine on the day of treatment. [712] During field spraying the air can contain up to 1.25 $\mu g/m^3$ of glyphosate, which is also found in dust inside homes. A survey carried out in the USA near treated fields shows concentrations of glyphosate and AMPA ranging from 0.97 to 9.1 ng/m^3 in 50% of air samples. [268, 270]

The foreseeable consequence of the widespread use of glyphosate is that it is found everywhere: in urine and in breast milk. In the non-occupationally exposed population, glyphosate can be found in 65% of mothers and 88% of children. Many studies show that glyphosate is found in higher concentrations in children than in their mothers. Overall over time, between 1993-1996 and 2014-2016, the percentage of urine samples with glyphosate has increased from 12% to 70%. [712] The population not professionally exposed takes up this herbicide through water (75 μg/L in the USA) and food (100 μg/kg in tomatoes): in the USA, 60% of wheat and soybean samples record glyphosate residues.

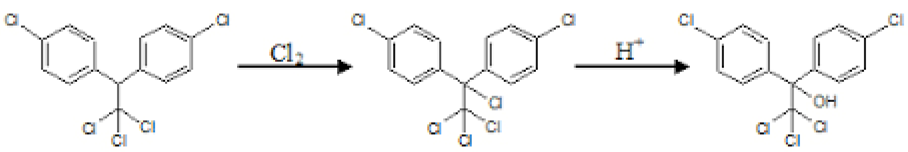
It is astonishing that a wise and rational community, in order to prevent the spread of a few species it considers undesirable, uses chemical means that contaminate the biosphere bringing death and disease even to itself. The effects of pesticides in the biosphere are largely unknown, we are gambling with the fate of all, although in truth the general effects are now widely documented. It goes without saying that we are subjecting the biosphere to an apocalyptic experiment, the harmful effects of which are still partly unknown. An intelligent and far-sighted society should espouse the precautionary principle and not use these molecules.

DDT: THE INSECTICIDE THAT HELPED TO DEFEAT MALARIA

Para-dichlorodiphenyltrichloroethane (DDT) is a chemical synthesized in 1874 but has been commercially available as an insecticide since the 1940s. [181, 248] The term DDT [specifically *p,p'-DDT (1,1'-(2,2,2-trichloroethylidene)-bis(4-chlorobenzene)*] *is* commonly known, but it is actually a complex mixture of its isomers and related compounds (e.g. *o,p'-DDT*, *p,p'-DDE*, and *p,p'-DD*). The latter two compounds (together with their ortho and para analogue derived from *o,p'-DDT*) in biological systems are also the two main breakdown products.

DDT, of which at least 1.8 million tonnes were distributed in the environment between 1963 and 2010, halves its concentration in soil between 2 and over 15 years. Its use peaked during World War II as it was used to control vectors of malaria, typhus, cholera, yellow fever and sleeping sickness, and as an insecticide in agriculture. DDT has been banned in many countries of the World since the 1970s: in Italy in 1978. Regulations on the use of DDT became extremely restrictive in 1981, and by 1986 the use of this compound was banned in the EU. Although banned in most countries of the World, DDT continues to be used to control mosquito vectors of malaria, as recommended by the World Health Organization. Malaria continues to affect millions of people: at least 405,000 deaths recorded in 2018, mainly children.

Although DDT has been recognized as very dangerous to the environment and humans, it is still produced. Moreover, it has been replaced by molecules that chemically are very similar and more toxic. Therefore, a dangerous substance has been replaced by more potent ones with a similar chemical structure. We report the synthesis of dicofol from DDT: note the chemical similarity, in fact dicofol is produced starting from DDT (so DDT may be a small unwanted fraction that remains during the process of dicofol synthesis). [183, 1282]



p, p' - DDT   Cl-DDT   Dicofol

As with other insects, the systematic and continuous use of molecules such as DDT (since 1950) or τ-fluvalinate has led to the emergence of resistant bee colonies. [181, 183] This lower susceptibility has been favoured by genetic factors that have been rewarded by artificial chemical selection by farmers and beekeepers.

DDT and its compounds are found in milk and eggs, and accumulate in domestic animals and fish. Due to its lipophilic properties and persistence in the environment, DDT and related compounds are prone to bioaccumulation and biomagnification in the food chain. Persistence and bioaccumulation of pesticides in the environment are well known. DDT is rapidly absorbed in humans and animals, and some DDT-related metabolites are generally more persistent than DDT itself.

The discovery of DDT and its metabolites after tens of years from the prohibition of its use is due to several factors such as illegal use and persistence in the soil for over 40 years (DDT, DDE and DDD). [541] In reality, in many areas of the Planet they are still used, and it is worth remembering that these molecules are lipophilic and can bioaccumulate in adipose tissues. The average concentrations of DDE in people over 60 are at least three times higher than those recorded in people aged between 20 and 39 years. Within the human body DDT has a half-life of 6 years and its metabolite DDE of 10 years. [541] Because of their remarkable persistence, these insecticides can be easily found in blood and breast milk. In Canada, DDT or its metabolites were found in the blood of all 1,696 subjects involved in the monitoring (aged between 20 and 79 years). The presence of DDT (found in 10% of the subjects, with an average concentration of 10 ng/g) and its metabolite DDE (in more than 99% of the subjects, with an average concentration of 326.9 ng/g) was correlated with the recording of pulmonary dysfunctions (e.g. reduced respiratory capacity), bronchitis and asthma. [541] Other studies have also confirmed this result. [542] In Spain it was found that being exposed to DDT as a child increases the probability of recording respiratory problems such as asthma. It should be remembered that DDT is transformed into DDE [1,1-bis-(4-chlorophenyl)-2,2-dichloroethene] and DDD [1,1-dichloro-2,2-bis(pchlorophenyl)ethane] by light (photolysis), microorganisms in soil, and metabolism in humans. In humans, the transformation is generated by the action of an enzyme complex called cytochrome P450. In turn, DDE and DDD are transformed (e.g. by oxidation) into other compounds.

Investigations detect the presence of organochlorinated insecticides in all the persons examined, despite the fact that only one of the 13 possible chemical conformations of DDT and DDE (1 of 13 possible isomers) was searched for. [542] Thus, mobility and persistence in the biosphere cause a widespread contamination of great concern.

The main target organs are the nervous system and the liver. DDT also has negative effects on endocrine homeostasis, reproduction, foetal development, and the immune system. The LD_{50}, i.e. the quantity that kills 50% of men if swallowed, is 10 mg/kg of body weight, therefore 700-800 mg kill a 70-80 kg man. [280] The IARC (International Agency for Research on Cancer) has classified DDT as a possible human carcinogen (group 2B). Exposure to DDT is essentially determined by the DDE assay. The dosage of the other important metabolite, DDD, is never carried out because, as has been known for some time, it is excreted very easily.

In conclusion, it is known that DDT is neurotoxic, carcinogenic and generates damage to the immune system and to reproduction. Although this knowledge is now public and validated, DDT is probably produced and used in at least the following countries: China, India, South Africa, Ethiopia and North Korea. [541]

DDT is one of the substances included in the Stockholm Convention on Persistent Organic Pollutants (POPs) and in the Protocol on POPs (LRTAP-POP) of the Convention on Long-Range Transboundary Air Pollution of the United Nations Economic Commission for Europe (UNECE).

THE ORGANOPHOSPHATE AND CARBAMATE INSECTICIDES

Insects such as pollinating insects (bees and butterflies) are being exterminated by the arsenal of poisons that is the basis of industrial agriculture. From 1985 to 1997, in rural areas of the USA, the number of bee colonies decreased by 57%, mainly due to pesticides. [175] In agriculture, the use of insecticides at the doses recommended on the label (e.g. chlorpyriphos, dichlorvos, malathion, profenofos, monocrotophos which are all organophosphates; deltamethrin which is a pyrethroid and thiamethoxam which is a neonicotinoid) generates the death of 100% of the honey bees (*Apis mellifera* and *Apis cerana*) exposed (by contact), in less than 48 hours. [866] Other insecticides (cartap hydrochloride, indoxacarb and imidacloprid), at the recommended doses, i.e. those indicated on the label and following the regulations approved by the Health Authorities, register a mortality rate between 50% and 90%. Therefore, if these molecules are used during flowering or near fields in bloom, they kill bees (and other insects) and no wonder, they are called insecticides.

Pesticides act by altering fundamental, very important physiological mechanisms. For example, they interfere with biological amines such as catecholamines, dopamine, serotonin, histamine and acetylcholine, which perform essential nervous, endocrine, and exocrine functions in insects and elsewhere. They are involved in behaviour such as aggressive behaviour, foraging, muscle contraction, learning and memorization processes. The destruction of the modulation mechanisms of these substances generates disastrous effects even at very low doses. Moreover, they can alter the functions of important centres such as mitochondria and can produce genetic damage.

Some insecticides such as organophosphates and carbamates act on the nervous system by inhibiting the activity of acetylcholinesterase, which is an enzyme used to inactivate the neurotransmitter acetylcholine that is present, for example, in the synapses between neurons in the central nervous system of bees (the lethal dose for 50% of exposed individuals or LD_{50} may be between 0.018 and 31.2 µg/bee). [107] These insecticides generate an accumulation of neurotransmitters and, having a similar mechanism of action, can produce additive and synergistic effects and are also very toxic to humans. [516] It must be remembered that acetylcholinesterase inhibitors were produced in the 1930s as nerve agents for use in warfare, so organo-phosphorates (phosphate esters or organophosphoric esters) are chemical compounds that arose from nerve gas studies during World War II. [934]

For some of these insecticides, bees may show an increased tolerance due to the presence of protective enzymatic mechanisms (e.g. cytochrome P450). Organophosphates can inhibit certain enzymes (such as esterases) even at very low concentrations. A study conducted in the United Kingdom showed that in 49% (57/117) of the bee poisoning events that occurred between 1994 and 2003, two molecules were responsible: an organophosphate (dimethoate) and a carbamate (bendiocarb). [107] Among the organophosphates, we must mention coumaphos, widely used by beekeepers to kill a parasitic mite: the *Varroa*. This molecule has proved to be dangerous: concentrations of 8 mg/L in the diet have been shown to increase the mortality of larvae. [107]

PYRETHROIDS

Pyrethroids are produced through chemical synthesis processes and act as insecticides and acaricides. They are the synthetic analogues of pyrethrins, natural constituents of the flowers of plants of the Asteraceae family (*Chrysanthemum cinerariifolium*). They act in the same way as the corresponding chemical compounds of natural origin, but overcome the main limitation of pyrethrins: their photolability. They are artificially produced active molecules that are much more persistent than natural analogues. The first synthetic pyrethroid, Fenvalerate, was placed on the market in 1978. [162]

Pyrethroids exert their action mainly by contact and their liposolubility allows their penetration into the epicuticular waxes. The mechanism of action is based on the interaction with the sodium channels (they cause a prolongation of the sodium flow during excitation). [516] Pyrethrins, despite their natural origin, are toxic to bees (LD_{50} between 0.05 and 21 µg/bee). [107] Synthetic pyrethroids, which have a similar structure to pyrethrins, are also very toxic to bees (LD_{50} between 0.017 and 20 µg/bee).

Contact pyrethroids are repellent and irritant: insects contaminated by low doses show the sub-lethal effect of irritation. Some pyrethroids are used as acaricides by beekeepers (flumethrin and τ-fluvalinate are used to control *Varroa*) and can remain in wax even at high concentrations (200 ppm).

ENDOCRINE DISRUPTING INSECTICIDES

Another category of insecticides alters insect growth by inhibiting exoskeleton chitin synthesis or metamorphosis. These molecules include diflubenzuron and fenoxycarb. These molecules are also very toxic to bees, especially to larvae: diflubenzuron has negative effects even at concentrations of 1 mg/kg. [107]

Important insecticides are those that alter hormonal functions such as pyriproxyfen, which mimics the action of the juvenile hormone. This molecule is active in the processes of metamorphosis, embryogenesis, reproduction and larval development of many species of insects. It is a molecule analogous to the neotenin or juvenile hormone. [217] In insects, this hormone regulates larval moulting, and among adult insects it is essential for gonad maturation and sexual behaviour. [35] In worker bees an increase in its production accompanies the transition from nurse bee to forager. The hormone must be produced in precise quantities during specific moments of the insect's reproductive life, otherwise it causes death. The administration of Piriproxifen favours a series of physiological and hormonal imbalances that lead to the death of the insect. This insecticide, although classified as moderately toxic, is capable of generating negative effects at very low concentrations (these effects are considered sub-lethal but are just as dangerous as lethal effects). [13] In Spain it is used to combat scale insects on citrus fruits; it is also used in cotton and tea plantations to combat fleas on dogs and cats, and to control the larval stages of mosquitoes. The application on citrus fruits of 0.25 kg/ha of the active ingredient Pyriproxyfen generates a residue in the fruit of 0.41 mg/kg (measured in the peel). It has been demonstrated that this molecule at very low concentrations, which correspond to those found in the waters of the agricultural areas where it is used, generates sub-lethal effects such as developmental and behavioural alterations. Studies carried out with predictive computer models on the (sub-lethal) effects generated by very low doses of pyriproxyfen predict colony collapse within one year after exposure to concentrations considered acceptable and safe; these predictions have unfortunately been validated in the field so the classification should be reviewed. [13] The procedures applied to examine and classify the toxicity of pesticides for the purpose of obtaining the marketing authorization do not provide the necessary guarantees.

THE USE OF PLANT INSECTICIDES: NEONICOTINOIDS

Neonicotinoid insecticides are derived from nicotine, a molecule produced by plants of the genus *Nicotiana*. The leaves of the tobacco plant (*Nicotiana tabacum*) can contain 90,000 ppm of nicotine, pollen 23 ppm, and nectar 0.1-5 ppm. [107] Nicotine mimics the neurotransmitter acetylcholine by stimulating postsynaptic neurons. [608] Neonicotinoids are synthetic analogues of nicotine that have a much higher affinity for acetylcholine receptors in the bee brain. They have been used since the 1990s and are now the most widely used insecticides in the World: in at least 120 countries and on 140 crops (thiamethoxam on 115 crops). [337, 525, 553]

Less than 10 molecules belonging to this category are widely marketed, but they represent more than 40% of the global agricultural insecticides’ market: thiamethoxam, clothianidin, imidacloprid, acetamiprid, thiacloprid, sulfoxaflor, dinotefuran, nitenpyram. [230, 355] Neonicotinoids (and fipronil) are worth one third of the market for insecticides: at least 20,000 tons of active substances were produced in 2010. [355] In 2008 the herbicide glyphosate was the most sold active ingredient in the World followed by imidacloprid (at least 5,450 tons were sold): imidacloprid, in 2009, was the most used insecticide in the World. [585] These insecticides are also registered for domestic use, e.g. as pesticides on dogs or cats.

Since the 2000s, they have been used on seeds and, as a result, their use has increased. Neonicotinoids enter the plant tissues (they are systemic) and persist for a long time. Very low concentrations of certain active molecules inside the seed are sufficient to ensure that, throughout the life of the plant, any insect trying to eat its tissues will be killed. They are sprinkled onto the seeds and will remain active in the plant throughout its growth, until harvest. So they are toxic at very low doses and are persistent. It is a new generation of poisonous or insecticidal plants created by human ingenuity. The strategy is to use them preventively, even before knowing if phytophagous insects will be present and without determining economic thresholds for phytosanitary intervention. It is like asking humans to start taking antibiotics from birth and for life in order to hope to prevent probable future diseases. Neonicotinoids in seeds, in order to increase the exterminating effects desired by farmers, are used in combination with other molecules such as fipronil. This is a crazy stunt, which has unfortunately already been implemented for years on millions of hectares.

In 2010, most neonicotinoids were used to treat seeds such as those of wheat, beet, soybean, rice, barley, corn, sunflower, canola and cotton. [359, 553] In the USA, already between 2009 and 2011, more than 18 million hectares (an extension almost twice the agricultural area used in Italy) were cultivated with corn seeds impregnated with neonicotinoids: at least 810 tons of clothianidin and 570 tons of thiametoxam were distributed in the environment each year (corn was alternated with soybeans). [414, 451] In 2010, in the USA, the following crops were treated with neonicotinoids (imidacloprid, clothianidin or thiamethoxam) 100% of the acreage planted with canola, 94% of the acreage planted with corn, 75% of the acreage planted with sorghum, 65% of the acreage planted with sugarcane, 51% of the acreage planted with rice, 42% of the acreage planted with wheat, and an equivalent percentage for cotton crops (other crops in which neonicotinoids are used are potatoes, peanuts, and alfalfa). [678] We are talking about more than 95 million hectares that, in the USA alone, already 20 years ago recorded a massive and unjustified use of very poisonous molecules (to have some terms of comparison, in 2016 the European utilized agricultural area was around 171 million hectares). Another estimate reports that in the USA, in 2011, between 34% and 44% of soybeans and between 79% and 100% of corn used seeds treated with these molecules (excluding 0.2% of organic corn). [349] In 2015, neonicotinoids were registered for 500 different applications in the USA. [349] Probably almost all corn seed planted in North America is treated with neonicotinoids and at least 90% of corn seed grown in the US. [482]

In Canada, neonicotinoids are used in 44% of the agricultural area (215,000 kg per year) and 95% of rapeseed is treated with these systemic insecticides (in 8.5 million hectares). [415] Also in India a large proportion of seeds are coated with insecticides. [175]

Neonocotinoids were first introduced in Europe and Japan in 1990 (imidacloprid). [193] Imidacloprid was introduced in France in 1991 in turnip cultivation, the following year in maize plantations and in 1993 on sunflowers. [307] The use of neonicotinoids in the treatment of seeds, such as sunflowers in France, has been suspected to be one of the causes of the decline of bees. [363] Imidacloprid was authorized in sunflower seeds in France in 1993 and its use was banned in 1999, partly due to protests from beekeepers, who reported losses of between 40% and 70%, compared to 10% in the years before 1994. [553] Five years later it was also banned on maize seed. [482]

In England and Wales, 11 years of monitoring showed a correlation between bee mortality and the use of imidacloprid. [483]

Neonicotinoid insecticides, used in Italy on seeds such as maize, registered an increase in bee mortality in 2003. [163] In 2008, in Italy, following the high mortality recorded in the spring, in conjunction with the sowing of maize, it was decided to ban the use of some neonicotinoids on seeds: imidacloprid, thiamethoxam, clothianidin and fipronil (not a neonicotinoid). [35] This ban generated the positive effect of reducing bee mortality in the following years: 12.5% in 2012 and 11.6% in 2013. [163] Also in Germany, the increase in bee mortality has been associated with the practice of sowing maize seeds containing neonicotinoids (clothianidin). [475]

The continuous, preventive and systematic application of pesticides on seeds generates an unprecedented contamination of the biosphere. Tens of millions of hectares of corn in the USA have been cultivated with plants containing thiamethoxam or clothianidin (the latter, in the body of insects, turns out to be a metabolite of the former) at doses between 0.25 and 1.25 mg per seed. At least 50 million hectares, already in 2010, were cultivated with maize, cotton or rape seeds treated with pesticides such as neonicotinoids. We must remember that the dose that kills by contact 50% of the exposed bees is between 22 and 44 billionths of a gram (ng) per insect. The dose capable of killing insects by ingestion is 10 times lower than that by contact: the LD_{50} for clothianidin is between 2.8 and 3.7 billionths of a gram per bee. [368] The amounts capable of exterminating insects are an order of magnitude a million times less than what is present in each of the more than 12,000 seeds planted in one hectare. Assuming that a corn seed contains 0.5 mg of clothianidin, it follows that each grain has the dose potentially sufficient to kill tens of thousands of bees. Through the seeds, each hectare is contaminated with the amount of clothianidin with the potential capacity to exterminate millions of bees, those that live in tens of thousands of hives during the summer. These theoretical calculations help us to have a measure of the destructive power put into the field and do not take into account the fact that some molecules are very persistent, so the harmful effects can last for a very long time.

These molecules kill insects at concentrations of millionths of a gram and cause sub-lethal effects at concentrations of billionths of a gram. In social insects such as bees, pesticides alter the ability to communicate: the insecticide imidacloprid reduces the possibility of communication through the bee dance. [589] This insecticide at very low doses has an excitatory effect but at higher doses it has an inhibitory effect. Among the effects recorded at doses below the lethal ones, there is the impairment of the foraging bees' ability to orientate, the performance of much longer flights and in longer times, and the reduced possibility of communication. Their memory is also impaired and they are unable to return to the hive. In fact, the vitality of the colony is seriously compromised to the point of collapse.

Bees can be contaminated during sowing by the dust produced. In the spring of 2008, 11,000 bee colonies in Germany were severely damaged by neonicotinoids in aor-borne dust during the sowing of maize. [484] It should be remembered that at that time, in Germany, it was even compulsory to treat maize with the neonicotinoid clothianidin to combat a worm that affects the roots (*Diabrotica virgifera*). Clothianidin was later withdrawn from Germany but the mandatory use of neonicotinoids on maize seed against rootworms has also been regulated in other states (e.g. Austria). All soil samples in which maize was planted record the presence of the neonicotinoids (e.g. clothianidin) and herbicides (e.g. atrazine) used in the field. [368]

Pesticides are being distributed everywhere and on a massive scale through seeds, without having the certainty of needing them. The economic thresholds for intervention are not assessed and, probably, taking out an insurance policy for any unacceptable economic damage from insects would be a better and cheaper alternative strategy. One of the principles that should be applied before deciding whether to distribute poisons is to assess the balance of advantages and disadvantages, including economic ones. Crop pests become dangerous only when they exceed certain thresholds that agronomists should determine on a case-by-case basis. With this strategy, a systematic poisoning of the environment is carried out, so there are certainly all the negative consequences such as pollution, destruction of biodiversity, diseases for bees and humans, and facilitation of the emergence of resistant pests. The insecticides will also kill the natural antagonists of the pests, so those that become tolerant to the molecule will also have fewer competitors.

NEONICOTINOIDS IN FLOWERS

Neonicotinoids can contaminate the soil, where they can remain for up to 19 years (this is probably an underestimate as insufficient information is available). [166] Residues of neonicotinoids have been found in woody plants six years after the last soil treatment. [362] In this context it has to be mentioned that about 70% of bee species build their nests in the soil.

Neonicotinoids can also be found in the reproductive organs of plants. In the reproductive parts of rice and potato plants, between 0.7% and 12% of neonicotinoids may be found which are initially distributed in the soil. [359] Translocation from the soil to the flowers and fruits then occurs. In sunflower flowers, 0.2% of the amount of fipronil used in combination with neonicotinoids can be found (in the seeds or in the field). [359]

Imidacloprid is translocated to the edible part so there is a risk for human health too. Neonicotinoids are found in 46.7% of fruit samples, as a consequence of foliar treatments, e.g. in melons, tomatoes, cucumbers and courgettes, or in 10% of lettuce, spinach and parsley samples. In tomatoes and apples, 21% and 28%, respectively, of the imidacloprid sprayed on the leaves may be found (at least one third of this amount is found on the leaf surface). [359] Therefore, concentrations of imidacloprid above the maximum permitted limits may be found in fruits.

These molecules will also be present in pollen, nectar and honey. In Austria, thiacloprid is found in honey at concentrations between 11.1 and 81.2 ng/g. Imidacloprid is found in nectar and pollen: The application of 0.79 mg of imidacloprid per sunflower seed results in 1.9 µg/kg in nectar and 3.9 µg/kg in pollen. [356] The average amount of imidacloprid in sunflower soil of 6 µg/kg is associated with concentrations in flowers of up to 2 µg/kg. In sunflower seeds, imidacloprid can be distributed in the amount of 0.7 mg per seed and is found in flowers at concentrations between 1 and 10 µg/kg: this is the concentration bees may be exposed to (other studies find it at concentrations between 5 and 30 µg/kg, using marked carbon). [363] Oilseed rape plants whose seeds were treated with neonicotinoids show concentrations up to 3.5 mg/kg of thiamethoxam in nectar and up to 2.4 mg/kg in pollen. [475]

In 83% of the 24 pollen samples, derived from treated sunflower seeds, imidacloprid was recorded with a concentration up to 11 µg/kg (in one work cited in this study, nectar is contaminated with 1.9 µg/kg of imidacloprid). [363] In maize pollen, imidacloprid can be found at an average concentration of 3 µg/kg and neonicotinoid concentrations as high as 20 ng/g (ppb) are found. Assuming that in 10 days a bee can consume 65 mg of pollen, it means that it will ingest 1.3 ng (65 mg x 20 ng/g), almost 50% of the LD_{50} which is about 2.8 ng/bee. In this study, most of the pollen collected by bees was represented by corn pollen. It must be considered that the LD_{50} is estimated with a single exposure while in this case the ingestion occurs over a period of ten days, it is a chronic exposure; other pesticides are found simultaneously in maize pollen too, such as: fungicides (trifloxystrobin, azoxystrobin, and propiconazole), herbicides (atrazine and metolachlor), and acaricides (coumaphos). [368] Additive and synergistic effects may greatly potentiate adverse effects, so overall toxicity increases. Neonicotinoids (chlotianidin) and all the pesticides just listed (except coumaphos) are found in wild flowers (*Taraxacum officinale*) grown in nearby fields. [368] Bees are exposed to a poisonous mixture that accumulates in the soil, moves into the biosphere contaminating nearby wild plants, and persists in subsequent years. Among the disconcerting conclusions reported in this paper is that in the commercial network there are no corn seeds not treated with neonicotinoids.

The following table shows the concentrations of some molecules recorded in pollen or in derived matrices produced by bees (pollen and nectar mixture). [359]

Insecticide *(in pollen and pollen-nectar mixture)*	**Percentage of positive samples** (%)	**Average concentration** (μg/kg)	**Maximum concentration** (μg/kg)
Acetamiprid	24.1	3	134
	45	4.1	26.1
Clothianidin	11	9.4	41.2
Dinotefuran	1	45.3	168.1
	100	11.2	147
Imidacloprid	87.2	2.1	18
	16.2	19.7	912
Thiacloprid	62	89.1	1,002
Thiamethoxam	12.8	28.9	127
Fipronil	3	1.6	29

Chemical pollution of flowers fundamentally undermines plant reproduction, generating effects that are largely unknown but predictably negative. The concentrations are so low that some analytical methods used are unable to detect them (HPLC/UV does not measure below 20 μg/kg). [363] Another alarming aspect is that in flowers during their formation there is an increase (over time) in the concentration of imidacloprid.
The following table shows the concentrations of some molecules found in nectar or honey. [359]

Insecticide *(in nectar or honey)*	**Percentage of positive samples** (%)	**Average concentration** (μg/kg)	**Maximum concentration** (μg/kg)
Acetamiprid	51	2.4	112.8
Clothianidin	17	1.9	10.1
	100	89	319
Dinotefuran	100	9.2	10.8
Imidacloprid	21.8	--	660
Thiacloprid	64	6.5	208.8
Thiamethoxam	65	11	20
Fipronil	6.5	70	100

The above tables show that pollen, compared to nectar, is more contaminated by neonicotinoids, while the opposite is true for fipronil, even though the latter is less soluble in water. The concentrations are obtained from very different situations since, for example, in some cases the neonicotinoids were distributed in the soil, in others with the seeds or with sprayers on the leaves. These differences partly explain the variability found. The distribution of the mixture of active molecules, using sprayers that atomise on the aerial part, is a greater source of contamination than the others. This research allows us to conclude that pollen, nectar and honey are sources of pesticide exposure, for bees and also for other insects. Bees will be chronically exposed to relevant concentrations of these molecules throughout their lives (e.g. through honey and pollen stored in the hive). The concentrations are up to hundreds of times higher than those showing sub-lethal effects (e.g. imidacloprid). [363] The situation is worsened by the simultaneous discovery of several active molecules, e.g. in nectar (e.g. 4 pesticides out of the 22 tested). [359]
Neonicotinoids are toxic to beneficial insects such as solitary bees reared for pollination, such as *Osmia lignaria*, *Osmia bicornis* (osmias are solitary bees that are widespread in Europe),

Megachile rotundata (solitary bee pollinator of alfalfa, legumes and carrots) and *Nomia melanderi* (solitary bee reared in the USA and New Zealand for the pollination of alfalfa). [362, 364, 365, 854] Neonicotinoids such as imidacloprid, clothianidin, dinitofuran and thiametoxam are also highly toxic to bumblebees. [362]

At least 5 neonicotinoid insecticides are used in Europe and they are among the most widely used pesticides in the World. For this reason, they are found in soils occupied by organic crops and in soils where they have never been used. Regarding their persistence in the environment there are no certainties and some data measure very different half-lives, for imidacloprid between 28 and 1,250 days. [1223] Due to their widespread use and persistence, neonicotinoids may be found in honey and other foods. In Europe, the maximum limits permitted in food are 0.05 mg/kg for clothianidin and imidacloprid and 0.2 mg/kg for thiacloprid.

In Ireland clothianidin, imidacloprid and thiacloprid were searched for in 30 honey samples, collected in 2014, from 30 apiaries located in three types of areas: urban, semi-natural and agricultural. 70% of the 30 honey samples contained at least one of the three insecticides and 48% recorded two. Neonicotinoids, with a lower frequency, are also found in honey samples taken from colonies located in areas classified as semi-natural. The two samples containing all three neonicotinoids come from urban or agricultural areas. Honey produced in urban settings in Ireland has the highest number of positive samples and clothianidin is found most frequently. This information confirms that neonicotinoids are also used in cities (sports facilities, public gardens, ornamental plants). In the UK, 70% of the ornamental plants marketed may contain traces of neonicotinoids, which are capable of harming bees at doses of a few billionths of a gram per insect, so even at low concentrations they are harmful. [1223]

Poisonous molecules are recorded in the most important foods for pollinating insects and in more than sufficient quantities to induce sub-lethal effects in bees, without considering the effects of metabolites and the concomitant exposure to other pesticides. Non-target species are exposed to toxic molecules that are bioaccumulative, water-soluble, and persistent (they produce harmful metabolites), even years later. Neonicotinoids will contaminate water, generating very dangerous contaminations far from where they are distributed.

NEONICOTINOIDS IN GUTTATION DROPS AND PLANT RESIN

Bees take resin from plants to produce propolis. Researchers found that the resin of sunflower plants, whose seeds had been treated with neonicotinoids, may be the cause of the finding of the same molecules in propolis: imidacloprid was determined at a concentration between 20 and 100 ng/g. [359]

Neonicotinoids may be found not only in nectar and pollen but also in droplets of liquid secreted by plants through transpiration (or guttation), which are sucked up by insects such as bees. Guttation is a natural phenomenon in which plants secrete drops of water containing organic and inorganic substances. They can be secreted from different tissues such as leaves and are used by bees and other insects. Through this mechanism, plants remove substances and regulate turgidity. Neonicotinoids distributed with irrigation water are also found in guttation droplets. Insecticides may be measured in guttation fluids at concentrations of hundreds of parts per million. [359] Corn plants produce these droplets primarily in the first few weeks after planting: between 0.1 and 0.3 mL per plant per day. [359] The guttation drops may contain concentrations of insecticides thousands of times higher than those capable of causing negative effects to bees. [260] Research carried out in Italy found neonicotinoids in guttation drops produced by maize (whose seeds had been treated) in the first two weeks after sowing, at concentrations between 10 and 100 mg/L for thiamethoxam and clothianidin, and up to 200 mg/L for imidacloprid. [400] Bees taking guttation drops at these concentrations died within minutes.

POISONOUS SUBSTANCES AT VERY LOW CONCENTRATIONS

The lethal dose by ingestion of imidacloprid, which can kill 50% of exposed bees, varies between 3.7 and 102 billionths of a gram per bee, depending on the experimental model applied. [363] Two aspects must be stressed:

- it only takes a billionth of a gram to kill an insect in a few hours;
- different studies may lead to very different results on the toxicological assessment, even by an order of magnitude.

Millionths of a gram per bee are equivalent to a concentration in the food (pollen, nectar or honey) between 0.1 and 1.6 mg/kg. [363] While the dose that kills 50% of the exposed bees by contact is about 24 ng per bee (experimental investigation lasting up to 48 hours). [363] The toxicity of neonicotinoids taken orally is greater than that by contact. Some compounds (nitroguanidine neonicotinoids including imidacloprid, clothianidin, thiamethoxam and dinotefuran) are very toxic to bees: the lethal dose for 50% of bees exposed by contact (LD_{50}) is between 0.004 and 0.075 μg/bee. [107] Measurements carried out in the laboratory record the following doses capable of killing 50% of the exposed bees by contact: [482, 607, 620]

- 18 ng/bee for imidacloprid,
- 22 ng/bee for clothianidin,
- 30 ng/bee for thiamethoxam,
- 75 ng/bee for dinotefuran,
- 138 ng/bee for nitenpyram,
- 7.1 µg/bee for acetamiprid,
- 14.6 µg/bee for thiacloprid.

The doses just indicated could represent an optimistic estimate, as these molecules can be potentiated by the presence of other pesticides. In the case of acetamiprid exposure, the simultaneous presence of the fungicide triflumizole increases the toxicity to bees by 244 fold and by 1,141 fold for thiacloprid. [482] An important but underestimated aspect is that some metabolites of neonicotinoids (olefin, nitrosamines, 5-hydroxyimidacloprid) are more toxic than the starting compounds.
Acetamiprid and thiacloprid (belonging to the category of cyano-substitutes) are less dangerous because they are inactivated by special detoxification systems present in bees: cytochrome P450.[I] In fact, the inhibition of cytochrome P450 increases the toxicity of these compounds (thiacloprid and acetamiprid) between 250 and 1,100 times. [107] Thiamethoxam is rapidly converted to clothianidin, which is thus an active and toxic metabolite. It takes only 2.5 billionths of a gram of clothianidin to kill, in less than 48 hours, 50% of exposed bees (deltamethrin has an equivalent acute toxicity). [355] Four billionths of a gram of imidacloprid generates a 50% probability of killing a bee in less than 48 hours; to obtain the same effect with an organochlorine insecticide such as DDT a dose seven thousand times greater is necessary. [260]

[I] The term cytochrome P450 indicates an enzymatic family of hemoproteins present in all living beings (more than 7,700 distinct macromolecules are known), belonging to the enzymatic subclass of mixed-function oxidases (or mono-oxygenases). Cytochromes P450 are the major players involved in detoxification, being able to act on a large number of different substrates, both exogenous (medicines and toxins of external origin) and endogenous (waste products of the body). [161]

Clothianidin is estimated to be 10,800 times more powerful than DDT: some studies note that it is enough to expose a colony to less than five billionths of a gram for half of the insects to die. [166, 181, 183, 354]

DDT effectively carries out the action desired by farmers, namely that of exterminating insects, when it is distributed at a dose of at least 200-600 grams per 10,000 square metres. The neonicotinoids, even if used with a dosage at least 10 times lower than DDT, show a lethal action up to 1,000 times greater. 7,5 g/ha for deltamethrin, 50 g/ha for clothianidin, 75 g/ha for imidacloprid are sufficient. [354] Therefore, neonicotinoids are much more toxic than earlier insecticides such as organochlorines, even when used at doses 10 times lower than the latter. Neonicotinoids kill the insect and persist in the environment, so the molecule remains in circulation in the biosphere and will encounter another insect or another living being and cause new damage. This creates a very dangerous chain that causes irreversible damage to humans as well.

THE SUB-LETHAL EFFECTS OF NEONICOTINOIDS ON BEES

The doses at which sub-lethal effects can be measured are much lower than those capable of killing bees in less than two days, either by ingestion or contact. Neonicotinoids, at sub-lethal doses, generate several adverse effects such as changes in behaviour, disorientation, memory impairment, and inhibition of foraging. The sub-lethal effects reported by neonicotinoids on bees are diverse: [260, 261]

- Reduction in adult life expectancy.
- Impairment of memory and learning ability. Thiamethoxam at a dose of 0.1 ng/bee reduces olfactory memory. [482]
- Reduced ability to communicate and consequently reduced effectiveness of foraging ability.
- Impairment of the immune system.
- Reduced orientation ability (one millionth of a gram is enough to reduce the rate of return to the hive by 30%, compared to untreated bees, and this exposure alone can be responsible for a significant decrease in the number of bees in the hive).
- Delayed larval development.
- Destruction of the intestinal flora which can, as a consequence, generate malnutrition.

Sub-lethal effects, i.e. those that do not kill the insect but alter important functions such as the ability to orient themselves or move, occur at concentrations at least 1,000 times lower than those that generate the death of 50% of the individuals in a few hours.

The amount of Imidacloprid that can show sub-lethal effects varies between 0,1 and 2 ng per bee, corresponding to a concentration between 1 and 20 µg/kg (millionths of a gram in a kilogram). [363] This means that very low doses are sufficient to record changes in insect behaviour, as is the case with Imidacloprid and its metabolites (Imidacloprid metabolites include: 5-hydroxyimidacloprid, 4,5-dihydroxyimidacloprid, desnitroimidacloprid, 6-chloronicotinic acid, olefins and urea derivatives). [378]

Clothianidin has a LD_{50} for honey bees of about 4 ng corresponding to the concentration in a sugar syrup of 20 ppb. Exposure of honey bees to neonicotinoids such as clothianidin has been reported to have several adverse effects: [1234]

- Alteration of mutual cleaning behaviour (grooming).
- Changes in gene expression in neuronal cells.
- Memory impairment.
- Alteration in the quality of the seminal fluid.
- Increased mortality of worker bees.

- Reduction in colony worker bee mass (by 21% at 20 ppb clothianidin).
- Increased carbon dioxide concentration within the hive (by 22% at 20 ppb clothianidin; this gas can affect memory, ovary development, and gene expression).
- Exposure of the colony to 5 ppb of clothianidin reduces the larval mass but this effect is not seen at higher doses (20 ppb).
- Clothianidin is still present in honey six months later.

Imidacloprid alters the colony's ability to thermoregulate (including bumblebees) and reduces social immunity. In general, exposure to neonicotinoid insecticides increases the severity of diseases caused by parasites (such as *Varroa* and *Nosema*). [1234]

60 ng of imidacloprid per bee, corresponding to 600 µg/kg, must be administered to kill 50% of bees in less than 48 hours; whereas 40 ng/bee, corresponding to 400 µg/kg, is sufficient to kill 50% of bees in less than 96 hours. [378] Among the metabolites tested in this research, 5-hydroxyimidacloprid and olefins have an acute toxicity similar to that of imidacloprid. To assess chronic toxicity, bees were fed for 10 days with a sugar solution containing very low concentrations of imidacloprid (0.1; 1; 10 µg/L). Fifty percent of the bees died within the first 8 days. Considering that the sugar syrup with the insecticide and its metabolites was consumed in the amount of 12 µL per bee per day, after 8 days they had consumed 0.01 or 0.1 or 1 ng per bee (equivalent to concentrations of 0.1 or 1 or 10 µg/kg). The alarming result is that mortality obtained by acute toxicity testing (of 50% of bees exposed in 2 or 3 days) can be achieved in as little as 8 days by administering doses between 30 and 30,000 times lower for olefins, between 60 and 60,000 times lower for imidacloprid, between 200 and 20,000 times lower for 5-OH-imidacloprid, and between 1,000 and 100,000 times lower for the other metabolites. [378] These data confirm that a chronic exposure to low doses generates the same effects produced, within hours, by doses hundreds or thousands of times higher. It only takes a billionth of a gram taken daily to kill a bee in less than 8 days. These amounts are so small that it is difficult to measure them instrumentally in the laboratory, as some of the most widely used analytical methods are not capable of determining such infinitesimal doses. It can be concluded that chronic toxicity is much higher than acute toxicity, as doses a thousand times lower still kill bees, albeit in a few more days.

It must be considered that plants treated with neonicotinoids (such as imidacloprid on maize or sunflower seeds) will contain these toxic molecules throughout their life. These substances will also contaminate crops and wild plants for subsequent years as they accumulate in the soil: insects and bees will be subjected to constant chronic exposure. The information on sub-lethal effects underestimates the real exposure as bees are in contact with hundreds of pesticides, in some cases more toxic than imidacloprid, such as fipronil. Thus, synergistic and additive effects could further reduce (by 10 or 1,000 times) the doses capable of killing bees following chronic exposure. The finding of these effects at very low doses, enhanced by synergistic and additive mechanisms, contradicts the principle that it is the dose that makes the poison. In this case, increasing the time of exposure or the number of molecules leads to greater negative effects. Unfortunately, the hazard assessment of pesticides carried out prior to authorization for use in the field does not take due account of such alarming results. In addition, the effects on wild pollinating insects could be more devastating, as they may be more sensitive than bees (they do not enjoy the benefits of sociality and are not in symbiosis with beekeepers).

The destructive power of the enormous quantity of pesticides distributed in a systematic and generalized way in the environment is difficult to perceive. We can have a vague idea by hypothesizing that each ton of active ingredient, in the first days following its distribution in the fields, could easily kill a number of insects equal to one followed by 13 or 14 zeros (10,000,000,000,000: ten thousand billion); considering that a colony of bees has 40,000 insects it means having a chemical arsenal potentially capable of exterminating 250 million hives in a few hours. So to measure the devastating power of tens of thousands of tons of pesticides

distributed every year we should use the units of measurement applied in astronomy: the bees that could be killed after contact with 20,000 tons of imidacloprid (assuming that 100% of the amount distributed reaches the target), placed side by side, could form a row so long that the Astronomical Unit, that is the average distance between the Earth and the Sun (about one hundred and fifty million kilometers) would not be enough.
In order to have a perception of gravity of the situation, it is possible to play with numbers by making another theoretical and approximate calculation, which can give a measure of the potential extermination capacity of imidacloprid. In England, in 2006, it was used on about 770,000 hectares for more than 82,000 kg in total. [697] Approximately 106 g of Imidacloprid was used per hectare. Let's assume that only 1% of this quantity reaches the target and that after killing an insect, at a dose of 20 ng (20 billionths of a gram), the active principle does not return to the environment and cannot damage other organisms. It means fielding a chemical weapon capable of potentially killing over 70 million insects per hectare; this enormous number corresponds to over 1,100 hives of 60,000 bees each, for each of the 770,000 hectares. Suppose we employ the same amount of DDT, 106 g per hectare, and that only 1% of this dose reaches the insects. If DDT kills a bee at a dose of 30,000 ng per insect (corresponding to 30 µg per bee), we could assume that we could exterminate 50,000 bees per hectare. In reality DDT was used in quantities 10 times higher, so it means that theoretically more than 500,000 bees could be exterminated per hectare. This comparison can give an idea of the destructive potential put into the field and how it has increased over time by more than 140 times, taking into account the greater quantity per hectare with which DDT was used, i.e. 10 times greater than neonicotinoids.
The lingering harmful action over the following weeks or years is hard to imagine, but certainly devastating.

A POISONOUS INSURANCE POLICY

With the use of neonicotinoids and other active molecules (e.g. fipronil) a systematic chemical attack on ecosystems is being carried out, which has never been recorded before. A devastating side effect of this kind of chemical insurance is that more than 90% of the fields, in which pesticides are distributed by sowing, did not need them. The plants that follow the treated crops, due to the permanence of the active molecules in the soil, will also be contaminated by these molecules. The preventive use of systemic and highly harmful pesticides does not increase the production of the crops on which they are used, as in the case of corn in Italy, but at the same time generates irreversible damage to ecosystems. [414] It would be more far-sighted to find other solutions, such as taking out insurance against crop losses from pests, rather than distributing poisons without any prior phytopathological assessment. Some concrete examples of the application of alternative insurance strategies have made it possible to spend 7 to 10 times less, compared to the use of seeds treated with neonicotinoids. [307] The use of neonicotinoids and other pesticides should be prevented. The old principles of integrated pest management have been forgotten. Assessing the presence and quantity of pests (e.g. with pheromone traps, leaf sampling, etc.) and estimating economic thresholds of damage should be the practice to be implemented before the distribution of poisons. Using pesticides, spending economic resources and polluting the environment, before being sure that they will serve a purpose, is also unwise from the point of view of agricultural budgets: they could only be used when the estimated economic damage exceeds reasonable safety thresholds and there is no alternative. If the monetary value of the environmental disaster and of the problems generated to human health is taken into account in the estimate of the economic damage, it would be easy to discover that they should never be used: the balance would always be negative (assuming

that it would be possible to give an economic value to the avoided diseases and anticipated deaths). Unfortunately, integrated agriculture management has, in the best of cases, become a reasoned method for pesticide management. In fact, this methodology, even when it has been applied, has not led to a drastic reduction in pesticide use.
As an alternative to crop loss insurance, different strategies could be designed, such as crop rotation and intercropping, i.e. the simultaneous cultivation of different plants in the same plot. Another strategy is that of biological control, i.e. that which employs and favours pest predators (e.g. other insects). The natural biological control of parasites could be favoured by the presence of areas with spontaneous vegetation (refuge areas, for example, for insectivorous birds). Another weakness of the agricultural system is that often the best agronomic consultant available to farmers is also a pesticide seller. The absence of an alternative culture is one of the critical points that should be addressed systematically from the top.
Tens of millions of hectares are invaded by highly toxic and harmful molecules unnecessarily, as distribution occurs before there are obvious signs of a pest attack. Neonicotinoids in seeds kill insects throughout the life of the plant (they impregnate all plant tissues), are persistent, remain in the soil and contaminate water. They kill insects but also harm birds, fish and amphibians. Many species of birds are insectivorous so they are extinct due to reduction of their food or poisoning. Reducing the massive use of neonicotinoids in a preventive manner would not harm farmers or reduce yields, but would avoid a lot of problems. The preventive use of systemic insecticides since sowing generates the following negative effects: [349]

- It affects non-target species (e.g. wild pollinators and birds).
- It favours the emergence of resistance phenomena in the target species.
- It promotes the development of non-target species that, in some cases, are benefited by the disappearance of competitors.
- It increases seed costs for farmers: between 2004 and 2011, corn and soybean seeds in the USA experienced a price increase of more than the double.
- Dependence on chemical agriculture increases as natural balances are irreversibly altered.
- It pollutes soil and water.
- It contaminates wild plants.
- It generates adverse effects on human health.

The agro-chemical industry encourages the use of seeds treated also with non-neonicotinoid insecticides and other pesticides such as fungicides and nematicides. It must be remembered that the sale of seeds is managed by a few companies (oligopoly), which have such power that they can afford to reduce or even eliminate from the market seeds not chemically treated with pesticides (or not genetically modified, for example for herbicide resistance).

Despite the widespread use of this strategy, which destroys the biosphere on which we depend, there is still uncertainty as to whether its benefits justify its many disadvantages. This is precisely what a study shows: in the US, between 34% and more than 50% of all soybean acreage is estimated to use seeds pre-treated with neonicotinoids. [583] Soybeans in the USA are treated with clothianidin, imidacloprid and thiamethoxam (neonicotinoid insecticides) and, together with maize, constitute one of the most important applications for this category of insecticides. Often the seeds are also simultaneously treated with fungicides. It must be remembered that these two crops can be genetically modified to be resistant to glyphosate, another non-negligible risk factor. The widespread use of neonicotinoids to kill arthropod pests in soya does not generate the desired benefits, such as increased production, which should justify the poisoning of the biosphere. The use of integrated pest management principles, such as monitoring the presence of pests and implementing targeted treatments only after economic thresholds for intervention are exceeded, produces greater benefits, in terms of increased production. The methodology of treatments with poisons in a preventive way, as a sort of insurance, generates costs and ecological damage not justified and not compensated by the increase in yields. According to this evaluation, carried out in the USA, the simultaneous treatment of soybeans with neonicotinoids and fungicides generates a yield increase of about 130 kg of extra soybeans per 10,000 square meters; the seeds were treated with quantities between 0.0756 and 0.2336 mg of fungicide and neonicotinoid active molecules per seed, and were sown at a density between 250,000 and 450,000 soybeans per hectare. This density of treated seeds means a field distribution of about 105 g of active molecules per hectare. [583] The use of neonicotinoids on seeds, compared to untreated seeds or those treated only with fungicides, generates a yield increase of not more than 190 kg per hectare, which corresponds to about 5% of the estimated production of 3,500 kg/ha per year. In conclusion, these small increases in yield do not generate a convenient increase in profitability. A major limitation that is typical of this economic analysis methodology is that no economic value is placed on environmental damage (e.g. to non-target species) and health. Despite this huge shortcoming, economic analysis recommends that this poisonous and expensive agronomic practice be reviewed. In this case, the use of neonicotinoids does not significantly and usefully increase the yields of soybean cultivation, but it does increase the costs and the environmental and health disadvantages. Application of good agronomic practices such as choice of sowing date, irrigation, use of the most appropriate varieties and use of integrated pest management principles can generate greater improvements and yield increases.

This pre-emptive agro-chemical strategy generates an unprecedented ecological disaster. The importance and need for immediate action is highlighted in a 2018 publication signed by 235 Authors calling on Governments around the World to ban the use of neonicotinoids. [337] It is necessary to suspend the application of neonicotinoids used today but it is also necessary to prevent the spread of new molecules and strategies that are entirely similar.

THE POISONOUSNESS OF CERTAIN INSECTICIDES TO BEES

The following table compares the concentrations of some insecticides capable of killing 50% of adult bees exposed by ingestion or contact. [585, 590, 591, 600, 678, 690] In addition, molecules that have a systematic action are reported, i.e. those that are absorbed and transported in all plant tissues, as in the case of neonicotinoid insecticides.

Molecule[1]	**Oral LD_{50}** (µg per bee)[4]	**LD_{50} by contact** (µg per bee)[4]	**Use on seeds**[3]	**Systematic action**	**Category**
Chlorpyriphos	0.25	0.059	YES	NO	Organophosphates
Clothianidin	0.0038 – 0.0028	0.044 – 0.022	YES	YES	Neonicotinoids
Cypermethrin	0.035	0.02	YES	NO	Pyrethroids
Deltamethrin	0.079	0.0015	YES	YES	Pyrethroids
Dinotefuran	0.023 – 0.0076	0.075 – 0.024	--	YES	Neonicotinoids
Fipronil	0.0042	-	YES	Moderate	Phenylpyrazoles
Imidacloprid[2]	0.081 – 0.0037	0.081 – 0.018	YES	YES	Neonicotinoids
Nitenpyram	--	0.138	--	YES	Neonicotinoids
Thiamethoxam	0.005 – 0.004	0.024 – 0.030	YES	YES	Neonicotinoids

[1] The lethal ingestion doses (LD_{50}) of other molecules are as follows:

- *Diazinon organophosphate insecticide: 0.09 µg/bee. [607]*
- *Pyrethroid insecticide lambda-cyhalothrin: 0.038 µg/bee. [620]*

[2] In the case of bumblebees, the LD_{50} of imidacloprid, i.e. the dose capable of killing 50% of the insects in less than 72 hours, has been estimated at about 0.02 µg per insect (and is equivalent to 2 mg/kg). [697]

[3] The seeds are impregnated so as to avoid subsequent treatments, as these molecules will spread, during the growth of the plant, in the plant tissues making it poisonous for all its life (e.g. corn).

[4] Mortality is usually measured in less than 48 hours.

The doses reported are approximate and are evaluated under laboratory conditions. Mortality is recorded in less than 48 hours. In reality, bees are exposed to complex mixtures on a long-term basis, and it is known that concentrations 10 or 100 times lower than those presented can generate an equivalent mortality, but over a longer period of time, quantifiable in days or weeks. The simultaneous presence of some fungicides or some parasites can enhance the toxicity of insecticides even by a thousand times, thus generating the same mortality effects at lower concentrations. Synergistic effects have been reported between the fungicide triflumizole and the neonicotinoid insecticide thiacloprid, and between the fungus *Nosema* and some insecticides such as imidacloprid. [678] Concentrations below 0.1 parts per billion may be capable of generating devastating effects. Some pesticides generate important sub-lethal effects at doses of billionths of a gram per bee: [13]

- The increase in the number of inactive bees was associated with exposure to fipronil (at a concentration of 2 µg/kg) and imidacloprid (at a concentration of 1.5 billionths of a gram per bee).
- The increase in the number of bees not returning to the hive was recorded due to exposure to 6 ng per bee of imidacloprid (neonicotinoid): 75% of bees do not return to the colony; and more than 79% do not return to the hive when exposed to two billionths of a gram per bee of clothianidin.
- The increase in flight time to reach the water and sugar dispenser was recorded when bees were exposed to half a billionth of a gram of clothianidin per insect.
- Abnormal movements were recorded when exposed to one billionth of a gram per bee of clothianidin.

The quantities necessary to register negative effects are so small that they are hardly detectable by analytical instruments in the laboratory, as they measure concentrations 10 or 100 times higher. [682] Theoretically, one gram of thiamethoxam (a neonicotinoid insecticide) or cypermethrin (a pyrethroid insecticide) provides a dose of poison capable of killing at least 25 million bees exposed by contact in a few minutes, while the same amount of imidacloprid or clothianidin (one gram) can be used to exterminate over 125 million bees exposed by ingestion in a few minutes. It is easy to understand why a few grams per hectare (on seeds) can be used to ensure an insect exterminating effect. This acute effect is carried out during the first hours after exposure, but if these molecules remain in the environment and circulate in the biosphere (e.g. in the food chain), the toxic action will be perpetuated. Therefore, the number of insects killed in the long term (weeks, months or years depending on the active ingredient) could be several orders of magnitude higher. Bees exposed through diet to a dose of thiamethoxam (neonicotinoid insecticide) equal to one tenth of what kills 50% of insects (LD_{50} of about 4.8 ng/µL) have a reduction in life expectancy of 41%. [590] In short, a few tens of grams per hectare, applied on the seeds, are sufficient to generate a devastating action for the whole life of the plant and also in the following years. We must also highlight the negative consequences on non-target species, such as solitary bees, which are generally more sensitive to these insecticides than honey bee colonies. Indeed, in many places on the Planet, such as in certain regions of Europe, wild pollinators no longer exist or are very rare. This information should be enough to alarm anyone with common sense and, therefore, an intelligent community would not use these chemical weapons. The systematic use of such harmful substances contributes to the transformation of the Planet into an increasingly inhospitable World.

PESTICIDES IN PLANTS AND BEEKEEPING PRODUCTS

PESTICIDE CONTAMINATION

Pollinators are exposed to different molecules because of:

- phytosanitary treatments carried out on the different crops;
- treatments carried out by beekeepers;
- widespread environmental contamination that exposes bees through the collection of pollen, nectar (including that from contaminated wild species), water, resin, and honeydew.

Bees are exposed to pesticides during distribution, from contaminated water (a family of honey bees consumes as much as 80-100 L per year) but also due to the absorption of systemic molecules contained in pollen, nectar, guttation droplets, honeydew, and resin. Exposure is always multiple and for each molecule it is possible to evaluate a risk quotient given by the ratio between the measured concentration (e.g. in pollen in ng/g) and the lethal dose that kills 50% of insects by ingestion (calculated in less than 72 hours in the laboratory and expressed in µg/bee). Another way of trying to quantify the level of risk is to calculate the percentage of the lethal dose for 50% of the insects or $\%LD_{50}$, i.e. the product of the maximum concentration measured (e.g. in nectar in ng/g) with the quantity of nectar ingested each day (mg/bee) divided by the LD_{50} (oral or by contact expressed in µg/bee). The review of the scientific literature shows that among the molecules that definitely constitute a high risk for bees due to exposure through pollen or nectar, there are 17 fungicides and 14 insecticides (in Europe and in the USA). [1200] Some molecules may be found at very high concentrations in pollen: the insecticide carbofuran (1,400 ng/g), the fungicide dicarboximide iprodione (524 ng/g), the organophosphate insecticides dimethoate (500 ng/g) and chlorpyrifos (100 ng/g), the insecticide spinosad (isolated from the bacterium *Saccharopolyspora spinosa*; 320 ng/g), and the fungicide chlorothalonil (265 ng/g). Nectar may also have high concentrations of some pesticides: dimethoate (1,595 ng/g), chlorothalonil (76 ng/g), the organophosphate insecticide phorate (53 ng/g) and the carbamate insecticide oxamyl (35 ng/g). [1200] Neonicotinoid insecticides can be found at dangerous concentrations, e.g. dinotefuran 34.7 ng/g in pollen and 7 ng/g in nectar. Unfortunately, many residues are found in plenty crops at the same time: 14 in rape (*Brassica napus*) or 11 in broad beans (*Vicia faba*). Pesticides are also found in pollen and nectar of wild plants, although often at lower concentrations (e.g. clothianidin, imidacloprid, thiacloprid, thiamteoxam). Overall, pollen is found to contain more active compounds than nectar. It follows that bees can be easily exposed to doses that are certainly dangerous: between 50% and 100% of the oral and contact LD_{50} for spinosad through daily pollen intake; higher than the oral and contact LD_{50} for dimethoate, imidacloprid and clothianidin through nectar. Bumblebees may be exposed to hazardous concentrations: greater than 100% of the LD_{50} for imidacloprid and dimethoate through nectar intake; equal to 70% of the LD_{50} for thiamethoxam in nectar; greater than the LD_{50} through contact with clothianidin, imidacloprid and dimethoate. The high concentrations of dimethoate in pollen suggest very dangerous effects also for other insects such as solitary bees (e.g. *Nomia*). No information on herbicides emerges in this study because insufficient data are available on these two matrices: pollen and nectar. In addition, only a few of the molecules actually used in agriculture are searched and no information is available on the other compounds present in commercial mixtures. [1200] Insects in a single feeding episode may be exposed to 14 different pesticides at the same time, whose additive and synergistic effects are significant but underestimated.

It is useful to remember that in many cases farmers carry out treatments with mixtures, as they can distribute several molecules simultaneously in the fields in order to reduce costs. The number of active molecules distributed in a single passage can be more than nine; an average of between 2 and 4 active molecules distributed at the same time has been recorded, depending on whether the crop is vegetable or orchard. [601] The fraction of cultivated areas treated with complex mixtures increased over time. Thus, there has been an increase in pesticide exposure; to give an example, in the UK, fungicides and insecticides are simultaneously distributed in more than 30% of the area planted with vegetables and 25% of the area planted with orchards (in 2007). [601] Honey bees can be exposed to dozens of molecules at the same time and throughout their lives. Those used by beekeepers such as acaricides and fungicides must be added to these molecules. Therefore, even in bee products such as honey and wax, it will be possible to find several active molecules at the same time (e.g. neonicotinoids and fungicides, or neonicotinoids and pyrethroids).

The negative effects of pesticides on bees are also officially recognized through the compensation mechanisms provided for by various regulations in many countries, such as Italy and the USA; in the latter country, compensation for pesticide damage has been programmed since 1967 and, until 1980, aid to beekeepers amounting to approximately 40 million dollars was provided. [974] This sum was much higher than the amount spent on bee research in the same period.

PESTICIDES IN POLLEN

The amount of pollen that can be collected by a colony may vary greatly. It has been found that hives placed near the bean crop can collect between 10 and 96 grams of pollen per day. [601] In the case of vine cultivation, 2,000 foraging bees can collect as much as 250 grams per day. It is possible to consider that for each kilogram of pesticide distributed in the fields, a concentration between 0.2 and 43 mg/kg can be found in the pollen. This quantity is defined as *residue per unit dose* and those presented are rough estimates that highlight how dangerous the exposure can be. To get an idea of the possible level of contamination, the concentrations of different pesticides measured in pollen collected by foraging bees at the entrance of hives are reported (in µg/kg):

- Carbaryl: 15 - 240.
- Carbofuran: 2 - 11,517.
- Deltamethrin: 7.5.
- Diazinon: 30 - 1,980.
- Endosulfan: 45 - 2,224.
- Fipronil and derivatives: 0.42 - 8.
- Imidacloprid: <0.5 to 36.
- Malathion: 240 - 7,640.
- Myclobutanil: 13.5 - 13.9.
- Ethyl Parathion: 19.2 - 3,700.
- Methyl Parathion: 8 – 6,630.
- Penconazole: 17.6 – 23.6.
- Tau-fluvalinate (anti varroa): 5 - 487.
- Tebuconazole: 12 – 16.5.
- Thiophanate methyl: 3,674.

This contamination of the bee colony can generate different types of negative effects, both in the short term (at higher concentrations) and in the long term. It is important to remember that pollen is the main food in the juvenile stages and is an important source of protein for the entire colony.
Through pollen, quantities of pesticides can be transported into the hive that are 1,000 and more times higher than the doses capable of killing 50% of adult bees exposed by ingestion. There are many studies demonstrating the danger of residues in pollen. In beehives located near apple orchards (in Slovenia), it is possible to find dangerous concentrations of pesticides used in orchards in the pollen. In particular, diazinon (organophosphoric insecticide) can be found in the pollen 10 days after the treatment in the field, at a concentration of 0.09 mg/kg, while 6 days after the treatment it is possible to measure in the pollen as much as 0.03 mg/kg of thiacloprid and 0.01 mg/kg of difenoconazole. [607] These results confirm the exposure to sub-lethal doses of both adult and juvenile bees.
The following table shows the concentrations measured in pollen collected from flowers (residue per unit dose). [601]

Active ingredient	**Application method**	**Pollen concentrations** (µg/kg)
Boscalid	Spray of 500 g active ingredient per hectare on oilseed rape	Day zero: 13,900 Day 1: 26,200 Day 7: 3,000
Captan	2,000 g active ingredient per hectare in apple orchards	Day 3: 18,970 Day 12: 40
Carbaryl	Spray of 1.1 kg active ingredient per hectare on alfalfa	240
Carbofuran	Spray of 1.1 kg active ingredient per hectare on alfalfa	Day 1: 10,183 Day 5: 233
Cypermethrin	On oilseed rape at a dose of 44 g active ingredient per hectare	Day zero: 1,900 Day 1: 110 Day 2: 70
Ethyl parathion	Spray of 1.1 kg active ingredient per hectare on sunflowers	Day 1: 3,700
Fluvalinate	144 g/ha	Day 1: 262 Day 5: 5
Iprodione	Two treatments in cherry cultivation (0.75 kg/ha pre-flowering and 0.188 kg/ha at flowering)	33.2 (maximum of 118)
Malthion	Spray of 210 mL of active ingredient per hectare on full flowering oilseed rape	One hour after distribution: 5,700
Methyl parathion (micro-encapsulated)	Spray of 1.1 kg active ingredient per hectare on alfalfa	Day 1: 2,168 Day 5: 43
Pyrethroids (PP321)	Spray of 5 g active ingredient per hectare on oilseed rape	Day zero: 200 Day 1: 500 Day 2: 10
Procymidone	0.75 kg active ingredient per hectare of strawberries	Day 3: 31.1 Day 9: 4.9
Thiacloprid	96 g active ingredient per hectare of apple orchard	Day 1: 90
Vinclozolin	375 g active ingredient per hectare of cherries	393

The male gametes of the flowers are found to be seriously contaminated with toxic substances. These data show that the concentrations in pollen may vary over time, decreasing and confirming the dangerous exposure of bees and pollinators (these molecules can also generate alterations in plants and reproduction).

In an agricultural area in southern Germany, monitoring was conducted to check the level of contamination of pollen collected by bees (*Apis mellifera*). A total of 260 pesticides (75 fungicides, 95 herbicides, 89 insecticides and 3 plant growth regulators) were searched for in freshly collected pollen from bees kept in an agricultural area where the predominant crops were apples, cherries, grapevine and plums (between April and July 2018). [1271] In the pollen collected daily for 102 days, 15 fungicides, 12 insecticides and 2 herbicides were detected. Of the 102 samples, only 13 did not record the substances sought and up to 13 pesticides were found simultaneously: the average was 5 active compounds per pollen sample. Concentrations ranged from 6 ng/g to 4,530 ng/g; the highest concentration was recorded for the fungicide tebuconazole (4,530 ng/g). Another fungicide, fluopyram, was also recorded at high concentrations (4,050 ng/g). The total concentration of all pesticides was found to be between 3,300 and 8,800 ng/g pollen. Two herbicides were found: pendimethalin (up to 1,810 ng/g) and dimethenamid (up to 120 ng/g). Some insecticides are found at high concentrations: tebufenozide (at a maximum concentration of 410 ng/g), fenoxycarb (at a maximum concentration of 370 ng/g), chlorantraniliprole (at a maximum concentration of 310 ng/g), thiacloprid, diflubenzuron and picaridin. The finding of 12 insecticides in pollen even at high concentrations suggests the use of these molecules during flowering (e.g. diflubenzuron and fenoxycarb). Pollinators are exposed to complex mixtures with little known but certainly negative synergistic effects.

POLLEN CONTAMINATION AS A RESULT OF THE USE OF SEEDS IMPREGNATED WITH PESTICIDES

One route of exposure is when sowing seeds impregnated with pesticides. Bees are exposed to dust and active molecules that are present in and on the seeds. In bees exposed during the sowing of maize treated with clothianidin (neonicotinoid insecticide), concentrations between 101 ng/bee (bees exposed at a distance of 9 m from the sowing machine) and 1,394 ng/ape (bees exposed at a distance of 1 m from the sowing machine) were found. [601] The active molecules distributed with the seeds remain in the plants throughout their life, making them toxic to phytophagous insects even when the maize plant is two metres tall. These molecules, like the neonicotinoid insecticides, are diffused throughout the plant and will also contaminate the pollen. The following table shows the concentrations distributed in rape, maize and sunflower seeds and those found in pollen.

Active molecules	**Application**	**Pollen concentrations** (µg/kg)
Clothianidin	0.03 mg/seed rape	2.59
Clothianidin	0.6 mg per maize seed	3.9
Imidacloprid	1 mg per sunflower seed	13 (average) 36 (max)
Imidacloprid	1 mg per maize seed	2.1
Thiamethoxam	1.2 mg/seed of maize	Between <1 and 15

For each kilogram of pesticide distributed in the impregnated seeds, concentrations between 0.1% and more than 4% can be found in pollen. For many of the active molecules used to impregnate the seeds, the concentrations found in the pollen may be higher than the doses capable of killing, in less than 3 days, 50% of the insects exposed by ingestion (LD_{50}).

PESTICIDES IN NECTAR AND HONEYDEW

Nectar is collected in the digestive tract in quantities of about 60 µL, of which 10% can be ingested by the bee itself. Concentrations of pesticides such as parathion can be found in nectar, at doses of 0.005 µg per flower in clover or 0.027 µg per flower in cabbage. [601] It is estimated that after the distribution of each kilogram of pesticide per hectare, concentrations between 5.3 mg/kg and 11.9 mg/kg may be found in the nectar. In the case of pesticides distributed through impregnated seeds, some studies report that for each milligram present in the seeds, 0.05 to 0.075 milligrams may be found per kilogram of nectar. [601]

Some phytophagous insects such as aphids produce a honeydew rich in sugars which, in some cases, has the function of attracting insects such as ants with which they establish symbioses (some aphids produce melezitose trisaccharide sugars). Bees feed on the honeydews produced by insects such as those belonging to the systematic group called Sternorrhyncha (insects of the order Rhynchota and suborder Homoptera). [659] This group includes aphids, scale insects, whiteflies and psyllids. These insects are known for the extent of damage they cause by phytophagy and sometimes also by the transmission of viruses. Other pollinating insects collect honeydew too, such as solitary bees (e.g. *Osmia*: this genus includes about 140 species of so-called solitary bees) and bumblebees.

The following table shows the concentrations of some pesticides found in nectar collected from plants or from the digestive tract of bees. [601]

Active ingredient	**Application method**	**Concentrations in nectar** (µg/kg)
Acephate (and its metabolite methamidophos)	Spray: 750 g active ingredient per hectare one day before the raspberries bloom	Day 1: 14,390 of acephate and 1,130 of methamidophos; Day 11: 800 of acephate and 90 of methamidophos
Acephate (and its metabolite methamidophos)	Spray: 750 g active ingredient per hectare two days before the blossoming of the cherry trees	Day 2: 2,840 of acephate and 270 of methamidophos; Day 7: 1,690 of acephate and 140 of methamidophos
Acephate (and its metabolite methamidophos)	Spray: 750 g active ingredient per hectare one day before the blossoming of the apple trees	Day 7: 1,400 - 8,440 of acephate and 150 - 650 of methamidophos; Day 14: 730 of acephate and 80 of methamidophos
Clothianidin	Rape seed treatment (0.03 mg/seed)	2.24 (maximum concentration)

The concentrations measured in the nectar freshly collected from the flowers or in the bees' stomachs are lower than those that will be measured in the nectar stored in the hive, because the bees carry out its dehydration, i.e. a reduction in its amount of water. During storage in the hive, the water content in the nectar will decrease and consequently the concentration of pesticides will increase.

PESTICIDES IN THE DROPLETS

Guttation drops, i.e. drops of liquid secreted externally by plants (e.g. on leaves), may also be a source of exposure to pesticides as this water is used by insects. The following amounts of guttation droplets may be collected in some plants: [601]

- wheat: 6.4 µL/leaf;
- barley: 64.8 – 5.6 µL/leaf;
- tomato: 45.5 µL/leaf;
- grape: 56.5 µL/leaf;
- rice: 62 - 110 µL/leaf.

To get an idea of the level of contamination possible as a result of different types of pesticide treatments, some concentrations measured in the guttation drops produced by the maize plant are reported. [601]

Active ingredient	**Application method**	**Concentrations in guttation drops** (mg/L)
Clothianidin	Maize seed treatment (0.5 mg/seed)	25 - 35 *(drops collected within 20 days after sowing)*
Imidacloprid	Maize seed treatment (0.5 mg/seed)	47 - 110 *(drops collected within 21 days after sowing)*
Thiamethoxam	Maize seed treatment (1 mg/seed)	11.9 *(drops collected within 21 days after sowing)*

The results presented in this and previous tables show that nectar, pollen and honeydew are vehicles for pesticides. Therefore, the distribution of pesticides causes contamination of the food chain and undermines the reproductive system of plants and insects. Already from the early stages of development, insects will be exposed to pesticides contained in foods such as pollen and nectar.
It is possible to consider, as an indication, that for each kilogram of active ingredient per hectare the following orders of magnitude can be found in the different matrices: [601]

- 4.4 - 15.3 mg/kg active ingredient in bees;
- 0.002 - 21.1 mg/kg in freshly collected pollen from foraging bees (up to 62.7 mg/kg in pollen stored inside hives);
- 0.05 - 11.3 mg/kg in the nectar;
- 0.4 - 1.2 mg/kg in the nectar (honey) stored in hives.

Bees and pollinating insects are exposed to high concentrations of pesticides and continuously through feeding. In this regard, it is estimated that bumblebees (the workers) may consume 30 mg of pollen per day and between 140 and 190 mg of nectar per day, while larvae consume about 22 mg of pollen per day and 42 mg of nectar, so they will take in dangerous amounts of complex and harmful mixtures.

PESTICIDES IN THE WAX

The following are the concentrations of some pesticides found in wax taken inside *Apis mellifera* hives. [601]

Active compound	**Application**	**Concentrations in beeswax** (µg/kg)
Acrinathrin		58-590
Amitraz	Acaricide used by beekeepers	<1 - 1,820
Azinphos methyl		228
Bromopropylate*	Acaricide used by beekeepers	1 - 1,612
Chlorfenvinphos	Acaricide used by beekeepers (200 mg/colony)	730
Coumaphos	Acaricide used by beekeepers (between 30 and 250 mg per hive)	1 - 235
Endosulfan		51-370
Fluvalinate	Acaricide used by beekeepers	<1 - 100
Lindane		18.8 - 130
Malathion	Acaricide used by beekeepers	<1 - 5
Rotenone	Acaricide used by beekeepers (1 g/colony)	22,200
Tau-fluvalinate*	Acaricide used by beekeepers	35 - 5,100
Tetradifon		32 - 580
Thymol	Acaricide used by beekeepers (4,500 – 6,000 mg/colony)	45,370

* It may be found in propolis too.

PESTICIDES IN BEES DUE TO EXPOSURE IN THE FIELD

The following table shows some pesticide concentrations recorded in dead bees following exposure to the active molecules used by farmers in the field. [601] It is worth noting that the concentrations measured in dead bees decrease day after day and that bees are killed even when the exposure is lower than what, in the laboratory, is capable of killing 50% of orally exposed insects in a few hours.

Active compound	**LD_{50}** (µg/bee and µg/kg)	**Exposure**	**Concentration measured in dead bees**
Carbofuran	0.16; 1,600	1.1 kg of formulated product per hectare of alfalfa	3,317 µg/kg on the first day. Between 33 and 250 µg/kg on day 6
Cypermethrin	0.37; 3,700	25 g active ingredient per hectare	54 ng/ape (540 µg/kg)
Ethyl parathion	0.175; 1,750	1.1 kg of formulated product per hectare of sunflowers	3,600 µg/kg on the first day
Malathion	0.27; 2,700	spray at a concentration of 159 mL/ha	Between 960 and 5,280 µg/kg
Methyl parathion (micro-encapsulated)	0.165; 1,650	1.1 kg of formulated product per hectare of alfalfa	Between 762 and 2,500 µg/kg on day 1. Between 2 and 134 µg/kg on day 6

LD_{50}: indicates the lethal dose that in the laboratory is able to kill, in few hours, the 50% of the insects exposed for ingestion.

Unfortunately, bees can be contaminated by drift, i.e. by pesticides used on crops that are not important for pollinators. Olive trees are not important from the point of view of beekeepers (bees can collect pollen, however pollination is anemophilous) but active molecules used to treat diseases in these trees can contaminate wildflowers and the environment, and be found in pollinators such as bees and bumblebees. A monitoring conducted in Greece (2017 and 2018) showed that the active molecules used to control the olive fly (*Bactrocera oleae;* the larva feeds on the olive fruit, and the adult on nectar, pollen and sugar solutions) may be found in the bodies of honey bees: dimethoate (organophosphate insecticide) up to 2.3 mg/kg (this concentration is higher than the LD_{50}, i.e. the dose that kills at least 50% of the exposed individuals in laboratory tests lasting 48 or 72 hours), omethoate (a metabolite of dimethoate) up to 0.06 mg/kg, and the pyrethroids beta-cyfluthrin and cyhalothrin up to 0.63 mg/kg (the concentration of beta-cyfluthrin measured in bees may even be higher than the LD_{50}). [1207]

The following maximum concentrations are measured in olives: up to 0.64 mg/kg dimethoate, up to 0.028 mg/kg thiacloprid, up to 0.017 mg/kg chlorpyrifos, up to 0.6 mg/kg beta-cyfluthrin and up to 0.024 mg/kg cyhalothrin. [1207]

In Greece there are plenty plants in olive groves attractive to pollinators which may be potentially contaminated: *Urtica spp. (*Urticaceae), *Parietaria officinalis* (Urticaceae), *Mercuria lisannua* (Euphorbiaceae), *Sonchus oleraceus* (Compositae), *Malva sylvestris*, *Uropermum picroides*, *Oxalis pes-caprae* (Oxalidaceae), as well as some aromatic plants (*Rosmarinus officinalis, Salvia officinalis, Thymus vulgaris, Cistus incanus*), and ornamental plants (*Lantana spp*.). Insecticides used as sprays or baits (the insecticide is mixed with proteins and other substances) may migrate to other plants and be transferred to bees and honey (e.g. cyhalothrin at a concentration of 0.14 mg/kg in honey; dimethoate at a concentration of 0.002 mg/kg). These molecules may also be detected in bumblebees: up to 0.7 mg/kg dimethoate, up to 0.06 mg/g omethoate, and up to 0.005 mg/kg cyhalothrin. In 2018, the highest concentrations of dimethoate and its metabolite were found in bumblebees, confirming that even wild species may be dangerously contaminated. [1207]

It must be pointed out that some insecticides, such as dimethoate, can be distributed in the form of attractive baits, i.e. they can contain substances, such as proteins, which attract other insects too, including honeybees. Therefore, these pesticides could also be consumed by arthropods other than the target insects, because they are mixed with attractants.

An emerging problem is the selection of insects resistant to organophosphates, as is the case with the olive fly and insecticides such as dimethoate. [1211, 1212, 1213] This is another good reason to change strategy.

Because of the danger (it is mutagenic) and the development of resistance by the target organisms, the use of dimethoate against the olive fly in Italy has been forbidden since 2020 (actually in 2020 a derogation was issued).

SPARKS OF AWARENESS: THE TOXIC EFFECTS OF PESTICIDES ON BEES AND OTHER BENEFICIAL INSECTS

INTRODUCTION

Pesticides, along with the reduction of biodiversity, are among the most important causes of bee deaths recorded worldwide. Agricultural productions and vegetables can be considerably damaged by pesticides that affect pollinators. A famous case is the reduction of the production of blueberries in Canada, a plant that is pollinated by more than 70 species of native insects, due to the use of fenitrothion in the 70s. [243, 361, 589]

In the USA, from 1947 to 2008, the number of beehives decreased from 6 million to 2.4 million (National Agency of Agriculture Statistics). In Europe, 30-35% of hives are reported to die every year. To confirm the extent to which pesticides could be involved in this mortality, it should be pointed out that more than 150 active molecules of pesticides are found in the pollen collected by bees. Among the molecules that some environmental associations (e.g. *Greenpeace*) identify as very dangerous for bees and therefore to be banned, there are neonicotinoids (imidacloprid, clothianidin, thiametoxam), chlorpyriphos, cypermethrin, deltamethrin, and fipronil.

Theoretically, only pesticides that, following toxicological evaluations, have recorded low levels of toxicity to pollinators (this is indicated on the label) can be used during flowering. Special precautions should also be taken for wild plants that may be in bloom in the vicinity of treated crops. Through wild flowers, bees may be exposed to phytosanitary treatments allowed in crops that are not in bloom. The risk may be so high that, in some cases, cutting or chemical weeding of plants that may be in bloom near or in cultivated fields is recommended. With the intention to avoid intoxicating or killing bees, things actually get worse: biodiversity is reduced, the availability of food for bees decreases and other pesticides such as herbicides are distributed.

Since at least 1991 (Directive 91/414/EEC), the use of insecticides during flowering has been banned, and all pesticides must be tested for toxicity to bees before they are marketed. [24, 291] Unfortunately, the measurement of sub-lethal, synergistic, cumulative, chronic effects on the colony and bioaccumulation in the environment and food chains is not given due importance. In addition to the harmfulness of the active molecules, there is the harmfulness of their derivatives, adjuvants and other substances in the mixtures used. At least 15 metabolites are reported in beekeeping products such as endosulfan sulfate, fipronil sulfone, 5-hydroxy-imidacloprid, DDE, and aldicarb sulfoxid. The latter is a metabolite of the insecticide aldicarb that belongs to the category of carbamates; it has been withdrawn since 2006 and its metabolite has an action on the inhibition of cholinesterase 76 times higher than the starting molecule. [45, 500] The highest concentrations of pesticides were found in wax (average concentration of 126 µg/kg) followed by pollen (average concentration of 66 µg/kg). The following active molecules were found in wax (in more than 50% of the samples): chlorfenvinphos, tau-fluvalinate, bromopropylate, coumaphos, and chlorothalonil; while the following molecules were found more frequently in honey (in more than 50% of the samples): thiacloprid, thiamethoxam, and acetamiprid. Neonicotinoid insecticides are the most frequently found molecules. The pesticides phorate, dimethoate, and carbofuran were found in more than 5% of nectar samples collected from treated plants. Pesticides used by beekeepers to control mites and fungi are also found in honey, indicating that honeycomb wax can release these substances (coumaphos or vinclozolin and to a lesser extent tau-fluvalinate). Concerning the hazard by contact and ingestion, the risk analysis carried out by this study shows: [45]

- that pollen presents a risk from contact with the following molecules: thiamethoxam, phosmet, chlorpyrifos, imidacloprid and clothianidin;
- that a high risk by ingestion comes from thiamethoxam and lindane contained in honey (these two molecules alone, at the concentrations measured in honey, could be responsible for a 50% increase in the mortality of foraging bees);
- that two neonicotinoid insecticides, clothianidin and imidacloprid, also represent a very high risk to bees (clothianidin is four times more toxic to bees than imidacloprid, but is found less frequently and at lower concentrations).

Another interesting result is that of the 124 pesticides found in pollen, 70 are found only in this matrix and not in honey. [45] In pollen, the most frequently found molecules (taufluvalinate, coumpahos, thymol, and chlorothalonil) are present with a high average concentration (100 ng/g).

Of the 77 active molecules found in honey, 23 were found only in this matrix and neonicotinoid insecticides are the most easily found. Honey contains the highest concentrations of hydrophilic compounds such as lindane and coumaphos. Usually hydrophilic compounds are more toxic from ingestion while hydrophobic compounds are more toxic from contact: pyrethroids, which are hydrophobic, have a toxicity by contact three times higher than by ingestion. [45]

Many data on various bio-indicators point to the state of suffering due to the use of poisonous molecules in agriculture. Pesticides at low concentrations affect a lot of species. Some effects recorded in several non-target species are reported as follows: [442, 597, 601, 675]

- Olfactory impairment in salmon.
- Damage to bats.
- Damage to amphibians by acting as endocrine disruptors.
- Imidacloprid reduces social interactions in termites such as trophallaxis, cleanliness (they become more susceptible to parasites) and tunnel construction. It also reduces the rate at which food passes through the gut and affects the microbial composition within the digestive tract.
- Destruction of the signals necessary for the formation of the symbiosis between the plant root system and the nitrogen-fixing bacteria in the soil.
- The herbicide glyphosate damages worms living in the soil and affects the growth of micro-algae and aquatic micro-organisms; it also disturbs the symbiosis and balance between roots and micro-organisms in the soil.
- Imazalil (a fungicide also used to preserve fruit; for example, it is distributed with wax on the skin of citrus fruits) generates neurobehavioural effects on reproduction in mice.
- Some pesticides, including neonicotinoids and fipronil, are believed to be responsible for lowering immune defenses and have been associated with the spread of infectious diseases in wildlife.

Different countries adopt different rules regarding the maximum tolerated concentrations of pesticides in honey and beekeeping products. Germany, Italy and Switzerland have regulated different maximum allowable limits for pesticides such as amitraz, bromopropylate, coumaphos, cyamizole, flumethrin, and fluvalinate. [41] The European Union has regulated the following limits for amitraz, coumaphos, and cyamizole: 0.2, 0.1, and 1 mg/kg, respectively. The EPA in the United States (USA *Environmental Protection Agency*) has set maximum permissible concentrations of 1 mg/kg for amitraz, 0.1 mg/kg for coumaphos, and 0.05 mg/kg for fluvalinate. The different regulations for the prevention of health risks also create various problems in trade: export or import must consider the fact that there are different rules in different countries.

LIMITATIONS OF THE PREVENTIVE TOXICOLOGICAL ASSESSMENT FOR BEES

Acute toxicity tests can measure the concentration that kills 50% of bees by ingestion; this method usually takes one to two days. Evaluation of contact toxicity is carried out by laboratory tests that measure effects within a few hours and up to 96 hours. [45] In general, the maximum duration of laboratory tests to verify toxicity, whether by ingestion or contact, is 96 hours, a single application is made or a single dose is administered, very serious effects are measured such as the dose capable of killing at least 50% of the bees treated. [717] Immediate lethal effects are measured, i.e. the most serious ones that can be recorded within a few hours.

The estimation of acute toxic effects considers the concentration of pesticides, for example, in pollen (risk by contact and ingestion) and nectar (risk by ingestion). Forager bees come into contact with about 1 g of pollen per day and eat 1.1 mg per day. Forager bees eat up to 80.2 mg of honey per day and larvae, which feed exclusively on pollen for 10 days, consume 6.5 mg per day. This information can provide valuable support for estimating exposure by ingestion and contact. [45]

Through the use of rapid methods, information on acute contact and ingestion toxicity is available for most pesticides. These survey methods do not measure chronic, synergistic, sub-lethal, and whole colony effects. Little and insufficient information is available on the transformation products of these molecules (e.g. metabolites). Some substances may have lethal effects on larvae at concentrations below those considered safe for adult bees. Sub-lethal effects may be recorded following exposure to concentrations below parts per billion, i.e. 100 times lower than those used to provide information on acute effects. [861]

Pesticides are classified into three categories based on their hazard to bees, as determined by laboratory measurements: [17, 25]

- Those that are hazardous (such as dimethoate, an organophosphate insecticide); in this category the lethal dose that kills insects is less than 2 µg/bee.
- Those that are not dangerous to bees (some fungicides and herbicides); in this case the LD_{50} (the dose capable of killing 50% of the exposed insects) is higher than 99 µg/bee.
- Those that are presumed hazardous. This intermediate category may not carry any label claims, resulting in ambiguity and uncertainty: DDT and chlordane (organochlorine insecticides) are classified in this category, although they have been shown to be harmful.

Serious situations have already arisen, such as that of endosulfan, which was declared harmless to bees by the manufacturer but turned out to be devastating. It was also thought that the neonicotinoids used for seed treatment could not pose a risk. History confirms that if scientific research is not independent of major economic interests, it produces results that are subject to the laws of profit.

Before marketing, it is difficult and costly to assess damage from chronic exposure, i.e. damage originating from small doses but over prolonged periods. To assess chronic toxicity in bees, experimental models have been proposed, such as that of administering increasing doses orally for ten days, in order to determine concentrations capable of killing 50% of the bees. [668] These experimental models are not yet widely used and in any case are not able to measure, under the proposed laboratory conditions, the countless chronic, sub-lethal and synergistic effects that could be generated by lower doses. Some preventive examinations are able to highlight sub-lethal effects such as immobility, loss of coordination of movements (e.g. the bee rotates around itself), the presence of abnormal movements (e.g. contracted abdomen), hyperactivity, aggressiveness and vomiting. [668] Following exposures of a few days, behavioural alterations can be recorded at doses lower than those capable of killing bees in a few hours.

An experimental model consists of placing small colonies of bees (6,000 individuals) in tunnels (10 m x 4 m) in which they are exposed to conditions that simulate what would happen in the field. Flowers attracting bees may be present in the tunnel and pesticides may be applied at the desired dose and in the desired manner. Colonies are placed in the tunnel for a few days and then exposed to the pesticides. [669] Seven days after pesticide application they are placed outside the tunnel. During these stages they are observed to record mortality, flight activity and colony condition such as larval development. A limitation of this method is that it requires the absence, for at least a 3 km radius from the experimental area, of crops that are attractive to bees and, moreover, it is not able to assess the complex exposures to which colonies may be subjected in reality.

SUB-LETHAL EFFECTS OF PESTICIDES ON BEES

Unfortunately, in the laboratory it is very complicated to study the behavioural alterations and diseases generated by low-dose pesticide exposures on individual species, and it is even more complicated to examine the effects on the intricate network of interactions between living beings and xenobiotics in natural or artificial environments such as agriculture. The effects of small doses and complex mixture interactions, which change over a lifetime, are largely unknown. Insecticides can persist in the environment for a long time, even more than 20 years for neonicotinoids and much longer for organochlorines. In addition, many factors can generate chronic stress to insects by enhancing the negative effects of pesticides. [673] For example, parasites that feed on hemolymph (the equivalent of blood in insects) weaken individuals by increasing the sub-lethal effects of pesticides.

To better understand what is meant by sub-lethal effects, an example is given for the neonicotinoid insecticide imidacloprid. Concentrations of imidacloprid of about 20 parts per billion impair the ability of bees (*Apis mellifera*) to obtain food (pollen and nectar), while 100 parts per billion, for 30 to 60 minutes or more, suppresses food-seeking activity. [690] Concentrations of 50 parts per billion reduce the ability to return to the hive (orientation and motor skills are impaired). Concentrations close to 10 parts per billion are recorded in sunflower, maize and oilseed rape pollen (the dose that can kill 50% of bees exposed via food is between 3.7 and 81 billionths of a gram per bee). [690] If the concentrations to which they are exposed exceed 600 parts per billion, 34% of the bees do not return to the hive (they vanish exactly as recorded in the disease called "*sudden and mysterious death of bees*, or *colony collapse disorder*"). Other research also shows the same effect. [743] Very low concentrations of imidacloprid (1.5 ng/bee which corresponds to about 115 ppb in nectar) or clothianidin (about 0.5 ng/bee) reduce the foraging activity (even very small doses of fipronil, 0,3 ng/bee, show the same effect). [743] In this study, bees were followed using special micro-appliances capable of recording the movements of individual foraging bees: for a few hours, they were observed in their movements within a few metres of the artificially fed colony (RFID-transmitter with a size of 2 x 1.6 x 0.5 mm and a weight of 4 mg). If they were exposed to 6 ng/bee of imidacloprid, i.e. to the dose present in sunflower nectar generated from treated seeds, at least one quarter did not return to the hive and tremors and increased immobility were recorded. [743] When exposed to 2 ng/bee of clothianidin, abnormal movements were recorded and only 20% of the bees returned to the hive: the disappearance of the bees becomes more evident at doses higher than 1 ng/bee. Clothiandin causes sub-lethal effects at doses lower than those of imidacloprid and, at the end of the publication, there are links to videos (lasting a few seconds) that show, in an unequivocal and sadly impressive way, the impairment of motor skills. [743] These disastrous effects were recorded by tracking bees in an artificial environment in which the dispenser of the sugar solution, at various concentrations of insecticides, was placed 7 metres from the colony and the bees were followed in their movements for a few hours. The negative consequences are probably worse under real conditions where, for example, movements are longer and exposures and stressors are many.

At very low concentrations of insecticides, bees have difficulty returning to the hive and take a longer time. These studies show that with very low doses the time interval between two visits to the same food source becomes longer, the number of visits per unit of time is reduced and the ability to obtain food is impaired. Doses of venom equal to 1/100,000,000 or 1/10,000,000 of the weight of bees are capable of generating severe sub-lethal effects (these are billionths of a gram per bee, which weighs approximately 100 mg). Concentrations per cubic metre 10 to 100 times higher (millionths of a gram) than those capable of generating sub-lethal effects are found in dust generated during sowing with seeds coated with neonicotinoids. Pollen and nectar may also contain doses 10 to 100 times higher than the minimum doses capable of producing adverse effects. [743] A coated seed may contain 0.5 mg of insecticide that could potentially harm (at a dose of one billionth of a gram per bee) 500,000 bees. The neonicotinoid insecticides present in each seed could be a sufficient dose to damage several colonies of bees irreversibly. These calculations are approximate and theoretical, but should give pause for thought about the potential poisonousness of these molecules.

We could ask which dose generates sub-lethal effects in birds or in humans. If we assume that the same adverse effects generated in insects are recorded in humans at doses one million times higher than those that harm bees, i.e. thousandths of a gram instead of billionths of a gram, then this does not change with respect to body weight. Doses equivalent to 1/10,000,000 of body weight may result in adverse effects in both humans and bees (5 - 20 ng in the case of bees and 5 - 20 mg in the case of humans).

Further evidence is provided. Thiamethoxam, which belongs to the neonicotinoid family, artificially administered at infinitesimal doses causes a reduction in the ability of bees to return to the hive. [243] Bees were artificially fed with a 1.3 ng (billionths of a gram) dose of thiamethoxam and were marked with an electronic thorax tag (RFID). [385] Tagged bees were moved to a distance of 1 km from the colony and their ability to return to the hive was measured (a detector automatically records their entry into the hive). Between 10.2% and 31.6% of bees exposed to thiamteoxam doses considered acceptable and safe did not return to the hive. By performing a computer simulation, the French researchers verified that the colony could have collapsed: following attendance (foraging) of rapeseed treated with the active ingredient, a reduction of more than 50% in the number of individuals could have been recorded in less than three months. The use of the molecule on oilseed rape was banned by the French Ministry of Agriculture, probably thanks to this evidence as well as to complaints by beekeepers. [243]

The sub-lethal effects of pesticides are not measured by the mandatory toxicological tests on bees before a pesticide is marketed, partly because they are killed by the higher concentrations used. [17, 35, 682] Sub-lethal effects of pesticides are those generated by doses that can be 10 times, 100 times or even smaller than those that are lethal in a few hours. They can also lead to the death of the colony, but in a slightly longer time frame (weeks). Pesticides are marketed without assessing these sub-lethal effects on target and non-target organisms, partly because otherwise most would have to be withdrawn from the market, an action that would be ecological, intelligent, altruistic, democratic and forward-looking.

Of concern is the fact that, in some cases, adverse effects are generated by molecules other than the active ingredients, but present in commercial pesticide preparations. This may partly explain some of the differences observed between the results obtained in the laboratory, where the active ingredient with a high degree of purity is used, and those recorded in the field, where all the molecules of the commercial preparations and other substances are present.

In the following pages we have tried to summarize and catalogue some of the problems caused to bees (*Apis mellifera*) at very low concentrations, even though the many interactions between substances and organisms are difficult to predict and largely unknown. However, the general effects are very evident and irrefutable.

REDUCTION IN OLFACTORY LEARNING CAPACITY AND MEMORY

Many pesticides are capable of altering olfactory memory: bees lose or are compromised in their ability to discriminate between a known odour and an unknown one. This ability is indispensable in a sensory World where chemical stimuli are essential for survival. Long-term memory leads to the generation of structural changes in nerve cells (e.g. creation of new connections) and, in the case of female fruit flies (*Drosophila melanogaster*), frequent learning processes increase energy consumption, to the point of causing a 40% reduction in egg production. [255]

Neonicotinoids cause a reduction in learning ability: bees do not distinguish between known and unfamiliar odours (thiamethoxam at a dose of 0.1 ng/bee). [13, 482] Imidacloprid at very low doses reduces medium-term memory capacity but not short- and long-term memory capacity. [695] This effect was measured in conditioned bees by assessing the responsiveness of the proboscis extension reflex. The association of a stimulus such as the olfactory one with a reward (e.g. water and sugar) is sufficient to make the proboscis extend when the learned stimulus is received (e.g. one can stimulate the sense of smell for 15 seconds with linalool, which is a mono terpene present in the essence of rosewood and is found free or combined in the essential oils of coriander, basil, lavender or bergamot). [696] Repeated associations between stimulus and reward can induce a lasting memory which results in 4 days in the case of foraging bees. The effects of conditioning, i.e. the response of proboscis extension following the olfactory stimulus, was measured at different time intervals, between 30 seconds and 24 hours. The dose of 12 billionths of a gram of imidacloprid, administered in a sugar solution, induces impairment in short-term memory (between 15 minutes and 1 hour) and increases oxidative metabolism in the bee brain. This dose of insecticide is able to destroy the functioning of particular nerve cells involved in medium-term memory, but not those involved in long-term and short-term memory. [695] It is therefore possible to say that the insecticide, at doses below those capable of killing, can generate a kind of amnesia.

Fipronil also impairs olfactory memory by reducing the ability to discriminate between a known and an unknown odour. [73, 178, 595] Sub-lethal effects have been measured at doses of 1/80 and 1/40 of the LD_{50} (lethal dose that kills 50% of individuals exposed by contact). [73] By administering fipronil by contact on the thorax of bees at concentrations of less than one billionth of a gram (0.5 ng/bee), it is possible to alter olfactory memory and olfactory learning. [594]

Pyrethroids (deltamethrin) also reduce the learning ability of bees. [593]

Another sub-lethal effect is attributed to emissions from engines burning diesel fuel. Substances produced by diesel vehicles reduce the olfactory capacity of bees, decreasing the efficiency of their ability to identify flowers (through volatile attractant molecules). [242]

IMPAIRED ORIENTATION AND COMMUNICATION SKILLS

Alterations in the orientation ability have been reported due to organophosphates and pyrethroid insecticides, such as permethrin and deltamethrin; for the latter substance it has been shown that bees lose the ability to return to the hive. [35] Deltamethrin can impair the ability of bees to orient at doses 27 times lower than those sufficient to kill 50% of exposed insects. [592] At very low doses, bees also show motor and coordination problems, as well as learning and memory problems. [592] This study also found that after exposing bees to concentrations of the insecticide capable of causing measurable alterations in behaviour and orientation, no residue of the active ingredient was recorded. Three hours after recording the behavioural alteration, it is not possible to measure the presence of the pyrethroid in the insect: active molecules can generate serious biochemical and physiological changes even if they degrade rapidly.

At sub-lethal doses the neonicotinoids (imidacloprid, clothianidin, and thiacloprid) impair the orientation ability of bees. [75, 256, 375, 482] At very low concentrations, imidacloprid, administered orally and as a single dose, impairs the communication ability of bees (*Apis mellifera*) and reduces mobility. [591] Surprisingly, the behavioural alteration recorded in the laboratory may occur 30 to 60 minutes after administration and this effect may disappear after a few hours. Exposure to a single dose (20 microlitres at 100 or 500 ppb orally) may generate temporary behavioural alterations.

In worker bees, low doses of the insecticide imidacloprid (1.25 ng per bee) generate an increase in mobility, whereas higher doses (2.5-20 ng per bee) cause a reduction in mobility: this effect has been observed for several neonicotinoids. [589, 595] The effect changes with dose but also over time, so that opposite behaviours are observed at different times.

The label of a thiacloprid-based neonicotinoid insecticide reads *"...is selective against pollinators (bees and bumblebees). In various laboratory and field studies ... has been shown to be safe or low toxicity to many beneficial arthropods*"; instead scientific evaluations have shown that 64 billionths of a gram per insect are capable of altering memory, and at doses of 170 billionths of a gram bees suspend the dance of the abdomen, i.e. the ability to communicate is undermined. [254, 257] The manufacturer of the active ingredient proposes a lethal dose between 80 and over 230 times higher than the amount capable of registering equally serious sub-lethal effects (the LD_{50} is 15,000 nanograms per bee). This experiment demonstrated a well-known fact in toxicology, namely that the chronic effects of a poisonous substance, in principle, do not have a threshold value.

An altered ability to fly and other abnormalities have been recorded for a lot of pesticides such as pyrethroids and neonicotinoids. Bees exposed to low (sub-lethal) doses of imidacloprid or deltamethrin reduce nectar and pollen-seeking flights by between 20% and 90%, and experience an impaired learning ability. [593]

Exposure for 11 days to concentrations of neonicotinoids below one-tenth of a billionth of a gram per bee are capable of generating harmful effects (contact LD_{50} of imidacloprid, thiamethoxam and clothianidin range from 18 to 30 ng/bee). [595] These pesticides harm bees when administered orally or by wetting the thorax. One tenth of a billionth of a gram of thiamethoxam per bee (0.1 ng/bee) is capable of impairing olfactory memory and learning ability; it should be remembered that the negative effects of thiamethoxam are partly a consequence of its metabolite: clothianidin. [595]

Very low doses of clothianidin (a neonicotinoid) applied once (0.7 ng/bee) impair the ability of bees to return to the hive. [73] Smaller doses (0.47 ng/bee) allow bees to return but fail to resume their foraging activity for several hours. [73]

Some insecticides (e.g. the neonicotinoid thiamethoxam) at very low doses increase the bees' movements towards the light. [666]

Fipronil (phenylpyrazole insecticide) at a dose of one-tenth of a billionth of a gram per bee, administered by contact or orally, induces the death of 100% of the bees after one week of exposure (0.1 ng/bee corresponds to one-fiftieth of the dose capable of killing 50% of the exposed bees: this is the LD_{50}). [595] Following the administration of even lower doses (0.01 ng/bee, which is equivalent to one-fiftieth of the LD_{50}) the bees remain immobile for longer (motor skills are impaired), ingest more water, and reduce the proboscis extension reflex. [595] The ability of bees to discriminate between a known and unknown odour is also impaired. Very low concentrations can produce the same effect as higher concentrations, but in a slightly longer time, and fipronil, in this research, was found to be more toxic and dangerous than neonicotinoids. [595]

Behavioural effects can be investigated by using artificial flowers that produce an odour that is attractive to bees, which can provide sugary substances with or without pesticides (in the laboratory). *Bacillus thuringiensis* toxin (Cry1Ac protoxin) reduces the number of visits to the flowers (in a unit of time). [593] This toxin is produced in genetically modified plants such as maize and is found in pollen at high concentrations. [658]

The herbicide glyphosate reduces bees' ability to fly but also sugar sensitivity (the ability to sense nectar), and interferes with learning abilities. [552, 718] Exposure to glyphosate may disrupt the bees' ability to return to the hive. [552]

Organophosphates generate damage to nerve transmission which can manifest itself in reduced ability to learn and memorize, and in problems with muscle contraction.
In bees, amitraz, formetanate and chlordimeform suppress foraging at very low doses. [482]
Alteration of the ability to communicate, such as impairment of the ability to locate food, has been observed in the case of organophosphates such as parathion, and neonicotinoids such as imidacloprid. [482] Since 1970, it has been known that exposure to parathion may promote an alteration of the dance behaviour that bees perform to communicate the location of the nectar and pollen source. [682]

The sub-lethal effects of pesticides, whether on individuals or the entire colony, can be devastating. Some of the limitations of laboratory studies are that one (pure) pesticide is used at a time, toxicity tests are carried out for only a few hours, and concentrations much higher than the most likely actual exposure are used. In bees the eyes are made up of thousands of units called ommatidia and are able to perceive certain colours. Photoreceptor proteins important for vision include opsin and rhodopsin. Bees actually have different types of opsin and rhodopsin that are specialized for seeing different wavelengths. So the proper expression of certain proteins is essential for insect vision. A study conducted in India, between the months of May and June 2014, measured the effects of some pesticides (endosulfan, cypermethrin, dimethoate) on the visual ability of bees (*Apis cerana*). [1228] The researchers reveal that exposure to pesticides generates several alterations:

- the ability to choose colours (e.g. blue) is impaired;
- the structure of the ommatidia changes (electron microscope observations);
- the expression of proteins essential for vision such as opsin and rhodopsin is reduced.

These results show that pesticides can impair one of the most important functions for bee survival: vision. [1228] Therefore, insecticides can impair many abilities such as colour choice, orientation and flight, contributing to lethal effects on the entire colony.

OTHER BEHAVIOURAL ALTERATIONS

Other adverse effects recorded in honey bees following exposure to certain pesticides are described hereafter in summary.

- Neurotoxic effects occur with erratic movements, tremors and paralysis following contamination by organochlorine and pyrethroid insecticides. [55]
- Aggressive behaviour is favoured by carbamate contamination.
- The presence of the insecticide chlorpyrifos has been associated with decreased appetite.
- Visual memory impairment was recorded for the neonicotinoid insecticides, fipronil and chlorpyrifos. [45]
- Alteration of the ability to regurgitate has been associated with the exposure to organophosphates (bees appear wet). The important phenomenon of trophallaxis, in which bees exchange food by regurgitating it, must be remembered (bees exchange nectar and enrich it with invertase: this enzyme, in an acid environment, breaks down sucrose into its two components, glucose and fructose).
- Pyrethroid insecticides (permethrin) generate an increase in time spent on self-cleaning and a reduction in time spent on activities such as trophallaxis (feeding capacity is altered).
- Exposure to certain organophosphate insecticides promotes lethargy, that is, a state of muscle relaxation and reduced sensitivity.
- Hypothermia has been detected following contact exposure to pyrethroid insecticides (deltamethrin). [193] Some fungicides also manifest these effects, but if bees are exposed simultaneously to insecticides (deltamethrin) and fungicides (prochloraz) hypothermia and an impaired thermoregulatory capacity occur at lower doses. [589, 592, 599, 866]

IMMUNE AND ENDOCRINE PROBLEMS

Very dangerous sub-lethal effects are those that can generate impairment of the ability to defend oneself and alteration of the delicate hormonal balances that regulate vital functions.

- Impairment of immunity - i.e. increased susceptibility to parasites such as bacteria, viruses, mites and fungi - has been observed due to exposure to the insecticide chlorpyrifos and the metals zinc, lead, and cadmium. [47] Cadmium may block calcium channels and damage honey bee muscles. [107] Reduced immunity may also be generated due to altered gene expression resulting from the presence of clothianidin. [483]
- Some pesticides, such as the fungicides prochloraz, triflumizole and propiconazole (they are ergosterol inhibitors), inhibit the bees' immune defences: they reduce the activity of the detoxification system consisting of the cytochrome P450 enzyme complex. [483]
- Reduction in the ability to detoxify some hazardous substances has been reported following exposure to coumaphos, thymol, and formic acid. [483]
- Azole compounds are fungicides used in agriculture and pharmaceuticals as anti-fungal agents. They are, for example, fluconazole, clotrimazole, propiconazole, and tebuconazole. These fungicides are considered to be endocrine disruptors because, for example, they disrupt the synthesis of steroid hormones in humans and animals; in bees they inhibit enzymes such as *CYP19 aromatase* and ergosterol synthesis. [46]

ADVERSE EFFECTS ON REPRODUCTION

Many pesticides, including acaricides used by beekeepers, can impair the reproductive ability of male honey bee colonies. Some insecticides (e.g. neonicotinoids) reduce the viability of the drones' spermatozoa. The reduction of reproductive capacity may, at least in part, explain the frequent need of beekeepers to replace the queen bee in their colonies, anticipating its natural death. Some alarming scientific evidence is summarized below, also because these effects could probably occur not only in bees but also in many organisms, including humans.

- Exposure to some molecules (e.g. thiamethoxam) causes a decrease in the number of eggs laid by the queen bee, resulting in colony collapse and/or killing of the queen bee by the worker bees. [35] The ovicidal effect in the ovaries of the queen bee and the larvicidal effect have been observed following exposure to diflubenzuron (which is an insecticide of the benzoylurea class). Some herbicides (2,4,5 T and 2,4D) also act as ovicides. [717]
- Neonicotinoids (e.g. thiamethoxam and clothianidin) result in reduced ovary size, reduced sperm count and reduced sperm viability in the queen bee spermatheca. [1179] Fipronil reduces sperm viability and sperm concentration.
- Pyrethroids (e.g. cypermethrin) alter egg composition (they lower the amount of vitellogenin) and reduce egg hatching rates (e.g. deltamethrin and bifenthrin). [717]
- Pyrethroid insecticides (deltamethrin and bifenthrin), at very low concentrations, reduce fertility in *Apis mellifera ligustica* and cause a prolongation of the time required for its development. [691] Deltamethrin also generates other sub-lethal effects such as forager paralysis, reduced foraging activities, reduced sperm viability, and altered orientation ability required to return to the hive. [45] Recommended doses of bifenthrin in the field (20-100 mg/L) generate a possible hazardous exposure, above the LD_{50}, and those recommended for deltamethrin (12.5-25 mg/L) are close to the LD_{50}. [691]
- Pyrethroids used by beekeepers against *Varroa*, such as tau-fluvalinate (detoxified by cytochrome P450), cause a reduction in size in queen bees, while males (drones) experience a reduction in their survival rate and produce fewer sperm. [602]
- Colonies exposed to phenoxycarb generate queen bees that do not produce eggs. [717]
- The fungicide chlortalonil reduces the size of queen bees, the number of worker bees, and the biomass of the entire colony. [718]
- Acaricides such as amitraz, coumaphos, and fluvalinate, as well as the pesticides chlorpyrophos and chlorthalonil, cause a drastic reduction in the number and viability of spermatozoa of honeybee drones and a near absence of motility. [991] Amitraz, formetanate, and chlordimeform can suppress reproduction. [482] Coumaphos reduces the weight of ovaries and queen bee.
- Acaricides authorized in organic beekeeping, such as oxalic acid, formic acid, also generate a reduction in sperm number and a reduction in wing length in male honey bees. [991]
- Queen bees and drones exposed to coumaphos and fluvalinate acaricides are smaller and have a minor sexual vigour. [54]
- Reduction in larval viability was recorded by administering some organophosphates such as parathion and methyl parathion.

LIFE CYCLE ALTERATION

Compromising the life cycle of bees is another important aspect as it has many negative implications.

- Reduced longevity has been observed following exposure to many different molecules: organophosphate insecticides, such as malathion and diazinon, carbaryl, and iprodione (a fungicide that can decrease bee life expectancy by 10 days). [39]
- Following the exposure to carbamate (carbaryl) and pyrethroid (resmethrin) insecticides, a variation in the age at which bees begin to become foragers was observed.
- Sub-lethal effects reported in honey bees following glyphosate exposure include an altered circadian rhythm (as well as impaired olfactory learning and reduced orientation skills). [86]
- The recycling of wax contaminated with low concentrations of pesticides showed sub-lethal effects such as reduced longevity and delayed larval development. [240] Pesticides present in the wax move into the other matrices of the hive and into the uncontaminated (new) wax, thus promoting the distribution of the molecules in the combs. Due to the use of pesticides in agriculture and beekeeping, wax may be simultaneously contaminated by several pesticides: in this research an average of 10 different active molecules is recorded, in different mixtures between 39 active molecules and 7 metabolites. [240] Among the most frequently found pesticides, there are the acaricides used by beekeepers, which can be found in wax at high concentrations: e.g. 22.1 µg/g for coumaphos.

PESTICIDES WITH AN ANTIMICROBIAL ACTION

Glyphosate is probably the most widely used herbicide in the World and also has an antimicrobial action on some microorganisms that have metabolic pathways similar to those of plants (it alters the synthesis of aromatic amino acids). The intestine of bees is mainly colonized by a few species of bacteria that are useful and necessary for the health of insects (e.g. bacteria of the genera *Lactobacillus* and *Bifidobacterium*). As in other animals, including humans, these gut microbes are useful for digestion and to counteract pathogenic microorganisms. The exposure of bees to environmental concentrations of glyphosate will reduce the abundance of gut microorganisms and increase mortality. [675] Bacterial strains that are resistant to glyphosate will be favoured: ultimately, some micro-organisms will be reduced in number and others will be favoured, similar to the effect of antibiotics. Exposure to glyphosate makes bees more susceptible to nutritional deficiencies and pathogens (thus also exerting negative effects in synergy with other factors). [675]

Bacteria in the digestive tract perform fundamental functions such as nutrition (e.g. pollen digestion), detoxification, and immunity. At least nine important bacterial species have been identified in the digestive tract of bees (*Firmicutes*, *Proteobacteria*, *Actinomycetes*). [705] Antibiotics have the ability to kill certain types of bacteria and have several undesirable effects such as the selection of resistant microorganisms. The administration of antibiotics to bees generates two other negative effects: it reduces the immune capacity, such as resistance to parasites such as the fungus *Nosema ceranea*, and it reduces the production of proteins with antibiotic action by the insect, as they cause immunosuppression (some proteins with this function are the following: abaecin, defensin1, and hymenoptaecin). [705] This observation confirms the knowledge that has been validated for decades also in humans, the administration of antibiotics enhances the pathogenicity of opportunistic parasites, making bees much more vulnerable. Bees will have a shorter life expectancy and pathogenic fungi will be present with a greater intensity. The presence of the endocellular parasitic fungus (*Nosema ceranea*) alone does not affect the microbial composition in the bee gut. Therefore, a synergistic effect between the health status of the gut microbial community of honey bees and a disease resistance is demonstrated. This phenomenon has also been observed in bumblebees, the fruit fly (*Drosophila melanogaster*), humans, and many other animals (e.g. mice). An interesting aspect is that bees exposed to antibiotics will have a shorter life expectancy than untreated bees, but the simultaneous presence of the fungus (*Nosema*) further reduces their survival.

The intestinal microbiome performs fundamental functions such as those for nutrition and defence against pathogens. Some microorganisms (*Acetobacteraceae*, *Lactobacillus* spp, *Bifidobacterium* spp, *Bartonella* spp, *Gilliamella* spp, *Snodgrassella* spp, *Frischella* spp) are involved in the digestion of monosaccharides, polysaccharides, and other important functions. The impairment of the intestinal flora affects the health of bees too: the ability to digest food or to defend themselves against diseases is reduced. Pesticides (e.g. glyphosate herbicides, organophosphates, neonicotinoids, and pyrethroids) can adversely affect the quality and quantity of the microbiome. [1229] This results in a reduction in the survivability of bees. Exposure for more than 5 weeks to very low concentrations of neonicotinoids, such as imidacloprid and thiacloprid, irreversibly alters the homeostasis of the microbiome. The abundance of some microorganisms (*Lactobacillus* spp and *Bifidobacterium* spp) in the intestines of honeybees decreases and their composition is altered. Neonicotinoid insecticides can affect microorganisms in several ways: [1229]

- It is known that some bacteria can use neonicotinoids to obtain carbon and energy, such as nitrogen-fixers (*Ensifer adhaerens* metabolizes thiamethoxam and *Pigmentiphaga* spp acetamiprid).
- In soil they can reduce the activity of microbial metabolism and proliferation (e.g. acetamiprid and imidacloprid).
- In the intestine, the modification of the microbial ecosystem, in favour of some species, generates imbalances in the immune defence capacity. In general, the intestinal microbial biodiversity of bees decreases following the exposure to these molecules, which will favour resistant microorganisms.
- Exposure of honey bees to thiacloprid may alter the composition of the intestinal microbial flora (e.g. *Lactobacillus* and *Bombella apis* decreases) as well as reduce body weight, sugar intake, and survival. [88] If the microbial flora is not irreversibly damaged it may recover over time, reaching concentrations prior to exposure to low doses of insecticides with bactericidal properties. [88]

These effects, at the colony level, may be devastating in the long run as well as altering the soil ecosystem.

OTHER ALTERATIONS

Bees may be exposed to very complex poison mixtures and synergistic effects may also be promoted by the presence of other factors such as certain parasites and malnutrition. We probably know only a minority of the possible effects; others are summarized below.

▪ Exposure to pyrethroids (cypermethrin) has been associated with changes in blood glucose and the activity of some enzymes (such as ATPases). [193]

▪ Deltamethrin and fipronil (at sub-lethal doses) alter the orientation ability during the flight of honeybees, which will more often move in the wrong direction, such as towards the sun rather than towards the hive. [589]

▪ Neonicotinoids (thiametoxam and imidacloprid) have been reported to damage intestinal cells and Malpighian tubules; their function is to free the haemolymph (in insects it is the equivalent of blood) especially from nitrogenous wastes that are discharged into the intestine. [193] Thiametoxam (insecticide) administered in the diet, at a dose equal to one tenth of the LD_{50}, causes problems to the intestinal cells, to the brain (morphological and histological damage to the important areas for vision and orientation), and reduces the life expectancy of the bees by 41%. [590] This neonicotinoid, at sub-lethal doses, reduces life expectancy, impairs orientation and learning ability, and reduces the sense of smell. Thiamethoxam, used in citrus fruits at permitted doses (in Brazil at a dose of 37.5 g of active ingredient in 100 L of water), kills 50% of honey bees in less than 214 minutes. [590]

▪ *Apis mellifera* and *Apis cerana*, exposed to sub-lethal doses of diflubenzuron (an insecticide of the benzophenylurea family), experienced weight reduction and development failure of the hypopharyngeal glands that produce the royal jelly necessary to feed the larvae and queen bee. [589] In nurse bees, exposure to low doses of imidacloprid also causes reduction of the hypopharyngeal glands. [35]

▪ Vitellogenin is a lipoprotein present in the haemolymph, it is involved in immune defence, reproduction, and is necessary to produce royal jelly. Vitellogenin is a protein with antioxidant properties and its presence in the body of bees is associated with longevity and good nutrition. [230] The concentration of this protein could be used to predict the ability of bees to survive the winter. Pyriproxyfen is a growth alterer that impairs the bees' ability to produce vitellogenin. [13]

▪ Amitraz induces cell apoptosis, also called programmed cell death. [482]

▪ In bees, the altered function of circulatory cells, with reduced frequency and force of contraction, has been associated with the presence of pyrethroids such as deltamethrin. [589]

▪ Some pesticides, such as thymol and menthol, used by beekeepers against mites (*Varroa*), are considered less toxic. In reality, these molecules can increase the mortality of the queen bee and promote the removal of larvae. [602]

▪ Oxalic acid and formic acid are considered low-toxic molecules to be used against mites parasitizing bees (e.g. *Varroa*): they may be found in small quantities in honey. Formic acid reduces life expectancy of bees and decreases the survival of larvae; oxalic acid can increase queen bee mortality and damage the brood. [602]

▪ During the first 3 days of life, honey bee larvae feed on royal jelly. Later, the larvae that will give rise to the worker bees will also receive pollen and honey, while those that will give rise to the queen bee will continue to receive abundant rations of royal jelly. Royal jelly consists of several nutrients such as proteins, carbohydrates, and lipids, including photosterols, which are important for the production of hormones and the formation of cell membranes. Bees that produce royal jelly must feed on pollen. It is generally believed that royal jelly, compared to pollen or honey, is much less contaminated with pesticides (according to the United States Environmental Protection Agency at least 100 times less). Indeed, labelled pesticides (carbofuran and dimethoate with the isotope 14 of the carbon atom) are mainly recorded in the digestive tract and are absent in the glands that produce royal jelly. [1202] However, even if they are absent in royal jelly, pesticides can generate indirect adverse effects such as the reduction of royal jelly-producing glands and a change in the composition of the royal jelly. Artificial exposure to pollen treated with a mixture of pesticides (acaricides, herbicides, fungicides, and insecticides) that reproduces the condition that may be recorded naturally generates variations in royal jelly production. Colonies fed with pollen enriched with pesticides (atrazine, azoxystrobin, carbaryl, chlorothalonil, chlorpyriphos, coumaphos, fenpropathrin, fluvalinate, and pendimethalin) were compared to others fed with unenriched pollen. [1202] After the addition of pesticides in pollen the following effects are recorded:

- a lower production of royal jelly;
- the variation in the composition of lipids and proteins such as those with antimicrobial action (at least 80 proteins have been identified in royal jelly including amylase, oxidase, defensin, 56 of which show significant variations compared to bees fed with pollen not enriched with pesticides).

These results show that even if pesticides are not measured in royal jelly or are present at lower concentrations than in pollen, there are still negative effects that affect the nutrition, mainly of queen bees. Indirect effects may be very dangerous too.

Studies based on laboratory tests have greatly contributed to the spread of the idea that fungicides are not dangerous for pollinating insects such as bees (*Apis mellifera ligustica*). As a result of this belief established over the years, fungicides may be distributed in crops during flowering. Therefore, pollinators are highly exposed to this category of molecules. Boscalid and pyraclostrobin are two fungicides used in almond cultivation in North America (they inhibit respiration in the mitochondria of fungi). Exposure through pollen (in North America in 2018) to very low concentrations of these two active molecules generates a reduced life expectancy of worker bees, a decreased colony size (21% fewer worker bees), an increased winter mortality (30%), and induces worker bees to become early foragers (bees may be exposed via food, during flowering, including through nectar): the exposure to the two fungicides increases the fraction of worker bees that collect pollen resulting in increased pollen collection. Some effects are measured at doses much lower than those causing the death of 15% of insects exposed under laboratory conditions (LD_{15}). In addition, reduction in the number of worker bees is also recorded at the dose equal to 10% of the lowest concentration recorded in pollen. [1231] The results show that the very low concentrations (boscalid between 1.5 and 6.1 ppm and pyraclostrobin between 0.4 to 1.7 ppm in pollen), to which bees can realistically be exposed in the field, cause significant effects on colony life. Therefore, fungicides can harm bees with significant sub-lethal effects and the possibility of using them during flowering should be reconsidered.

Other fungicides have also been reported to have negative effects on pollinators, such as chlorothalonil which causes a reduction in the generation of worker bees and the emergence of smaller queens in bumblebee colonies. [1231] Thus, fungicides may manifest direct adverse effects on pollinators by contributing to their accelerated decline.

Finally, the problems that can be generated by mutagenic substances are mentioned. The effects of genetic alterations may differ considerably depending on the type of mutation and the location where it occurs. A mutation may not lead to any consequences. If the mutation alters coding sequences, i.e. genes, there may be a visible change in the phenotype. Some pesticides are mutagens, i.e. they alter genes and therefore may change the phenotype. Also in insects, as in the human species, some pesticides favour the appearance of genetic defects and the alteration of genetic expression, such as that in the larvae of bees. [47, 54,184] An example is the appearance of bees with malformations due to the exposure to growth inhibitors such as pyriproxyfen: at a dose of 54 ng/larva, 20% of the bees show malformations. [13]

INFINETISAMAL QUANTITIES DAMAGE THE BUMBLEBEES

Some sub-lethal effects recorded in bumblebees are summarized below.

- Neonicotinoids can alter the thermoregulatory ability of the bumblebee (*Bombus impatiens*) colony and the ability to build and insulate the nest with wax (at doses of billionths of a gram per worker bee). [667]
- Also in bumblebees (*Bombus impatiens),* neonicotinoid insecticides (imidacloprid) alter the ability to modulate the immune response. [398]
- Doses of imidacloprid of 1 µg/L, i.e. 10 times lower than that which can be found in pollen or nectar, reduce larval production in bumblebees (*Bombus terrestris*) by one third. [418, 861]
- Imidacloprid (a neonicotinoid insecticide), at sub-lethal doses, in bumblebees (*Bombus terrestris*) reduces growth speed, impairs foraging ability, decreases the generation of new

queens by 85%, and the colonies experience a lower growth rate. [54, 368, 371, 442] Social interactions are also altered (*Bombus impatiens*). [665] Very low dose exposures can significantly affect the colony development of these pollinating insects.

- Imidacloprid reduces the ability of bumblebees to obtain food and pollen, and generates a reduction in the size and an alteration of the composition of the colony (e.g. it causes a change in the numerical proportions between different individuals, such as larvae and drones compared to other castes such as workers). Imidacloprid at concentrations of 0.7 ppb in sugar water and 6 ppb in pollen reduces the amount of pollen collected by bumblebees by 31%. [420]

- Imidacloprid has been used in many crops such as sunflowers, oilseed rape, maize, peas, beans, and orchards which are visited by bees, therefore it can be found in plant pollen at concentrations up to 10 ppb. [674] The artificial administration of imidacloprid to bumblebees (*Bombus terrestris*) at concentrations which may be found in pollen has resulted in the following sub-lethal effects: [674]

  - the colonies exposed to the insecticide, after 42 days, were populated by fewer bumblebees;
  - in colonies exposed to the insecticide, birth rates were lower, while mortality rates were higher (these signs become evident 3 weeks after initial exposure).

The negative effects on the survival capacity of the colony are not observed immediately after exposure, but several days later: in this case the effects are very evident since the third week. Doses that, in a few days or a few hours, do not allow us to record the increase of mortality, after more than three weeks are able to produce devastating effects on the functionality of the colony, compromising its survival. [674] These effects are visible after weeks and only at the level of the colony, therefore they cannot be measured by acute toxicity tests, which analyse mortality rates at doses that kill insects in less than two days and which are carried out on a few adult individuals. The situation is exacerbated when other factors such as flower shortage and the presence of disease are added.

Neonicotinoid insecticides are also very dangerous for bumblebees (*Bombus terrestris* and *Bombus impatiens*) as they can perform several negative actions such as:

- interactions with nicotinic acetylcholine receptors as they are agonists and are not inactivated by acetyl cholinesterase, so they generate a much longer ion channel stimulation;
- the alteration of DNA methylation;
- mitochondrial dysfunction;
- the variation of the expression of some genes (e.g. the production of some fatty acids and hormones is reduced);
- the reduction of immune defences;
- the inhibition of the ability to recognize sugar (the ability to recognize a food source is impaired);
- the alteration of the reproductive capacity (generating smaller oocytes and reducing the number of eggs produced);
- the impairment of the thermoregulatory capacity;
- the modification of food-seeking behaviour and orientation. [1233]

Therefore, neonicotinoids also have lethal and sub-lethal effects on bumblebees, which may compromise their ability to survive.

LOW-DOSE EXPOSURES IN BIOLOGICAL CONTROL INSECTS

Sub-lethal effects occur in reared bees but also in wild pollinators and in natural enemies; for example, negative consequences have been reported among Neuroptera of the family Chrysopidae such as *Chrysoperla carnea*. [625] This species is used in biological control because its larvae are active against aphids. Also in the case of the wolf spider (*Lycosidae*), which is a skilled ambush hunter, sub-lethal effects, i.e. at very low doses, have been recorded. [589, 626]

Insecticides used to kill aphids on which ladybirds feed generate malformations in adults that have been exposed in the larval stage. The same effect has also been recorded in other insects used in biological control such as *Chrysoperla carnea*. [589, 625]

Pesticides at sub-lethal doses can affect the reproduction of beneficial insects in several ways: chlorpyrifos alters the ratio of males to females in some hymenopterous insects that feed on other insects. In *Aphytis melinus* it causes a reduction in the number of females. *Aphytis melinus* is native to China and was introduced into Italy in the late sixties. It is a parasitoid of the red mealybug (*Aonidiella aurantii*), a parasite of citrus fruits. The adult is similar to a small yellow-orange wasp about 1 mm long. [632] The female, after mating, lays her eggs in the citrus mealybug. This mealybug (*Aonidiella aurantii*) is native to South-East Asia from where it has spread to all citrus-growing areas in the World. [638] The female *Aphytis melinus*, in addition to parasitizing its host, kills sometimes the mealybug with an ovipositor in order to feed on the hemolymph (which comes out of its body). The control of the red mealybug, by means of *Aphytis melinus*, is carried out by applying the principles of integrated pest control, which schedules the monitoring of the males of mealybug with special traps. Depending on the degree of infestation, several tens of thousands of individuals per hectare are distributed.

Following exposure to chlorpyrifos, the *Trichogramma brassicae* wasp (entomo-parasitoid hymenoptera), which is used in biological control, also generates progeny whose sex ratio is altered in favour of the females. [638, 654, 655] The males of the wasp *Trichogramma brassicae*, exposed to very small doses of chlorpyrifos, are less attracted to the hormone produced by the females (at the $LD_{0,1}$ or lethal dose for 0.1% of exposed insects). [589, 633] Thus the possibility of reproduction is altered for insects considered useful because they feed on plant parasites. This wasp is used to control the European corn borer i.e. the nocturnal moth *Ostrinia nubilalis*, as it lays its eggs in the eggs of the corn borer. [635] Even though it is called the corn borer, this moth (the caterpillar) is able to feed at the expense of at least 250 different plants including sorghum, pepper, eggplant, bean, and some ornamental flowering plants.

The *Trichogramma brassicae* wasp is also able to control other pests such as the cabbage moth (*Mamestra brassicae*), which is a nocturnal moth that can damage cauliflower, beet, tomato, pepper, potato, sunflower, tobacco, cereals, ornamental and pome plants. The insect can be bought in packs of hundreds of capsules, each of which contains 3,800 eggs that will generate 60% female individuals. [634, 636, 637]

Even some biological control systems can generate serious problems for beneficial insects. The wasp *Trichogramma platneri* is used in biological control all over the World as it is able to attack pests (e.g. eggs and larvae of moths) of crops such as apples, pears, grapevine, avocado, and is damaged by biological control systems such as soaps, oils and *Bacillus thuringiensis*. [652] *Bacillus thuringiensis* is a sporigenous bacterium that lives in the soil. The bacterium and its toxins can be distributed in organic farming to kill certain insects. When ingested by phytophagous insects, the bacterium sporulates in the host releasing the so-called Bt toxins or delta-endotoxins that are able to damage the digestive tract of the larvae of Diptera, such as mosquitoes, or to cause a paralytic disease in the caterpillars of many Lepidoptera (or butterflies and moths). [653] These biological toxins (they are proteins) are used in the formulation of insecticides for organic farming, but are also produced independently in the tissues of transgenic plants (such as MON 810 maize). If sugar is added to the commercial formulation before it is sprayed on the green parts of the plants, it is more attractive to the larvae. The toxins of *Bacillus thuringiensis* distributed on crops as well as killing the prey (eg. moths) of the wasp useful for biological control (*Trichogramma platneri*) also generate problems for the wasp itself, immobilizing it. [652] This wasp is sold in packages containing 100,000 eggs, to be used in crops for biological control.

The wasp *Aphidius uzbekistanicus* (Hymenoptera Aphidiidae) is used in biological control of cereal aphids. When adults are exposed to pyrethroid insecticides (deltamethrin and lambda-cyhalothrin) they generate a progeny with a reduced number of females. [638] It must be remembered that in many insects such as this one and bees, females are generated from fertilized eggs while males from unfertilized eggs (they are haploid). Some insecticides can alter the ability of fertile females that have mated to fertilize their eggs and produce other females. [589]

Pesticides can reduce the olfactory and orientation abilities important for reaching food. Therefore this ability can also be reduced with dramatic effects already at low concentrations of some active molecules. An example is the reduction in the ability find the honeydew of aphids on which a wasp (*Aphidius rhopalosiphi*) feeds following exposure to low concentrations of deltamethrin. Pesticides reduce the likelihood that predators (wasps parasitizing insects such as honeydew-producing aphids) will recognize odours that should attract them to their prey. [589] Also in the honey bee some pesticides (e.g. 1 ng of fipronil applied to the thorax of each honey bee) reduce the ability to recognize a sugar solution (measured by the proboscis extension reflex).

Some active molecules reduce the intensity with which parasitoid insects (e.g. *Aphidius ervi*) use their antennae to explore their prey, without reducing their mobility. This behaviour is important for the recognition of the prey before the laying of the eggs in the host. [589]

Azadirachtin A is extracted from the seeds of the Neem tree (*Azadirachta indica*) and is used in organic farming as an insecticide, acaricide, and nematicide. Exposure to azadirachtin A reduces the ability of some parasitoid wasps (e.g. *Hyposoter didymator*) to track down their prey. [589, 656, 657] Even wasps (e.g. *Hyposoter didymator*) exposed to azadirachtin A may move away from their prey, exactly the opposite of what they should do to feed.

Pesticides can also alter reproduction through another effect. Sub-lethal doses of some pesticides can compromise the capacity of some insects to oviposit, as happens in the case of the females of the wasp *Aphidius ervi,* when exposed to lambda-cyhalothrin at the LD_{20}, i.e. that which generates the death of 20% of the exposed insects. This is a hymenopteran (Braconidae) used in biological control as it is a common parasitoid of aphids of different genera. [589, 643] The impairment of the oviposition capacity of the *Trybliographa rapae* wasp has also been recorded.

Some insecticides can generate a reduction in developmental timing in females and an increase in this timing in males, i.e. they may show different effects depending on gender. This phenomenon has been recorded for the insecticide permethrin on some beneficial bugs (*Supputius cincticeps*), as they are predators of many plant pests. [589, 627]

Some pesticides such as phenoxycarb act as insect growth regulation hormones and also harm beneficial insects such as *Chrysoperla carnea*. [589]

Females of the parasitoid wasp *Neochrysocharis formosa* show a reduced oviposition and reproductive capacity when exposed to low doses of imidacloprid; in this insect, imidacloprid has a likely repellent effect too. [647, 648] This wasp is able to parasitize the eggs and larvae of more than 100 species belonging to at least 4 orders (beetles, diptera, hymenoptera, and lepidoptera such as the tomato moth or *Tuta absoluta,* which arrived in Italy from South America a few years ago).

The oviposition capacity of the wasp (parasitoid hymenoptera) *Colpoclypeus florus* is impaired by certain pesticides such as spinosad, whose active ingredient is derived from a soil bacterium (*Saccharopolyspora spinosa*). [649] This wasp attacks butterflies whose larvae affect fruit crops and grapevine (*Vitis vinifera*): it is able to parasitize the moth or vine leafroller tortrix (*Sparganothis pilleriana*) widespread in Eurasia. [589, 650, 651] The vine leafroller also attacks many other species such as chestnut (*Castanea sativa*), elderberry (*Sambucus nigra*), quince (*Cydonia oblonga*), potato (*Solanum tuberosum*), alfalfa (*Medicago sativa*), and beans (*Phaseolus vulgaris*). [650] Other pesticides, such as diflubenzuron used in apple orchards, also generate sub-lethal effects on this useful wasp. [652] Thus a wasp used to naturally counteract the growth of moths harmful to crops such as grapevines or apples is damaged by insecticides used to kill their prey.

The wasp *Microplitis croceipes*, which lays its eggs in the body of caterpillars of pests such as those of maize (*Helicoverpa zea*), when exposed to imidacloprid (a neonicotinoid insecticide) or aldicarb (carbamate), used in cotton crops, registers a reduction in the ability to respond to odours of 71% with the first and 62% with the second active ingredient.
Alteration of the sense of smell reduces the ability to orient themselves. This effect has been observed in some beneficial insects at sub-lethal doses of deltamethrin (*Trissolcus basalis*) and lambda-cyhalothrin. [589] The wasp *Trissolcus basalis* parasitizes the eggs of the green bug (*Nezara viridula*) which is a phytophagous insect. [640, 641] This bug has a distinct polyphagy at the expense of more than 30 plant families, both monocotyledons and dicotyledons. The green bugs spend the winter among the dry leaves, in the hedges, waiting for the spring climate to allow them to start their activities: they reproduce and lay their eggs by stinging the host plants. The green bug is probably native to Ethiopia and is now widespread throughout the World. As in the generality of Heteroptera, this insect emits a rather annoying odour so that predators do not approach it. It is also for this reason that its predators attack its eggs rather than the adults. The *Trissolcus basalis* wasp has the ability to limit the damage generated by the bug but this ability is compromised by sub-lethal doses of various pesticides. [589]

The monarch butterfly (*Danaus plexippus*), famous in North America for its long migrations (even over 2,000 km), and the Painted Lady butterfly (*Vanessa cardui*), also known for its migratory talents (from North Africa to England), are harmed by low concentrations of imidacloprid in nectar (between 29 and 54 µg/kg). [697]

Sub-lethal effects recorded on some beneficial insects include a reduction in the intensity with which they attack their prey. The insect *Acanthaspis pedestris* (a heteropterous hemipteran) exposed to cypermethrin shows a reduced attraction to prey: its hunting abilities are reduced and sexual communication important for reproduction is compromised. A parasitoid wasp (*Diaeretiella rapae*) also becomes less efficient in predation following exposure (e.g. it will reduce the number of attempts at oviposition). [589, 646]

Unfortunately, many pesticides (e.g. carbaryl, cyfluthrin, deltamethrin, endosulfan, baytroid, sevin) do not have a repellent effect so bees do not avoid entering treated fields. [193] Some pesticides (e.g. fipronil) reduce the frequency of flower visits by pollinators such as honeybees. [589] In some cases, however, pesticides also have a repellent effect (e.g. pyrethroids and dimethoate) on insects that eat other insects such as the seven-spot ladybird *Coccinella septempunctata.* Beneficial insects, in this case, will not head for food, i.e. the insects they are supposed to be feeding on (if they have not been killed by poisoning). Therefore they will feed much less on their prey (e.g. aphids) or even regurgitate them because they have become poisonous. Between 53% and 80% of *Nebria brevicollis* beetles that have eaten aphids on plants treated with deltamethrin regurgitate them. The common ladybird (*Coccinella septempunctata*) is an insect in which both larvae and adults are predators of aphids or other small insects. Organic farming uses ladybirds in the biological control of aphid infestations, resulting in a significant reduction in pesticide use (eggs and larvae can be purchased online).

Pesticides at sub-lethal doses can alter the ability of beneficial insects to move. Some insects, when irritated by the presence of insecticides, walk and attend to each other more frequently (chemioreceptors on the outside of the body are probably stimulated). This behaviour has been observed in the ladybird (*Coccinella septempunctata*) following the exposure to deltamethrin. [589, 628]

The insecticides monocrotophos, dimethoate, methyl parathion or quinalphos, at sub-lethal doses, generate abnormal development of the digestive tract, testes and ovaries of another useful insect, as it preys on insect larvae: *Rhynocoris kumari*. [589, 629] In this insect, monocrotophos and methyl parathion, at a dose of one tenth of that capable of killing 50% of the exposed animals, generate a reduction in the capacity of the immune defences (they decrease the number of plasma cells in the haemolymph).

The insecticides dieldrin and endosulfan, applied at a dose that kills 30% of fruit flies (*Drosophila melanogaster*), reduce immune defenses against the larvae of a parasite (the parasitoid wasp *Leptopilina boulardi*). [589, 630, 631]

It was observed that a solitary bee (*Megachile rotundata*) exposed to doses of pyrethroids equal to the LD_{10} reduced the number of eggs by 20%. [691]

CONSIDERATIONS FOR SUB-LETHAL EFFECTS

These observations allow another reflection: reared honey bees are not a good bio-indicator of the negative effects of pesticides on beneficial insects such as solitary bees, butterflies or insect predators harmful to farmers. In honey bees, some effects, such as those on reproduction, may be mitigated by the fact that the queen bee remains within the colony, protected by thousands of bees forming a kind of protective filter. Furthermore, a queen can be easily replaced by purchasing it from the other side of the World. Honey bees can also travel by plane and have an ally who makes all the difference, the beekeeper.

In general, social insects are more resistant to some negative effects of pesticides than solitary insects. In the case of neonicotinoids, colonies of honeybees (more than 10,000 individuals) were less susceptible to low doses than colonies of bumblebees, which in turn were more resistant than solitary bees. Solitary bees showed the most severe effects at the same doses of neonicotinoids. In addition, in the same honey bee or bumblebee colonies, identical sub-lethal doses of insecticides lead to greater adverse effects when the colony size is reduced. [665] Thus, it is shown that an increasing colony size has a protective effect in social insects and that the same doses are more damaging to smaller colonies and even more damaging to solitary insects.

The effects of pesticides on insects, in addition to depending on factors such as genetic and environmental ones (temperature, mode of distribution of toxic molecules, etc.), depend also on the age of the bees. It has been demonstrated that the sensitivity of bees to a pyrethroid insecticide called cypermethrin increases with age: the LD_{50} varies 2-fold with age. The type of distribution and the formulation can also greatly influence the hazard of the active ingredient. For example, methyl-parathion distributed as a micro-encapsulated formulation, i.e. in tiny plastic (e.g. nylon) or rubber granules (with a size of 20-40 µm, i.e. the size of pollen grains), can be toxic in the hive even 27 months after its last use. [35, 974] Pesticides distributed inside these micro-containers (e.g. nylon capsules) are released very slowly and therefore persist longer. When these microcapsules are distributed during flowering, they can be collected as if they were pollen. [974]

Several negative effects have been presented that manifest themselves in different ways, such as the generation of diseased insects destined to die, the reduction of the capacity to procure food, the reduction of fecundity, the alteration of the capacity of communication necessary for reproduction, the alteration of the numerical proportions between the sexes, the reduction of the sense of smell, the inability to orient themselves, the reduction of mobility and of the capacity to fly, the reduction of the ability to search for prey and to hunt, and the reduction of the capacity of communication; this review summarizes what we know, but the effects we do not yet know may be much more troubling. Each of these alterations is capable of generating effects equal to or greater than those measured with concentrations capable of killing insects in a few hours, the difference being that they are measured over longer timescales: days, weeks or months and at much lower concentrations. Low doses of pesticides compromise the survival capacity of insects, affecting their health and killing them slowly.

PESTICIDES USED IN ANIMAL HUSBANDRY

In veterinary medicine some pesticides, such as pyrethroid insecticides and organophosphates, are used to control parasites such as scabies or vectors such as flies. The active molecules are distributed on manure, in the premises or on animals through showers. Some of these compounds may be eliminated in livestock manure and contaminate the environment (e.g. water).

Beekeepers in the south of France, at the beginning of the winter 2008-2009, recorded the death of more than 4,000 colonies located in the mountains, far from cultivated fields but near sheep and cattle farms where compulsory veterinary treatments were carried out (e.g. insecticides to fight disease vectors such as bluetongue virus). Pyrethroids (permethrin and deltamethrin) used to control insect vectors of parasites (bluetongue virus) were recorded in dead bees. [1172]

A number of insecticides are authorized in France in the livestock sector, using mainly the following substances: amitraz, cypermethrin, deltamethrin, dicyclanil, dimpylate, fenvalerate, flumethrin, phoxime. In addition, medicines against parasites are used which may contain the following molecules: ivermectin, doramectin, éprinomectin, moxidectin, clorsulone, closantel, praziquantel, triclabendazole. Several anthelmintics are used and may contain: albendazole, closantel, febantel, fenbendazole, levamisole, netobimine, nitroxinil, oxfendazole, oxyclozanide, piperazine, praziquantel, pyrantel, and triclabendazole. [1172] Some of these substances are known to have toxic effects on bees: cypermethrin, deltamethrin, flumethrin, ivermectin, and moxidectin. Others are known to have adverse effects on coprophagous insects and aquatic organisms.

Livestock manure is treated with insecticides (larvicides such as cyromazine and diflubenzuron) against fly larvae (*Musca domestica*). Manure is used to fertilize plants and, in this way, the dangerous substances it contains are also distributed.

The treatment of sheep, cattle or pigs with ivermectin (a molecule toxic to bees) results in high concentrations of this substance in faeces (one week after subcutaneous injection). [1172] Cypermethrin can also be found in high concentrations in the faeces of treated cattle, three months after administration. Some of the substances used and their metabolites can degrade very slowly in the environment: 217 days in soil for ivermectin. Cattle manure containing ivermectin takes much longer to degrade: 340 days instead of 80 days. Thus, the rate of decomposition of organic matter is slowed down. Negative effects on coprophagous insects are also recorded, as is reasonable to expect from insecticidal molecules.

Bees need several dozen litres of water per year (between 30 and 70 L). They can find it within a radius of about one kilometre from the site of their colonies. It has long been known that bees prefer water sources rich in sodium, ammonium and magnesium, as in the case of puddles filled with decaying organic matter, such as sewage and slurry. Thus, water contaminated with insecticides and chemicals is used by bees, which can also drink directly from the droppings.

Substances used in veterinary medicine are added to the molecules distributed to treat plant diseases. Therefore, potential synergistic and additive effects, largely unknown, are favoured. As a proof of the existence of these risks, in the winter of 2013-2014, French beekeepers recorded an increase in the mortality of colonies where substances such as: neonicotinoids (used in agriculture) and pyrethroids (used in agriculture and also animal farming such as cypermethrin) were found.
Sub-lethal effects recorded from very low doses of some of these substances are:

- the reduction in the olfactory memory due to the exposure to ivermectin;
- the loss of orientation and reduced fertility due to the exposure to deltamethrin.

In conclusion, the risks to beneficial insects from the exposure to veterinary medicines and in synergy with pesticides used in agriculture are largely unknown but predictably negative. We are certain that some of the substances used by farmers are very toxic to insects such as bees and persist in the environment posing a real danger.

GENETICALLY MODIFIED PLANTS WITH AN INSECTICIDAL ACTION

GMP (genetically modified plants) have been modified by making them capable of producing toxins. The ability to produce poisons is introduced in the form of genes, so that the plant becomes poisonous on its own, reducing the need to distribute insecticides. As with pesticides distributed with sprayers in the field, insects have become resistant to these self-produced toxins from artificially modified plants (they are called transgenic plants). These toxins, which are proteins chemically, may be found in pollen, even if at very low concentrations (<90 ng/mg). [107]

Bacillus thuringiensis is a bacterium that lives naturally in the soil and is able to produce various proteinic toxins that can be used as insecticides (e.g. against lepidopterans and beetles). [159] Thanks to biotechnology, the toxins can be purchased and used as insecticides in organic farming.
Genes conferring the ability to produce *Bacillus thuringiensis* toxins have been artificially introduced into cotton (*Gossypium hirsutum*), maize (*Zea mays*), potato (*Solanum tuberosum*), and tomato (*Lycopersicon esculentum*): already in 2008, these genetic modifications were tested in field experiments on 30 different plant species. [602]
Bacillus thuringiensis toxins present in genetically modified plants (e.g. in pollen and nectar) could harm bees and be ingested by humans through honey. [107] These bacteria live in the soil and produce proteins (crystalline δ-endotoxins or Cry) which, in the intestines of certain insects, generate molecules capable of killing them. One of these proteins has been used to control a moth, the bee wax moth (*Galleria mellonella* or greater wax moth). [107, 158] Therefore, these proteins have also been used to control a moth that harms beekeepers.

It would be necessary to have more information on the possible alterations in the composition of nectar and pollen consequent to genetic modifications or, however, generated by artificial selection. Nectar does not contain, or contains very low concentrations of proteins unlike pollen. It might be useful to have more information on changes in the quality and quantity of pollen and nectar, and on changes in the composition of volatile substances attractive to pollinating insects.
Due to the spread of plants genetically modified to be resistant to herbicide phytotoxicity, the amount distributed in agriculture that may generate adverse effects is increasing. It might be interesting to have more information on the likely transfer of genes for herbicide resistance or other modifications to microorganisms (bacteria and yeasts) in the gut of bees. A particularly interesting topic in the field of public health is that of antibiotic resistance in the gut bacteria of

bees (as in human intestinal microorganisms and those of farmed animals such as cattle, pigs, and chickens). Little information exists on these topics. These are very interesting areas of research that could demonstrate the existence of risks that are currently underestimated or unknown.

UNAVOIDABLE MULTIPLE EXPOSURES

Bees may come into contact with pesticides used by beekeepers (e.g. acaricides against *Varroa*) or with pesticides distributed by farmers, breeders, and in urban environments (e.g. to control weeds). Assuming that during each flight a bee may come into contact with a plant surface of 5 cm^2, and that each bee makes 5 flights per day, the foragers will come into contact with a surface of 25 cm^2 per day. Multiplying this surface by the number of foraging bees in flight, which may be equal to one third of all the worker bees present, we obtain an estimate of the plant surface they could come into contact with: more than 33 m^2 per day; therefore, the passive exposure to pollutants (e.g. products distributed on plant leaves) may be considerable. [13] Considering that bees easily move in a radius of at least 1.5 km around the hive means that they will be able to visit an area of a few square kilometres (in reality they can move more than 10 km, in an area of 100 km^2). [184] The area of the average farm in Europe is about 0.16 km^2 so bees may be exposed to pesticides used in hundreds of different farms and crops at the same time. [32] In addition, beekeepers are forced into nomadism because of intensive monoculture. So bees are inevitably exposed to complex and unpredictable mixtures.

Beekeeping products may contain various pollutants at various concentrations that can be exchanged through diffusion within the hive. Fat-soluble pesticides (such as bromopropylate, coumaphos, t-fluvalinate) accumulate in the wax, where they can be found after years, while water-soluble molecules can be found in honey (formic acid, oxalic acid, and cymiazole) and can also alter its taste. The larvae stay 21 days in the cells, so they can passively absorb dangerous substances present in the wax: coumaphos (an acaricide used by beekeepers) can be transferred from wax to honey in less than 30 days. [13]

There ia huge evidence of chronic exposure to mixtures consisting of many different substances, not just pesticides:

- More than 150 different pesticides have been found in samples obtained from apiaries around the World. [41]
- Another scientific work shows that 161 pesticides have been found in beehives worldwide, of which 124 in pollen, 95 in wax, and 77 in honey or nectar; the most frequent are insecticides (83 active molecules), followed by fungicides (40 active molecules), herbicides (27 active molecules) and acaricides (10 active molecules). [45]
- More than 120 different pesticide molecules can be found in hive products: an average of 6 molecules per sample. [602]
- Forty-six different pesticides may be found in pollen samples of the 171 searched for in 108 samples (in the USA in 2007). [603] Some of the pesticides found most frequently were (in descending order): insecticides, fungicides, and herbicides. The most frequently found molecules were: fluvalinate (in more than 70 pollen samples), coumaphos, chlorpyrifos, endosulfan, and atrazine (in more than 20 samples). [603] In pollen, insecticides are found together with fungicides. In wax, fluvalinate, coumaphos, and chlorpyrifos are the molecules found most frequently: the first two in 100% of the samples. These data confirm how systematic and serious is the contamination of important matrices for bees.
- Another study goes so far as to determine 17 molecules of different pesticides in the same pollen sample. [603]

- In the 887 pollen, wax, and bee samples tested in North America, an average of 6.5 different pesticide molecules are found per sample: a total of 121 different pesticides are found (of the 200 searched). [597]
- Ninety percent of the commercial pollination service in agriculture is obtained from honey bees, which are suffering from an alarming increase in mortality. In North America, pesticides are found in beekeeping products at high concentrations and up to 39 residues simultaneously with an average of 8 active molecules per wax sample. [1203] Among the molecules most frequently found in wax are fluvalinate, coumaphos, coumaphos oxon (a metabolite of coumaphos), and chlorpyrifos. The first three residues are a consequence of the actions taken by beekeepers to control the parasitic mite *Varroa destructor*. Bees are exposed to very dangerous complex mixtures as 87 active molecules can be found in wax (out of 121 searched) and 98 in pollen (with an average of 7 active molecules per sample and up to 31 molecules in pollen simultaneously).

In Italy, 130 molecules of pesticides, marketed in at least 1,280 products, are most frequently used (in fact, more than 400 molecules of active molecules are authorized, which are marketed in a mixture with hundreds of different substances on which little information is available). [32] The situation becomes more complicated if we consider derivatives such as metabolites of active molecules and substances used in the commercial mixture (e.g. adjuvants that have different functions such as to facilitate the dispersion of active molecules). There are at least 15 metabolites that have certainly caused negative effects, among which aldicarb sulfoxide, endosulfan sulfate, fipronil sulfone, 5-hydroxy-imidacloprid, DDE, and omethoate. [45]

The synergistic effects generated by these mixtures are mostly unknown, but the limited information available suggests harmful effects on non-target organisms, such as wild pollinators. Most of these molecules are found at concentrations lower than the dose capable of killing 50% of adult bees, but it must be remembered that pollen is the main nutrient of juvenile bees and sub-lethal and synergistic effects can be as devastating, or more so, than those measured in a few hours. Exposure to complex and hazardous mixtures will last a lifetime. This condition of risk should cause the regulation of the use of hazardous molecules to be reviewed, as the effects are difficult to measure in laboratory tests.

Unfortunately, wild pollinators are in decline and one of the most important causes are pesticides such as insecticides. Among the most dangerous and at the same time most used molecules on the Planet there are the neonicotinoid insecticides that are authorized on more than 140 crops in the World. They are persistent and water-soluble, so they can easily move away from the site where they are used. Insects can make long migrations (e.g. the monarch butterfly, *Danaus plexippus*) and be exposed to dozens of different molecules. Others, such as some solitary bees, are unlikely to move more than one kilometre far.

Research was conducted in North America (Missouri) in 2016 and 2017 to assess the contamination of wild bees, butterflies, and queen bumblebees. [1225] Live insects were collected in areas characterized by having a portion of the area in a natural condition near cultivated fields. These are therefore habitats with a high level of diversification. The predominant crops are maize (*Zea mays*) and soybean (*Glycine*), with seeds coated with neonicotinoids such as imidacloprid and clothianidin. In 2016, 101 wild bees (including 16 queen bumblebees), 7 caterpillars, 62 butterflies and moths were collected from four areas; in 2017, 467 wild bees (including 16 queen bumblebees belonging to 5 different species) were collected from the same 4 sites as in 2016 and one more area. Bigger insects such as bumblebees can be analysed individually while multiple individuals (of the same species and from the same site) are used for smaller ones. Overall, 90 wild insect samples could be obtained in which 168 pesticides and degradation products were searched for. [1225] Before being used for analysis, the insects were cleaned of the pollen on their bodies. In the 43 wild bee samples, 9 pesticides were detected:

metolachlor (in 19% of the 43 samples), flumetralin (16%), atrazine (14%), tebuconazole (12%), tebupirimfos (9.3%), bifenthrin (5%), fludioxonil (5%), oxadiazon (2%), and imidacloprid (2%); herbicides (in 23% of the samples), fungicides (16%) and insecticides (14%). Seventy-nine percent of bee samples record one active ingredient, 16% two and 5% three: tebuconazole was found in 100% of positive samples and atrazine in 80%. Many of the wild bees captured in this research build their nests in the soil and can therefore be easily contaminated by the active molecules found in the soil. In fact, 75% of the bees recording tebupirimphos build their nests in the soil (e.g. *Melissodes*). [1225] Forty-four percent of the 23 butterfly samples contained no pesticides, but in the others, 8 pesticides were recorded: 35% contained herbicides, 30% insecticides and 17% fungicides. The molecules found in the positive butterfly samples were: metolachlor (26%), bifenthrin (22%), p,p′-DDD (22%), imidacloprid desnitro (17%), atrazine (17%), tebuconazole (13%), azoxystrobin (4%) and p,p′-DDT (4%). 74% of the 24 samples of queen bumblebees recorded the presence of at least one pesticide: 25% contained 3 active molecules. The molecules recorded were: tebuconazole (46%), desnitro-imidacloprid (33%), metalochlor (33%), atrazine (25%), flutriafol (13%), cyproconazole (8%), fludioxonil (8%), oxadiazon (4%), and bifenthrin (4%). [1225]

The results of this research confirm that wild pollinators are exposed to mixtures of poisonous substances, some of which are much more toxic than others, such as the organophosphate insecticide bifenthrin, the organochlorine degradation product p,p′-DDT and imidacloprid. The last one was detected at the highest concentration of 22.5 ng/g (in the honeybee *Melissodes*), while bifenthrin was detected at the highest concentration of 32.8 ng/g. These concentrations are higher than the concentrations that are likely to cause adverse effects.

INTRODUCTION TO SYNERGISTIC EFFECTS

A major problem is the simultaneous exposure to hundreds of molecules and their derivatives such as metabolites. [375] Substances other than the active substance contained in the commercial formulation may also generate dangerous and largely unknown effects (e.g. surfactants, stabilising agents, dispersants, adjuvants). [717] Surfactants, which are capable of destroying the outer layer of the insect's body (the cuticle forming the exoskeleton), may be added to the mixtures with which pesticides are marketed. In most cases we do not know what kind of interactions may arise from lifetime exposure to very heterogeneous and variable mixtures of compounds.

Synergistic effects are nothing more than negative consequences greater than those that would be caused by the sum of the effects recorded for the individual molecules: the negative effect of the mixture is much greater than the sum.

It is difficult and expensive to try to assess the synergistic toxicity generated by the simultaneous presence of 2 or 6 or more molecules: in England, in 2015, an average of three active molecules (2.6) per hectare were distributed with each single distribution (aerosol generated with the sprayer). [474] It is unrealistic to think of comparing the effects of combinations derived from the thousands of pesticide molecules, and their additives and adjuvants, to which living beings, including bees and humans, may be exposed.

The toxicological evaluation of a single toxin does not represent reality, as one may be exposed to hundreds of different molecules at the same time. Preliminary studies, mainly managed by the pesticide companies, assess the acute toxicity of one (pure) substance at a time. Inside the hive, 121 different pesticides may be found, some of which are used by beekeepers. [473] If one wanted to try laboratory tests to measure the synergistic effects of 121 active molecules, evaluating them two at a time, it would be necessary to try several thousand different combinations; this is a considerable investment of time and resources. In any case, an evaluation would still be incomplete, since the number of substances with which insects come into contact at the same time is more than two, and there are many more than 121.

Some researches have tried to test binary combinations and have measured several synergistic effects. Imidacloprid is one of the most widely used insecticides in the World: soybean and cotton are probably the crops in which it is used to a greater extent; in these crops as many as 17 pesticide distributions are made per year. It has been demonstrated that there are synergistic effects and, therefore, an increase in bee mortality following the exposure to imidacloprid and one of the following active molecules: tetraconazole, sulfoxaflor, oxamyl (in *Apis mellifera*). [675] In practice, an increase in mortality greater than the sum of the mortalities generated by the exposure to the single active molecules at the same doses is recorded. The coexistence of imidacloprid and one of the three molecules mentioned above leads to an increase in mortality, measured in 48 hours, between 15% and 26%.

The simultaneous administration of 8 pesticides [imidacloprid (insecticide), tetraconazole (fungicide), sulfoxaflor (insecticide), oxamyl (carbamate insecticide), acephate (organophosphate insecticide), glyphosate (herbicide), clothianidin (insecticide), λ-cyhalothrin (pyrethroid insecticide)] kills all exposed bees within 48 hours: each active ingredient is used at the concentration equivalent to the lethal dose for 20% of exposed individuals (LD_{20}). [675]

The inhibition of the detoxification system (cytochrome P450 inhibited with piperonyl butuoxide) increases the toxicity of imidacloprid by 5.2-fold: tetraconazole has this effect. Piperonyl butoxide is used as a synergist in combination with insecticides such as pyrethrins. [230, 236, 675] The simultaneous presence of these molecules also potentiates the effects of pyrethroids.

Synergistic effects may occur due to the alteration of fundamental physiological functions: some fungicides (thiophanate-methyl) and insecticides (the neonicotinoid imidacloprid) decrease the ability of bees to produce energy. [237] Another synergistic mechanism is due to molecules that alter the transport mechanisms of pesticides within the insect body. [473]

The synergies between hundreds of pesticide combinations are largely unknown but certainly negative. The chronological order of exposure may have different effects: exposure to sub-lethal doses first to tau-fluvalinate or coumaphos and then to amitraz does not (in laboratory studies) result in increased amitraz toxicity. On the contrary, if bees are exposed first to amitraz and then to tau-fluvalinate or coumaphos, the toxicity of amitraz increases. [1203] Thus, time succession may also influence, in different ways, the adverse effects at very low doses.

For most toxic molecules such as pesticides, the metabolic sites where synergistic effects are generated have yet to be discovered and the situation is worsened by the fact that new molecules and usage strategies are continuously being produced. In this regard, it is possible to highlight that little information is available on the use of nano particles (they may be the size of microorganisms), which are used to facilitate the dispersion and persistence of active molecules.

Synergistic effects may also occur between xenobiotics and substances naturally present in flowers. Plants can produce bee attractants such as quercetin or bee repellents such as quinine, an alkaloid produced by plants of the genus *Cinchona*, or caffeine produced by plants of the genus *Rutaceae* and *Rubiaceae* (caffeine can improve the memory of bees). [718] Quercetin stimulates the germination of pollen in many plants. This molecule may be found in honey, pollen, propolis, and it has been discovered that in bees it favours the activation of 12 genes, which increase the production of protein defence systems such as cytochrome P450. Thus, natural substances such as quercetin can increase the ability to metabolize and inactivate certain pesticides. [718] A deficiency in the intake of these substances, such as that generated by the use of herbicides, may easily lead to negative effects, as they reduce the number and diversity of flowers.

Herbicides and fungicides are molecules that are not used to kill insects and usually the acute toxicity measured for bees in the laboratory is low. [107] Also for these reasons herbicides and fungicides have no restrictions (e.g. on labelling) on reducing bee exposure. This means that insects may be exposed to high concentrations during flowering. For some herbicides a strong effect of reduced life expectancy and increased mortality of exposed colonies has been demonstrated (e.g. paraquat, an herbicide banned in Europe where, however, it is produced). [107, 160]

Some factors, which can enhance the adverse effects of pesticides and are difficult to assess preventively, are listed below.

- The variation of toxicity to different environmental conditions such as humidity, wind, rainfall, and temperature. Environmental conditions also affect persistence and dispersion.
- The method of distribution can greatly influence effectiveness:
    - The same amount of active substance can be distributed in different ways and in different dilutions (e.g. in 100 L of water or in a few litres of solvent), generating different consequences.
    - The atomization system can generate droplets of different sizes even up to 100 times. In some cases, pesticides are distributed inside micro-particles (e.g. plastic).
- The different genetic susceptibility of bees or of their different stages: larvae and adults suffer different effects following exposure to the same doses.
- The spread of pathogens: for example, pollen can carry some viruses and the *Nosema* fungus and, also for this reason, in laboratory tests, it can be replaced by feed sources of proteins (e.g. yeast extract).
- The insect resistance to disturbing factors is influenced by many factors such as malnutrition (e.g. pollen deficiency), the presence of parasites such as the *Nosema* fungus, and the inhibition of immune defenses or detoxification systems.
- The density of apiaries in the area which may favour the spread of diseases and erode local genetic diversity.
- The simultaneous presence of several active molecules, such as amitraz and pyrethroids, may lead to additive and synergistic effects. [107] Synergistic effects have been reported between tau-fluvalinate insecticides and organophosphates such as coumaphos, between neonicotinoids and fungicides, and between neonicotinoids and pyrethroids. [474] It is known that synergism exists between fungicides, which are usually considered to be of low toxicity to bees, and neonicotinoid insecticides and pyrethroids. In this regard, it is useful to recall a figure already highlighted: in Italy, on average in 2014, more than 5 kg of active molecules were used per hectare (in Spain more than 3 kg/ha, in France and Germany more than 2.5 kg/ha). [213] In some crops such as vineyards and apple orchards, the number of treatments and quantities may be much higher: in the Autonomous Province of Trento (Italy), 50 kg/ha per year are exceeded, in the Province of Bolzano 43 kg/ha per year, in Valle d'Aosta 22 kg/ha per year, and in Veneto 14 kg/ha per year. [500] In apple orchards peaks of 90 kg/ha/year may be recorded. [532]

Little information is available on synergistic effects, but some studies confirm that very low doses, which are considered to be non-dangerous, can actually have negative consequences. Bees that survive the winter live longer and therefore are potentially more damaged by low concentrations of complex mixtures. One study investigated the synergistic effects of three molecules: the insecticide imidacloprid, the fungicide difenoconazole and the herbicide glyphosate. Bees were artificially exposed by feeding to concentrations that could be measured in pollen and nectar of the three molecules, either individually or in combination. [1216] Bees were artificially fed for 20 days with these three pesticides individually, in binary combination or all three together, at concentrations for each of 0.1, 1 or 10 μg/L. The study demonstrates the existence of synergistic effects because the mortality resulting from the combination of several pesticides is greater than that generated by the individual molecules. Herbicides and fungicides are considered not very dangerous but a chronic exposure for 20 days to very low doses of combinations of these molecules generates an increase in mortality of more than 50%. Detoxification processes are altered, the nervous system is damaged, defence mechanisms against oxidative stress, metabolism and immune defences are disturbed. [1216] Therefore, the synergistic effects at doses considered safe by acute toxicity tests, following chronic exposures at lower concentrations, are equally lethal.

Synergistic effects may increase toxicity by as much as 100 or 1,000-fold. Therefore, preliminary studies carried out under laboratory conditions can provide information that is probably a considerable underestimate. In conclusion, toxicity tests have this other limit, they do not measure synergistic effects, i.e. those due to simultaneous exposure to different molecules. The multiple exposure, besides being able to generate negative effects higher than those expected by the simple sum, can generate new and more dangerous ones. Pesticides belonging to categories considered not very dangerous for bees, such as some fungicides and herbicides, in the presence of molecules such as insecticides and acaricides, can generate non-negligible negative effects. These effects can be as dangerous and devastating as the acute effects measured by the manufacturers before marketing.

SOME SYNERGISTIC EFFECTS

The scientific literature has gathered evidence of the existence of synergistic effects sufficient to trigger the precautionary principle, i.e. behaviours for self-protection. The following pages summarize some of these.

Synergies between fungicides and insecticides: inhibition of poison protection systems

Many substances act as inhibitors of the three main detoxification systems possessed by bees, so the damage can be enhanced by the alteration of defence systems. In this regard, it is necessary to highlight that bees have detoxification systems that are, in some cases, less effective than other insects (e.g. *Drosophila melanogaster*, known as the fruit fly, and *Anopheles gambiae*, or the mosquito that can transmit malaria). The three main systems of detoxification in bees are as follows: [601]

- The enzyme glutathione-S-transferase.
- The protein complex called cytochrome P450 mono-oxygenase. This system performs several reactions such as oxidations or demethylations, and is responsible for the transformation of the neonicotinoid thiamethoxam into clothianidin. It is also capable of carrying out the detoxification of organochlorine insecticides and pyrethroids.
- The enzyme carboxyl cholinesterase.

The inhibition of these systems increases the toxicity of many pesticides (e.g. tau-fluvalinate and coumaphos). [107] In the presence of inhibitors of the cytochrome P450 mono-oxygenase protein complex, tau-fluvalinate or lambda-cyhalothrin become more toxic, as the dose capable of killing 50% of insects is decreased (LD_{50}).

Clothianidin and dimethoate act as antagonists on *Apis mellifera*, *Bombus terrestris*, and *Osmia bicornis*, as they probably compete for the same enzymes. [474] Both of these pesticides interact with the cytochrome P450 enzyme complex: dimethoate is activated, so its toxicity is increased, while clothianidin is metabolized so that it becomes less toxic. It is easy to foresee synergistic effects between molecules such as some fungicides capable of inhibiting the cytochrome P450 mono-oxygenase enzyme complex, and insecticides that should be detoxified by this system. Consequently, the concentrations that will kill 50% of the bees exposed will be, in real conditions, much lower than those calculated in the laboratory: in the presence of prochloraz the LD_{50} is reduced even tens of times for alpha-cypermethrin, deltamethrin or fluvalinate. [601]

Many studies report synergistic effects between acaricides and fungicides, i.e. between the molecules used by beekeepers and those used by farmers (they may be contained in pollen; fungicides, such as myclobutanil, and may be found in pollen at a concentration of 1 ppm).

- Synergistic effects between deltamethrin (insecticide) and prochloraz (fungicide) were reported in the 1980s. [243] In the presence of the fungicide, which is not considered dangerous for bees, the same harmful effect of the insecticide is obtained, but at a concentration 50 times lower. [859]
- One study found that 14% of the 60 wax samples tested contained at least 2 of the 120 pesticides sought, and 5% contained 3 to 4. In some wax samples, insecticides (diazinon, fenpyroximate or the metabolites of amitraz) and fungicides (difenoconazole, fenbuconazole or azoxystrobin) may be found simultaneously at average concentrations of 6.4 µg/kg for insecticides and 10.1 µg/kg for fungicides, respectively. [67] The fungicide tebuconazole inhibits the detoxification enzyme system (cytochrome P450) by enhancing the adverse effects of insecticides.
- Fungicides (triazole) can generate synergistic effects with pyrethroid insecticides. [33, 39] When the fungicide propiconazole is present, the toxicity of pyrethroids is enhanced at least 16-fold, that of thiacloprid is increased 560-fold, and that of acetamiprid is increased 100 times. [45]
- Synergistic effects were demonstrated by the simultaneous presence of tau-fluvalinate (a pyrethroid used against the *Varroa destructor* mite) and fungicides: prochloraz increases the toxicity of the pyrethroid tau-fluvalinate by 100 times, probably due to the inhibition of the detoxification system (cytochrome P450). [474]
- Some fungicides, used in almonds (boscalid and pyraclostrobin), alter transport mechanisms that in bees are useful for detoxification, increasing the toxicity of the pyrethroid tau-fluvalinate by at least 3 times (tau-fluvalinate is used against the mite *Varroa destructor*). [473]
- On adult bees, neonicotinoids and azole fungicides show synergistic effects as they generate a 200 to 1,000 times increase in toxicity; for example, triflumizole increases the toxicity of acetamiprid and thiacloprid. [35]
- A synergistic effect between the fungicide propiconazole and the neonicotinoid insecticide thiamethoxam has been demonstrated, with at least an 8.3-fold increase in toxicity to bees. [67] The honey bee toxicity of the neonicotinoid insecticide thiacloprid increases by three orders of magnitude when trifumizole is present. [600] Tebuconazole may increase the toxicity of neonicotinoids, such as thiacloprid, through the inhibition of the detoxification enzyme complex (cytochrome P450 monooxygenase). [184, 601]
  The toxicity (LD_{50} by contact) of acetamiprid (a neonicotinoid) is increased 6 times when butoxide is simultaneously present, 105-fold by propiconazole or 244 times by triflumizole. [600] The toxicity (contact LD_{50}) of thiacloprid is increased 154, 559 and 1,141 times, respectively, in the presence of the same molecules (butoxide, propiconazole or triflumizole). [600]
- Fungicides can increase the toxicity of acaricides by more than 100 fold. [243, 601] The fungicide chlortalonil has reported synergistic effects with the pyrethroid acaricide tau-fluvalinate used by beekeepers to control the *Varroa* mite. [718]
- The toxicity of the acaricide tau-fluvalinate (pyrethroid) is increased if a fungicide such as prochloraz is present at the same time. Prochloraz also increases the toxicity of coumaphos and fenpyroximate (acaricides). [706] As already mentioned, this effect may

be explained by the fungicide's inhibition of the detoxification system (cytochrome P450), which is able to inactivate the acaricides used by beekeepers. Amitraz also increases the toxicity of three other acaricides whose detoxification is carried out by cytochrome P450 (that of tau-fluvalinate is increased 5-fold). These acaricides are among the most widely used molecules to control the damage caused by the parasitic mite *Varroa destructor*. The mite sucks the hemolymph of the bees, can carry viruses and generates immunosuppression damaging mainly the male population of the colony. Due to the widespread use of some of these pesticides, mites (*Varroa*) resistant to tau-fluvalinate and coumaphos have been reported. These two active molecules are lipophilic and accumulate in the wax, potentially causing synergistic effects for years. In this study, the level of toxicity recorded was, in a descending order, as follows: amitraz > phenopyroximate > tau-fluvalinate > coumaphos. [706]

- Some fungicides, such as prochloraz, triflumizole, and propiconazole (they are ergosterol inhibitors) reduce the activity of the detoxification system formed by the cytochrome P450 enzyme complex. [483] This inhibition increases by hundreds of times the acute toxicity of molecules such as acetamiprid and thiacloprid, and by several times that of coumaphos, tau-fluvalinate, and fenpyroximate. Inhibition of the activity of detoxification systems enhances the toxicity of molecules such as organophosphates (e.g. the acaricide coumaphos). [483]
- In maize fields, the exposure of bees to fungicides increases the acute toxicity of neonicotinoids. [387] In particular, the fungicide boscalid halves the LD_{50} of clothianidin or thiamethoxam.
- In some pollinators, *Bombus terrestris* and *Osmia bicornis*, the presence of propiconazole (fungicide) potentiates the toxicity of clothianidin (a neonicotinoid insecticide) by a factor of three. [474]

Simultaneous exposure to fungicides used by farmers and acaricides used by beekeepers increases the toxicity of the latter to a significant extent. This could be a reason to oblige farmers to inform beekeepers of the treatments they intend to carry out. The best solution is always another: to design a new food production system that does not require pesticides.

Synergies between insecticides

- Synergistic effects were also recorded by the simultaneous presence of acaricides such as coumaphos and tau-fluvalinate: the toxic effect of tau-fluvalinate increases by 3 times, if the hive was previously treated with coumaphos. [67, 243] The simultaneous presence of coumaphos and tau-fluvalinate potentiates some negative effects on the queen bee. [68]

- The simultaneous presence of organochlorines and pyrethroids generates greater toxic effects (they are found simultaneously in honey). [178]

Synergies between pesticides, pests, and microorganisms

- It is known that the exposure to pesticides may increase the susceptibility of bees to certain parasites. [107] The gut parasite *Nosema ceranea*, in combination with neonicotinoids (e.g. thiacloprid and imidacloprid) or the insecticide fipronil, generates synergistic effects. [235, 243, 386, 482] It follows that the presence of neonicotinoids increases susceptibility to the intestinal parasite (a fungus) and increases mortality. [360] These studies demonstrate the existence of additive and synergistic phenomena at very small doses.

- The persistence of certain molecules inside the hive (e.g. in the wax) may favour a greater susceptibility to infections such as those by the *Nosema* fungus. [32, 35, 706] The reduction of defence capabilities, through the inhibition of systems such as cytochrome P450, will favour parasites such as mites (*Varroa destructor*) or fungi (the intestinal parasite *Nosema ceranea*).

- The combination of contact exposure to neonicotinoids, such as clothianidin, together with the presence of the parasitic mite *Varroa destructor* generates complex synergistic effects. [367] Clothianidin alone and at the lowest doses reduces the grooming behaviour that sends the ecto-parasitic mite away from the bees' bodies. Clothianidin increases the occurrence of wing-deforming virus infections. Either alone or together, these two factors alter the gene expression in the brain.
- *Beauveria bassiana* is a cosmopolitan entomopathogenic fungus, used in biological control as it is capable of causing damage to different types of insects. [238] The fungus causes silkworm disease. When the spores of the fungus come into contact with a susceptible insect they germinate and grow inside the body, using it as a source of nutrition. After the death of the insect, the mycelium develops on the corpse, producing new spores. The parasitized insect, but not yet dead, spreads the fungus to other specimens. This fungus in association with pyrethroid insecticides generates synergistic effects. [230]

Some interactions between diseases and pesticides have been observed in bees, and knowledge about other pollinators is even more scarce (e.g. solitary bees, butterflies, bumblebees).

- Some fungicides and herbicides can inhibit the intestinal flora of bees, promoting negative consequences. [32, 54]
- The simultaneous presence of microorganisms, used for their insecticidal capacity such as *Bacillus thuringiensis* (used in organic farming), and molecules such as pyrethroid or neonicotinoid insecticides generates synergistic effects. [230]
- Neonicotinoids, such as clothianidin or imidacloprid, administered to bees (separately by contact and at very low doses) inhibit the immune system and promote infection with deformed wing viruses. [35, 395]
- Some insecticides, at very low concentrations, enhance the production of an immune-inhibiting protein in bees. Feeding bees with a sugar solution containing clothianidin or imidacloprid (at concentrations between 0.1 and 10 ppb) promotes infection by the wing deforming virus. Insects were exposed to 21 ng of imidacloprid or clothianidin per bee, i.e. between 1/10 and 1/1,000 of the lethal dose by contact (capable of killing 50% of them in a few hours). Thus, at very low doses the ability to defend against viruses is reduced. [35, 395]
- The presence of the chronic bee paralysis virus increases the toxicity of cypermethrin. [601]
- Pyrethroids alter the metabolism of lipids such as those that form intestinal barriers by promoting the passage of viruses from the gut to the hemolymph. [601]

Other synergies

- The use of antibiotics (used against American foulbrood) has resulted in increased sensitivity of bees to the acaricides fluvalinate and coumaphos. [473]
- A pollen-poor diet accentuates the adverse effects (increased mortality) reported following the use of Fipronil. [35]
- Herbicides and fungicides can potentiate the toxic effects of acaricides, such as fluvalinate and coumaphos. [55]
- Molecules such as fumagillin (used by American beekeepers against the *Nosema* fungus, but banned in Europe) or quercetin (naturally present in the pollen and nectar of some plants) may increase by several times the toxicity of neonicotinoids such as acetamiprid (these molecules act by inhibiting transport mechanisms useful for detoxification). [473]

Synergistic effects also occur in other organisms

In aquatic organisms (such as crustaceans: *Daphnia magna*) the simultaneous presence of fungicides and insecticides increases the toxicity of the latter. [598] In particular, the fungicide prochloraz (inhibits ergosterol biosynthesis, thus it is an endocrine disruptor) increases the toxicity of the pyrethroid insecticide alpha-cypermethrin by 12 times, while the fungicide epoxiconazole increases the toxicity of the insecticide by six-fold and propiconazole increases the toxicity of the insecticide of seven times. These synergistic effects (in the range of 3 to 16 times toxicity enhancement) have also been demonstrated in bees (*Apis mellifera*). [598] Fungicides (epoxiconazole and propiconazole) are often distributed in the field mixed with pyrethroid insecticides (simultaneously and together to control fungi and aphids). This information confirms the existence of worrying synergistic mechanisms between fungicides and insecticides, even in non-target organisms such as aquatic organisms. The alarm is supported by the fact that water monitoring detects several pesticide molecules simultaneously. Synergistic effects can be generated by sub-lethal doses of pesticides, but also by the simultaneous action of pesticides and parasites. There are many other pollutants that we distribute in the environment about which we have little information and the interactions between combinations of factors are largely unknown.

The recording of increased bee mortality, together with the reduction in biodiversity and the results of environmental monitoring (e.g. of the presence of pollutants in water), allows us to confirm the presence of underestimated risks.

RISK ASSESSMENT IS NOT SIMPLE

There is a close, and obvious, correlation between the number of pesticides found in wax or pollen and the health status of the bee colony. In practice, there is a relationship between the molecules to which bees are exposed throughout their lives and their health. One way to estimate the level of risk at which bees are exposed to pesticides is to calculate the sum of the hazard of individual pesticides. Adding up the measured amounts of each pesticide (e.g. in pollen or wax in µg/kg) and dividing the result by the doses capable of killing 50% of the bees by ingestion (in µg/bee, determined over 24 or 72 hours) gives an estimate of the level of risk. This model assumes that there are only additive and not multiplicative effects, and is unable to weigh long-term and metabolite effects.

Research in the USA found 93 different pesticides in bee colonies: 13 active molecules in bees, 61 in the larval food (a mixture of honey, pollen, and a secretion by worker bees called bee bread) and 70 in wax. [719] Up to 4 active molecules are found in bees at the same time and only 15% of the bee samples were free of pesticides (5/33). Acaricides used by beekeepers were found in most bee samples (coumaphos and tau-fluvalinate). Applying the methodology of summation of risk analysis of pesticides in bees showed that the most dangerous pesticide is fipronil (found at concentrations up to 9.9 ppb). All samples of food prepared by bees for larvae have at least one pesticide and insecticides are the category that contributes most to the risk sum. Molecules considered less dangerous are found at high concentrations: the fungicide chlortalonil is found up to concentrations of 26,000 ppb, and the acaricide coumaphos up to 3,260 ppb. It emerges that bees are exposed to many different active molecules at high concentrations already in their juvenile stages. Up to 39 active molecules were measured in a single wax sample, with an average of 10 molecules per sample and no sample containing less than 3 active molecules. Neonicotinoid insecticides were also measured in the wax. In this matrix, the calculation of the contribution to the risk summation sees acaricides in the first place, followed by insecticides. [719] Thus, all the colonies examined are simultaneously exposed, by contact and ingestion, to dozens of different molecules. The beekeepers practiced nomadism so the colonies were moved between citrus, apple, blueberry, cucumber, and pumpkin fields. Colonies also moved to provide pollination services were more contaminated than those used exclusively for honey production. Due to the high mortality rates, beekeepers were in some cases forced to replace colonies. The beekeeper then compensates for the damage caused by this massive exposure by artificially reproducing the colonies. The number of active molecules found (mainly in wax and larval food) could be used to predict the death of the colony or the need for queen replacement 30 days in advance. Among the active molecules clearly associated with an increased probability of colony death, fipronil and chlortalonil are reported, also because they were present at high concentrations.

The calculation of the relative hazard summation is a very approximate method and the level of contamination measured in this research is sufficient to raise concern and suggest the necessary choices: do not use pesticides either in beekeeping or in agriculture. In this study, only a few active molecules were searched for and, while presenting a very alarming scenario, the picture provided is an underestimate of the real exposure.

The level of pesticide contamination in honeydews produced by insects such as aphids, scale insects, whiteflies, and psyllids (systematic grouping Sternorrhyncha) is poorly understood. It would be interesting to know more about how different xenobiotics behave in the food chain consisting of soil, plants, phytophagous insects and honeydew-feeding pollinating insects such as ants, honeybees, solitary bees (genus *Osmia*) and bumblebees (and insectivorous and carnivorous animals such as birds).

Adult worker bees are usually used to test the toxicity of pesticides, but depending on their age they live differently and have different physiological characteristics. Therefore, the same level of exposure may generate very different effects depending on the age of the bees and this is what a study on honey bees aged between 4 and 42 days has shown. The exposure to insecticides generates different effects with age and the cumulative effect on mortality increases with age. [1235] Therefore, laboratory tests to assess acute toxicity cannot effectively represent all possible consequences for the whole colony.

By the time (assuming it is possible) we get to know the additive and synergistic effects of various combinations of active molecules and other factors destabilizing insect health, it will almost certainly be too late. Instead of focusing on the idea of choosing the least dangerous combinations of pesticides, it is much more far-sighted to try to organize a pesticide-free and more sustainable system of plant cultivation.

RISK ASSESSMENT FOR OTHER POLLINATORS

The presence of pesticides generates even more evident problems in geographical areas where biodiversity has not yet been irreversibly compromised, such as agricultural areas near primary forests (e.g. in the Amazonia). We are used to thinking of honeybees as the pollinators par excellence, but there are tens of thousands of other species of insects that are indispensable for food production and that we are eliminating without knowing what benefits they provide. For example, stingless bees (Hymenoptera Apidae Meliponini) are important pollinators for many plant species: between 40% and 90% of trees in tropical environments. [1199] These bees appeared before the stinging bees and have developed important symbioses with plants, the result of a co-evolution of at least 65 million years. The damage generated by pesticides to insects is mainly studied on honey bees but the effects on other pollinator species are somewhat different and more serious. The honey bee model cannot provide useful guidance for implementing risk analysis on other pollinators. Some stingless bees are social insects with biological characteristics that make them potentially useful for crop pollination. There are over 600 known species of meliponine (stingless) bees that can build nests using wax, plant resin and mud. They can also collect petals, leaves, feces and seeds. Therefore, compared to honey bees they also collect other materials such as soil which is a risk factor not present in honey bees. They can also collect pollen and nectar, but they travel much shorter distances than honeybees (2 km at most as opposed to 10 km). Stingless bees are generally smaller (in some species, worker bees can weigh over 60 times less than honey bees) and can generate eggs that serve as food instead of procreation. The life cycle from egg to adult is usually much longer than that of honey bees (twice as long, so larvae will feed for a longer period: 12-15 days instead of 5 days), they produce fewer eggs, and some species may steal food from other bees (*Lestremellita*). [1199] These differences add other variables for pesticide risk analysis that are not negligible. In addition, artificial breeding is not possible for the majority of species and protocols to be applied in the laboratory are not available.

The importance of pollinating insects other than bred ones is underestimated or, in some cases, unknown. Some solitary bees are useful for pollinating crops such as rape and strawberries in Europe, sunflowers in North America, orchids in Asia and coffee in Africa. [1238] The impact of pesticides on solitary bees is not known in detail but is certainly negative. The European Food Safety Agency suggested (2013) to include *Osmia bicornis* in the pesticide risk assessment. This solitary bee is easily reared and feeds on nectar and pollen. The latter, also for this insect, constitutes the main food of the larvae. Exposure of *Osmia bicornis* to concentrations of pesticides lower than those recorded in pollen generates negative effects. Colonies of solitary bees fed with pollen contaminated by insecticides (e.g. by the organophosphate chlorpyrifos or the pyrethroid cypermethrin) record a reduction in larval survival and a decrease in larval mass at very low concentrations: a few billionths of a gram per gram of pollen, i.e. at concentrations much lower than the highest recorded in pollen. [1238] This study demonstrates that higher concentrations of insecticides may be recorded in naturally collected pollen than those capable of compromising the survivability of *Osmia bicornis*.

Honey bees are used as a reference for obtaining toxicological information of pesticides on pollinators. Due to genetic, environmental and biological differences, risk assessment results obtained with honey bees are not useful for risk estimation for other non-target organisms. The example of *Melipona scutellaris* is given, which is a stingless social hymenopteran (family Apidae) native to Brazil. It lives in colonies of up to 6,000 individuals and produces up to 10 kg of honey per year. This insect is able to perform a type of pollination that honey bees do not do: they generate vibrations that can induce the mechanical separation of pollen from the anthers. At least 50 genera of bees have been identified that are able to promote pollen detachment through vibration. [1237]

Exposure of *Melipona scutellaris* to the neonicotinoid insecticide thiametoxam generates much more severe effects than those recorded by *Apis mellifera,* in fact the same mortality is caused by a dose 4 times lower (in Brazil thiametoxam is used in coffee, cotton, citrus and soybean cultivations). [1237] Therefore, the honey bee is not an appropriate model for establishing safety thresholds for all pollinators and other non-target insects. On the other hand, if we continue to wait until we have adequate scientific information on the certainty of adverse effects on other pollinators, it will be too late: we will have exterminated them. We must take preventive action by adhering to the precautionary principle: until it is proven that pesticides do not harm non-target organisms, they must not be used.

PESTICIDES THAT ARE CERTAINLY VERY DANGEROUS FOR BEES

At the end of this elaborate and incomplete discussion it is possible to try to summarize, in a list, some pesticides that are definitely dangerous for bees. The regulations state that pesticides that are very toxic to bees are those that belong to *Group 1*, i.e. those that register a lethal dose of less than 2 millionths of a gram per insect (LD_{50} <2µg/bee). [35, 173, 500, 866] Unfortunately, however, some pesticides can cause damage to living beings such as bees at concentrations measured on a lower scale, i.e. in billionths of a gram per bee. For example, imidacloprid, which belongs to the family of neonicotinoids (i.e. synthetic molecules derived from nicotine), can kill 50% of bees by contact at a dose of less than 18 ng per bee. [13] Over time, the old pesticides have been replaced with others that are much more poisonous: the quantities of insecticides capable of generating the same negative effects have been reduced by a factor of 1,000.

The determination of concentrations, which should help the cataloguing of pesticides into hazard classes, actually generates very variable results, as it depends on a multitude of factors, mostly unknown and difficult to investigate in the laboratory. Therefore, the classification is very poor because the knowledge available to us is incomplete. This is another reason why the precautionary principle should be applied: in default of a confirmation of the absence of danger, they should not be used. The list ia as follows: abamectin, acephate, acrinathrin, aldicarb, aldrin, aminocarb, arsenicals, avermectin, azinphos-ethyl, azinphos-methyl, azodrin, bifenthrin, bendiocarb, bufencarb, cadusafohyl, carbaryl, carbophenothion, carbofuran, carbosulfan, cartap hydrochloride, cephate, chlordane, chlorfenvinphos, chlorpyrifos ethyl, chlorpiriphos methyl, clothianidin, cyantraniliprole, cyfluthrin, cypermethrin, DDT (dichlorodiphenyltrichloroethane), dinetofuran, decamethrin, deltamethrin, diazinon, dichlorvos, dichrotophos, dieldrin, dimethoate, dimeton-S-methyl, disulfoton, d-phenothrin, EPN, emamectin, endosulfan, etofenprox, etrimfos, famphur, fenamiphos, fenazaquin, fenitrothion, fenoxycarb, fensulfothion, fenpropathrin, fenthion, fenvalerate, fipronil, flucythrinate, flubendiamide, formetanate, formothion, fosthiazate, glyphosate, heptachlor, heptenophos, hexaflumuron, imidachlorprid, indoxacarb, iprovalicarb, isophenphos, lambda-cyhalothrin, lindane, malathion, methamidophos, methidathion, methyl demeton, methiocarb, methomyl, methyl parathion, mevinphos, monocrotophos, naled, nicotine, omethoate, oxamyl, oxydemeton-methyl, parathion, permethrin, phentoate, phorate, phosmet, phosphamidon, phoxim, pyrethrins, pyrimicarb, pirimiphos-methyl, profenofos, propoxur, pyridaben, pyrimethanil, quinalphos, rotenone, resmethrin, spinetoram, spinosyn A and D, stirophos, tefluthrin, tenephos, tetraethyl pyrophosphate, tetrachlorvinphos, thiamethoxam, thiodicarb, toxaphene, trichlorphon, vamidothion.

Instead of trying to compile a list of substances that are definitely poisonous, we should change our perspective and ask ourselves if there are substances that can exterminate living beings, at doses of millionths of a gram or billionths of a gram, which are harmless to non-target species. These are substances that irreversibly alter mechanisms fundamental to life and therefore ubiquitous among living beings (e.g. alteration of nerve transmission or hormone balances). The negative changes caused in non-target organisms, even if they act on different physiological mechanisms, may be just as important for life and their alteration generates devastating effects. We allow ourselves to be deceived by a methodological error favoured by the more or less conscious desire not to accept limits.

The lethal doses that kill 50% of bees in 48 hours by contact, by ingestion, and the estimated half-life (in days) for some molecules in soil are reported below. [1168] A longer half-life suggests that the molecule may bioaccumulate easily.

The three numbers indicate, respectively: [1168]
LD_{50} contact (μg/bee) - LD_{50} ingestion (μg/bee) - Half-life in soil (in days)

Insecticides			
Beta-cyfluthrin	**0.031**	**0.050**	13
Bifenthrin	**0.015**	**0.20**	**87**
Carbofuran	0.16	-	14
Chlorpyrifos	**0.072**	**0.24**	50
Clothianidin	**0.039**	**0.004**	**121**
Cypermethrin	**0.034**	**0.064**	69
DDT	8.8	5.1	**6,200**
Diazinon	**0.38**	**0.21**	18
Endosulfan	6.4	21	**86**
Fenthion	**0.22**	-	22
Fipronil	**0.007**	**0.001**	**142**
Imidacloprid	**0.061**	**0.013**	**174**
Malathion	0.47	9.2	1
Mevinphos	**0.094**	-	1
Pyrethrum	**0.18**	**0.057**	-
Acaricides			
Acrinathrin	0.17	**0.12**	22
Amitraz	50	-	1
Coumaphos	20	4.6	-
Phenpyroximate	11	-	49
Tau-fluvalinate	8.7	45	4
Tetradifon	1,250	-	**112**
Fungicides			
Azoxystrobin	200	25	78
Boscalid	200	166	**118**
Captan	215	91	4
Carbendazim	50	-	22
Chlorothalonil	135	63	44
Myclobutanil	40	34	35
Propiconazole	50	77	**214**
Tebuconazole	200	83	47
Herbicides			
Metolachlor	-	110	**90**
Simazine	879	-	**90**

BEES BIOINDICATORS OF UNSUSTAINABLE CHEMICAL AGRICULTURE

WORLDWIDE BIOMONITORING ON PESTICIDES AND OTHER MOLECULES SUCH AS PCBs (polychlorinated biphenyls)

The results obtained from some biomonitoring studies described in scientific publications are summarized below. The studies are reported in chronological (or publication) order and summarize the results on pesticide residues recorded in beekeeping products in foreign countries. Most of these studies show that bees are exposed to dangerous concentrations of dozens of molecules at the same time. We are carrying out a great experiment whose overall effects are obvious: the reduction of biodiversity and the increase in mortality of even bred insects. With this dense and sad set of data, we hope to promote proactive thoughts and actions, which are essential for the necessary change.

- The content of pesticides in beeswax (*Apis mellifera*) was investigated at five different sites in France. [68] The research started in 2002 and ended in 2003. A total of 125 bee colonies from 25 apiaries were sampled and 16 insecticides and 2 fungicides were searched for. In each apiary, 5 colonies were chosen to be sampled in October-November (each wax sample weighed 15 g and the laboratory used 2 g: analyses were carried out using a gas chromatograph and a triple-quadrupole mass spectrometer). [76] The wax was selected from the sample free of honey, pollen, larvae or else so as not to have interferences. In two years, 47 wax samples were analysed (obtained by mixing wax from 5 colonies per apiary from 5 different sites). None of the apiaries were nomadic.

33 wax samples of the 47 tested, i.e. 70%, contained at least one of the molecules searched. Of the 18 molecules sought, 14 were found: 34% of the wax samples contained only one molecule and 36.2% contained between 2 and up to 8 different pesticides simultaneously. Residues of tau-fluvalinate (used against *Varroa*, but also by farmers), coumaphos and endosulfan were found most frequently, in 62%, 52%, and 23% of the samples, respectively. Also in a work carried out in Canada (2003) tau-fluvalinate was found with a high frequency: in 92% of samples of wax with brood and in 38.5% of samples of wax obtained from honey combs. [68]

In France, coumaphos is found at the highest average concentration of 792.6 µg/kg (maximum concentration of 4,113 µg/kg). Residues of cypermethrin, lindane and deltamethrin were found in 21.9%, 4.3%, and 2.4% of the samples, respectively. [68] Fenitrothion was detected in only one sample, but it was the active ingredient found at the highest concentration after coumaphos (511 µg/kg). At the time of the study, coumaphos and lindane were banned for agricultural use on plants, and the only formulation containing coumaphos permitted for use against *Varroa* in 2002 and 2003 was not sold in France. Lindane was found in 2 samples at concentrations of 5.3 and 32.2 µg/kg. Therefore, the wax samples were contaminated mainly by pesticides used by beekeepers and secondarily by those used by farmers (organophosphates and pyrethroids) and, unfortunately, molecules not authorized for years were also found (e.g. lindane). The Authors point out that in Europe the presence of acaricides in wax is not regulated and that for 62% of the active molecules examined there is not enough toxicological information available on the larvae.

Research carried out in Germany (in 1999) also found that coumaphos may be found in 62.5% of wax samples, and can be determined at high concentrations even 6 months after a treatment of the colony (529 µg/kg). [68] The concentration of coumaphos does not decrease after a 2-hour heat treatment at 140°C, so that the practice of beekeepers to recycle wax leads to an increased risk to bees.

Bee colonies raised in Asia have recorded the presence of acaricides such as bromopropylate, coumaphos, amitraz, fluvalinate, and tetradifon (an insecticide) in beeswax. [68] Wax is not used as food by bees but bees, after secreting it from their abdominal glands, can chew it, exposing themselves to the pesticides it contains. In addition, the wax cells contain honey, pollen, eggs, larvae and the entire colony.

Some of the results of analyses carried out on beeswax in France are summarised below. [68]

The name of the molecule, the category to which it belongs and any prohibition on its use are indicated. [68] I = insecticide; A = acaricide; F = fungicide; **P** = prohibited.	The four numbers indicate. Respectively, the pesticide **residues** in the wax samples (µg/kg); the **frequency of positive samples** (% out of 47), the **maximum** and **average concentrations**	LD_{50} in larvae (µg/larva)	LD_{50} in adults (µg/bee) Exposure O = oral C = contact
Azinphos-methyl, organophosphate I A	2 **10** **817** 446.1		0.43
Chlorpyrifos, organophosphate I A	3 **7,3** **19** 14.9		0.11
Coumaphos, organophosphate I A **P**	24 **52.2** **4,112** 792.6		3-6 O
Cyfluthrin, pyrethroid I A	5 **12.2** **31.9** 20.1		-
Cypermethrin, pyrethroid I A	7 **21.9** **76.3** 36.3	0.066	0.06
Deltamethrin, pyrethroid I A	1 **2.4** 14.7 --		0.7 O
Endosulfan, organochlorinated I A	11 **23.4** **243** 88.8	28.1	21.8
Phenythrothion, organophosphate I A	1 **2.1** 511 511		0.28
Fenthion, organophosphate I A	0 0.0 N/A N/A		0.3
Lindane, organochlorinated I **P**	2 4.**3** **32.2** **18.8**		-
Malathion, organophosphate I A	4 **8.5** **18.1** 15.1	0.74	0.73
Methidathion, organophosphate I A	0 0 ND ND	0.27	0.27
Mevinphos, organophosphate I A	0 0 ND ND	0.44	0.31
Parathion, organophosphate I A	1 **2.1** **99** --	-	0.07-0.1 C 0.09-0.13 O
Methyl-Parathion, organophosphate I A	0 0 ND ND		0.29
Tau-fluvalinate, pyrethroid I A	13 **61.9** **422** 196.4		65.85
Procymidone, dicarboximide F	1 **2.1** **27.7** --		-
Vinclozolin, dicarboximide F	1 **2.1** **21.5** --		-

Note: the analytical method used here has a limit of quantification for methyl-parathion and deltamethrin of 20 µg/kg. LD_{50} indicates the concentration that under experimental conditions kills 50% of insects exposed orally (by ingestion) or by contact.

- Some results of a monitoring of beekeeping products conducted in France, between 2001 and 2005, are summarized as follows. [243] Pesticide concentrations (in µg/kg) in pollen, honey, wax, and live bees are given.

Pesticides (µg/kg)	**Pollen**	**Honey**	**Wax**	**Live Bees**
6-chloronicotinic acid	1.2	1.2	--	0.9
Azinphos-methyl	--	21.8	**228.2**	--
Carbaryl	**142.4**	30.8	--	**214.3**
Carbofuran	**32.7**	16.1	--	12.9
Coumaphos	**423.5**	**37.9**	**647.5**	**1,546**
Deltamethrine	**39**	2.6	14.7	**16.9**
Endosulfan	**45.8**	--	**50.95**	8.3
Fipronil	1.2	--	--	0.5
Fipronil dèsulfynil	0.9	--	--	1.2
Fipronil sulfone	1.7	--	--	0.4
Inidacloprid	0.9	0.7	--	1.2
Lindane	**7**	**8.5**	**18.8**	**10.5**
Pensonazole	17.6	--	--	7.5
Procymidone	--	--	27.7	--
Propiconazole	--	--	--	--
Tau-fluvalinate	**334.1**	**44.7**	**220**	**65.5**
Tebuconazole	16.5	--	--	18.2
Tetraconazole	--	--	--	17.3
Vinclozoline	--	**109.4**	21.5	--

The results reported in this paper show that the bee colony is exposed to dozens of different molecules and at dangerous concentrations. Molecules used by beekeepers such as coumaphos and tau-fluvalinate are present in greate quantities. Contamination of pollen and honey should also be highlighted. The former is one of the most important foodstuffs in the juvenile stages and the latter is used not only by bees but also for human consumption. Some molecules are found in dangerous concentrations such as coumaphos, deltamethrin, endosulfan, and tau-fluvalinate. Bees are therefore exposed to lethal concentrations, both by ingestion and by contact, even in their juvenile stages. Finally, it should be noted that banned molecules are found: in November 2006, the use of lindane was banned in 52 countries and subject to restrictive regulations in 33 other countries. The Stockholm Convention on Persistent Organic Pollutants in 2009 established a further international restriction on the use of lindane in agriculture. [251]

- A study published in 2010 recorded the presence of 258 pesticides in hive products in Germany: only 24% of the samples did not record any residues (among those searched for). [484] Fungicides were the most frequently found category and coumaphos (an acaricide used by beekeepers) was the most frequently found substance. The same work shows that the fungicide chlorthalonil was found in pollen in the USA up to concentrations of 1,300 ppb. In France and the USA, 100% of the wax samples were contaminated by acaricides used by beekeepers (coumaphos and fluvalinate)., Due to the widespread and systematic worldwide use of acaricides, mites (*Varroa*) resistant to these molecules have been recorded and, unfortunately, this can lead beekeepers to the temptation of using unauthorized molecules.

- In Spain, 197 samples were examined for acaricide contamination in wax in the years 2003 - 2008. [1209] The results obtained are shown in the table below.
The name of the active substance, the number of samples, the number of positive samples (and their percentage), and the concentration range (µg/kg) are listed in order.

Chlorfenvinphos	197	189 (**96%**)	19.6 - **10,640**
Tau-Fluvalinate	157	147 (**94%**)	27 - **88,659**
Bromopropylate	33	29 (**88%**)	13.6 – 22.7
Amitraz	114	16 (14%)	12 - 63
Endosulfan sulfate	20	2 (10%)	144 - **231**
Coumaphos	134	5 (3.7%)	17.3 - **195**
Malathion	117	2 (1.7%)	160.5 - **184**
Flumethrin	15	1 (6.7%)	
Acrinathrin	15	1 (6.7%)	
Chlordimeform	15	1 (6.7%)	
Chlorpyrifos	18	1 (5.6%)	

It can be observed that practically all wax samples are contaminated, that concentrations may be high and that unauthorized molecules such as chlorfenvinphos (probably used illegally to control the *Varroa destructor* mite) are detected. The molecules chlordimeform, chlorpyrifos, malathion, and endosulfan are used in agriculture.

- In France in 2008 and 2009, pesticide monitoring was carried out on live bees (141 samples), honey (141 samples) and pollen (128 samples) taken from 18 apiaries located in four different areas. [39] A total of 80 molecules were searched for and the results showed that 95.7% of the honey samples, 72.3% of the bee samples, and 58.6% of the pollen samples contained at least one pesticide. The frequency of finding was highest in honey, followed by pollen and then bees, but the highest concentration was found in pollen. The most frequently found active molecules were the fungicide carbendazim and the acaricides amitraz and coumaphos. To treat mites (e.g. *Varroa distructor*) and fungi (e.g. ascospheriosis) beekeepers use several dangerous molecules that may remain in the honey such as the acaricides amitraz, celazole, bromopropylate, coumaphos, flumethrin, taufluvalinate, and some fungicides like carbendazim.
Apiaries in rural areas were found to be the most contaminated one and the highest concentrations were recorded in late spring.
A total of 20 different molecules were found in bees, with samples containing up to 6 active molecules at the same time (36.2% of samples contained at least 2 residues and 18.4% contained at least 3 residues).
A total of 28 molecules were found in honey, with samples containing up to 8 molecules at the same time (an average of 3 residues per sample analysed). Of the honey samples analysed, 80.8% contained at least 2 residues, while more than 3 residues were found in 57.4% of the samples.
Twenty-three molecules were found in pollen with samples that could contain up to seven residues simultaneously. 30.5% of pollen samples contained at least two active molecules and 11.7% contained at least three of them.
In this French monitoring, 80 molecules were searched for and 37 (46%) were found, of which 12 were found in all the matrices examined: 3 acaricides (2 metabolites of amitraz, amitraz I and amitraz II, and coumaphos), 3 fungicides (carbendazim which is also a metabolite of thiophanate-methyl, flusilazole, and thiophanate-methyl) and 6 insecticides (carbaryl, phosmet, piperonyl butoxide, pyriproxyfen, tau-fluvalinate, and triphenylphosphate). The most frequently found pesticides were:

- in live bees: carbendazim (41.1%), triphenylphosphate (24.8%), coumaphos (17.8%) and amitraz II (16.3%);
- in honey: coumaphos (78.0%), amitraz II (68.8%), carbendazim (64.5%), phosmet (12.8%) and cyproconazole (11.3%);
- in pollen: carbendazim (34.4%) and amitraz II (14.8%).

The highest concentrations were:

- in live bees, the fungicide thiophanate-methyl (2,419 ng/g) and the insecticide chlorpyrifos (180.2 ng/g);
- in honey, the acaricide amitraz II (116.1 ng/g) and the fungicide carbendazim (87.9 ng/g);
- in pollen, the fungicides thiophanate-methyl (3,674 ng/g) and carbendazim (2,595 ng/g).

Among the molecules with dangerous effects on bees found at high concentrations (therefore with lethal effects) the insecticides chlorpyrifos and phosmet are highlighted.
In this monitoring, molecules banned in France are found, such as the acaricide coumaphos, found in 78% of honey samples (110/141). Triphenylphosphate is also forbidden in France, but it is found in 2% of honey samples. It is important to underline that it is also used as a flame retardant and therefore it is possible that it is spread in the environment through industrial activities (e.g. in the atmosphere).
Apiaries were most contaminated in the months of highest pesticide use (April-June). In this study, honey was the most contaminated matrix, while other studies have shown that pollen was the most contaminated matrix. However, in this study pollen recorded the highest mean and maximum concentrations.

- In the USA during the years 2006-2009 about one third of the colonies of bees (*Apis mellifera*) died (in winter). Due to this alarm a demanding monitoring was financed, which achieved worrying results. [597] In North America in 887 pollen, wax and bee samples, 121 different pesticides of the 200 searched were found. 60% of the 259 wax samples and 350 pollen samples contained at least one molecule (2007/2008 season). Overall, an average of 6.5 different pesticide molecules were found per sample in the 887 samples. 47% of the wax and pollen samples contain the acaricides used by beekeepers (fluvalinate and coumaphos) and a fungicide (chlorothalonil). 98% of the wax samples were contaminated: up to 204 ppm by fluvalinate, up to 135 ppm by bromopropylate, up to 94 ppm by coumaphos, up to 46 ppm by amitraz and its metabolites, and by lower amounts of chlorothalonil. Overall, 87 active molecules with their metabolites were identified in wax. In the 259 wax samples on average six different pesticides were found with a maximum of thirty-nine. Wax is the matrix that constitutes a very important and therefore dangerous source of contamination in the long term.
In the 350 pollen samples, 98 pesticides and their metabolites are found. A very alarming fact is that on average the examined pollen samples contain 7.1 different pesticides and single samples are detected containing up to 31 pesticides. So negative synergistic effects are to be expected. In pollen at least ten pesticides exceed the concentration of one tenth of the LD_{50} (lethal dose that kills 50% of the bees within a few hours), so it is not surprising that bees die. The fungicide chlorothalonil in pollen collected by bees reaches concentrations of 99 ppm, while the insecticides aldicarb, carbaryl, chlorpyrifos and imidacloprid, the herbicide pendimethalin and the fungicides boscalid, captan and myclobutanil are all found at concentrations in the order of 1 ppm. It should be stressed at this point that pollen is one of the most important foods for the development of larvae.
An average of 2.5 molecules were found in the 140 bee samples, with a maximum number of 25 active molecules in a single sample: 83.6% of bees contained fluvalinate, 60% coumaphos

and 8.6% chlorpyrifos. Fluvalinate and coumaphos are very persistent in the hive, the half-life in wax is more than 5 years.
This study shows that 98.3% of pollen and wax samples are contaminated with two or more pesticides, 91% with three pesticides, 80% with four pesticides. Fifty percent of these samples contain at least one systemic active ingredient. The acaricides fluvalinate (a pyrethroid) and coumaphos (an organophosphate acaricide) are the most frequently combined pair of molecules, in 77% of the samples, followed by the pair fluvalinate and chlorothalonil (a fungicide) in 41% of the samples, fluvalinate and chlorpyrifos (an organophosphate insecticide) in 39.4% of the samples and coumaphos and chlorothalonil in 39.1% of the samples. It is necessary to highlight that it is possible to distribute fungicides even during flowering as there are no limitations, unlike insecticides. Therefore, it is not surprising that fungicides are among the molecules found most frequently in pollen (e.g. chlorothalonil) and that those such as vinclozolin and iprodione are recorded at high concentrations: 32 and 5.5 ppm, respectively.
In the whole of the wax, pollen and bee samples, the molecules found most frequently, in a descending order, were: fluvalinate and coumaphos, followed by chlorpyrifos, chlorothalonil, amitraz, pendimethalin, endosulfan, fenpropathrin, esfenvalerate, and atrazine.
Neonicotinoids were not found in bees but in wax and pollen: thiacloprid in 23 samples, imidacloprid in 14, acetamiprid in 11, and thiamethoxam in one. In some cases, it was possible to associate bee mortality with the presence of high concentrations of some molecules (in dead bees): permethrin at a concentration of 19.6 ppm (LD_{50} about 1.1 ppm) and fipronil at a concentration of 3.1 ppm (LD_{50} of 0.05 ppm). Thus, the concentrations were, respectively, 20 times and 62 times higher than those that under experimental conditions kill 50% of the exposed bees in a few hours.
Acaricides (fluvalinate, coumaphos, and amitraz) are more concentrated in wax than in pollen, while pesticides used in agriculture are found at equivalent or higher concentrations in pollen than in wax (aldicarb, chlorothalonil, chlorpyrifos, endosulfan, pendimethalin, fenpropathrin, azoxystrobin). Some pesticides used in agriculture, such as pyrethroids and insect growth regulators (methoxyfenozide), tend to bioconcentrate in wax. These molecules were more concentrated in wax than in pollen, although they are not used by beekeepers. Some metabolites of acaricides (lipophilic metabolites of fluvalinate and amitraz) are found at higher concentrations in bees than in wax.
This information makes it possible to predict potentially disastrous synergistic effects, not least because overall concentrations of 200 ppm are exceeded in many samples. Most of the samples that contained a fungicide recorded the simultaneous presence of an insecticide/acaricide of the pyrethroid family or of the organophosphates. In reality, the possible synergies are much more complex, since on average 6-7 different molecules are found in the same sample, reaching 31 molecules simultaneously present in pollen or 39 in wax. Despite the fact that the scientific depth of this investigation has shed light on the origin of the ecological alarm for pollinators and others, the article refers to an increase in bee mortality as a result of a disease whose causes have yet to be discovered: *Colony Collapse Disorder* or sudden bee death. And yet, as we have already written, in the dead bees the researchers found concentrations of pesticides (fipronil) up to 62 times higher than the dose that in laboratory tests can kill 50% of the bees exposed in a few hours (and 19 times higher than the LD_{50} for permethrin); in the bees fluvalinate was found at a concentration equal to 50% of the LD_{50}; in the pollen 10 pesticides exceed the concentrations that certainly have sub-lethal and lethal effects (a tenth of the LD_{50}). While these results leave no room for doubt, it is suggested that the causes of the increase in bee mortality should be sought elsewhere: *Colony Collapse Disorder* or bee death, which according to the Authors is not related to pesticides. This contradiction between the quality of the results and the conclusions is unbelievable: the Authors declare that they have no conflict of interest. [597] It is

also incredible that many colonies have survived this deluge of poisons present in deadly concentrations.

- In Spain, chlorfenvinphos is recorded in 100% of the 32 wax samples tested (at an average concentration of 449 μg/kg: range between 20 μg/kg and 3,182 μg/kg), tau-fluvalinate in 96.8% of the samples (at an average concentration of 996 μg/kg and a maximum concentration of 12,978 μg/kg). [606] An alarming finding of this study is that all samples of the mixture used by bees to feed the larvae (bee bread) contain pesticides: a total of 16 active molecules were found. This means that bees are already systematically exposed to complex and harmful mixtures in their larvae.

- A study published in 2010 determined the presence of acaricides, used by beekeepers at the recommended doses, in different hive matrices (amitraz, fluvalinate and coumaphos). [692] The hives were located near apple orchards in Slovenia where the insecticide diazinon was used. Among the results of this research it is highlighted that: [692]

- The acaricide coumaphos was found in royal jelly (it is secreted by special glands of young bees) at a concentration of 400 ng/g (5 days after the treatment).
- Fluvalinate was found at high concentrations in the head of bees (228 ng/g), where important glands are located, and in larvae (1,038 ng/g).
- Doses of acaricides that are considered safe for agricultural use in reality are not safe because they generate (sub-lethal) effects that are harmful to the colony.
- Products considered safer such as oxalic acid and formic acid kill the intestinal cells and those of the larvae's salivary glands.

- In India, among the molecules most frequently found in honey, there is lindane (or γ-hexachlorocyclohexane or gamma-HCH) and in second place dichlor-diphenyl-trichlorethylene (DDT). [41] These molecules have been banned for decades in most parts of the World because of their persistence and hazardousness. Malathion was found at concentrations above the maximum permissible limits in India (2011).

- A research published in 2011 and conducted in the USA investigated the presence of pesticides in wax where, on average, 10 active molecules were found at the same time and overall in this matrix there are 39 pesticides and 7 metabolites. [240] Thus, wax constitutes a highly contaminated matrix and synergistic effects are possible. Pesticides can move from wax into other matrices contaminating also larvae, pollen, and honey in honeycombs. Among the active molecules found most frequently and at the highest concentrations, there are those used by beekeepers such as the acaricide coumaphos (measured up to a concentration of 22,100 ng/g) and the pyrethroid insecticide fluvalinate (measured up to a concentration of 24,340 ng/g). Pesticides, in the combs, generate many negative effects such as a reduction in the life expectancy of adult bees by at least 4 days and a delay in larval development that favours the parasitic mite *Varroa;* these are sub-lethal effects that may contribute to fatal colony weakening. Unfortunately, contaminated wax is also recycled and these molecules (and their metabolites) may remain there for years.

- In France, the analysis of the content of pesticides in wax from 125 colonies reveals the presence of 14 of the 18 pesticides searched (16 insecticides and acaricides, and 2 fungicides), (article published in 2012). [41] Tau-fluvalinate, coumaphos, and endosulfan are the molecules found most frequently. Wax allowed the detection of molecules used by beekeepers and farmers.

- In Belgium in 2012, the presence of 300 pesticides, including organochlorinated and organophosphates (used for agricultural and livestock purposes), was monitored in wax from 10 beehives in different locations throughout the country. [70] Chemical analyses performed on the wax (using GCMS/MS and LC-MS/MS methods) showed the presence of 18 of the 300 pesticides tested (6%), with a frequency of 3 to 13 active molecules per sample and, unfortunately, none was found to be free of residues.

- In Germany, a 5-year monitoring (2012-2016) investigated the content of pesticides in 281 samples of pollen collected by bees (special mechanical systems were used to collect pollen when the foragers returned to the hive). [188] In this investigation, 282 active molecules of pesticides were searched at three different sites:

1. One characterized by 60% permanent grassland and orchards.
2. One occupied mainly by grains such as wheat, oats, canola, corn, and barley.
3. The last one occupied by 30% permanent crops like grapevine, pome fruit and 40% cereals like maize. Pollen was collected from this site for only three years (2012-2014) instead of five.

The pollen, 5 g per sample, was collected between March and August from stationary hives and was analysed using the technique of gas chromatography combined with mass spectrometry. The following procedure was applied to define the level of danger of the residues found: divide the concentration of the molecule found in the pollen (in µg/kg) by its LD_{50} (the dose sufficient to kill in a few hours 50% of the insects exposed by ingestion). The LD_{50} was obtained from several databases accessible on the internet. [189, 190, 191] If the ratio between the recorded concentration and the LD_{50} is greater than 50 and the larvae consume 9.5 mg of pollen per day, it means that during the 10 days of feeding with pollen they are exposed to a daily dose of pesticide equal to 0.5% of the LD_{50}, so the risk is considered high (risk quotient). In this research they also implemented palynological analysis under the microscope. Among the results obtained it is interesting to highlight some of them:

- Only one-fifth of the samples were found to be uncontaminated.
- Of the 282 active molecules sought, 73 were found.
- In pollen samples from site number three, 58 pesticide active ingredients were identified, in site number two 37 and in site number one 24. So site number three was found to be the most dangerous to bees.
- In area number three the average number of molecules per sample was 3.8, while in area two it was 2.
- At all sites, fungicides are the molecules found most frequently (more than 50%).
- In area three, the highest frequencies of occurrence and concentrations were recorded: up to 7,177 µg/kg of pesticides.
- The hazard level (risk quotient greater than 50) was not exceeded for pesticides found in area one, but was exceeded six times in site two (methiocarb and dimethoate) and 12 times in site three (dimethomorph, fenhexamid, fluazifop and indoxacarb).
- At site number three, the neonicotinoid insecticides (clothianidin and imidacloprid) exceed the value of 50 by a factor of 4, resulting in a risk quotient of up to 600.

- Considering the sum of the risk quotients of the pesticides found simultaneously in the different pollen samples, 4.9% (7 samples) of the pollen taken from site two and 23% (13 samples) of the pollen from site three exceed the (arbitrary) threshold value of 50.
- 27 pesticides out of the 73 found exceed the maximum allowed concentrations: 3 in site one, 10 in site two and 25 in site three. 15 samples per year from site three exceed the maximum limits for pollen (2 from site one and 8 per year from site two).
- Pollen collected from cultivated fields with vineyards record the highest concentrations of pesticides (e.g. dimethomorph found up to 3,747 μg/kg).
- The highest concentrations are recorded in spring.
- In some crops, treatments were carried out during flowering.

The following substances were found with concentrations very dangerous to bees: methiocarb, dimethoate, clothianidin, imidacloprid, fluazifop-butyl, and fenhexamid.
In conclusion, orchards were the most contaminated crops, as no sample was free of pesticides. Unfortunately, when beehives are located near pesticide-treated monocultures and bees have no other means of feeding, it is easy to find concentrations in the order of mg/kg (very high) in the various beekeeping matrices. Other studies reported in this publication record risk quotients in orchards such as citrus orchards above 2,000, in apple orchards above 4,000 and in some cases the value of 40,000 is exceeded.

- A 2013 study by Spanish and Argentine researchers examined the presence of 120 pesticides in 60 samples of beeswax from 60 different apiaries (using liquid chromatography with a mass spectrometer). [67] Each sample consisted of a piece of wax (10 cm x 10 cm) randomly extracted from a hive in one of the 60 selected apiaries (each apiary had from 30 to more than 360 colonies). To test repeatability and reproducibility, each sample was analyzed 5 times (dimethoate was used as the internal standard).
All 60 wax samples contained pesticides and overall 31 of the 120 molecules searched (25.8%) were found. 14% of the samples contained at least 2 pesticides and 5% contained from 3 to 4 of them. Metabolites (DMPF and DMF) of amitraz, an acaricide used by beekeepers to control a parasite (the *Varroa* mite), were found in wax at concentrations between 5 and 466 µg/kg (sum of DMPF and DMF), organophosphate insecticides at concentrations between 1 and 464 µg/kg, fungicides at concentrations between 1 and 23 µg/kg, and herbicides at concentrations between 1 and 5.9 µg/kg. Insecticides were found at higher concentrations: an average concentration of 16.2 µg/kg (14 insecticides of the 64 sought were detected). The most frequently detected insecticides/acaricides were: diazinon, phenthoate, pirimiphos-methyl, and profenofos (organophosphates found in 17.3% of the samples), and carbaryl and fenoxycarb (carbamates found in 15.3% of the samples). The insecticides found less frequently (less than 6% of samples) were: acetamiprid, imidacloprid, and fenpyroximate.
Ten of the 46 fungicides searched (21.7%) were detected: azoxystrobin, difenoconazole, myclobutanil, and spiroxamine were found in 4% of the samples.
In Europe, imidacloprid, thiametoxam, and chlotianidine were banned since December 2013 (for seed treatment, soil and leaf applications) with the intention of protecting pollinators (this is the year in which this work was carried out). Thiametoxam and chlotianidine were not found, while imidacloprid was found in 5 samples at concentrations between 3 and 5.1 µg/kg.
Fipronil was also found in a sample: in Europe, in 2013, this molecule was banned too.
Some insecticides (diazinon, fenpyroximate or the metabolites of amitraz) and fungicides (difenoconazole, fenbuconazole or azoxystrobin) may be found simultaneously in wax at average concentrations of 6.35 µg/kg for insecticides and 10.07 µg/kg for fungicides. The simultaneous presence of these molecules could cause synergistic effects due to the inhibition of the detoxification system of the digestive tract (cytochrome P450 enzyme complex).

Synergistic effects were recorded by the simultaneous presence of acaricides such as coumaphos and tau-fluvalinate: the toxic effect of tau-fluvalinate is increased by 3 times if the hive was previously treated with coumaphos.
Neonicotinoids (acetamiprid, imidacloprid, and thiacloprid) were found in 6% of the samples analysed. The simultaneous exposure to 2 neonicotinoids (imidacloprid and thiacloprid or acetamiprid) was recorded in 2 samples. Also 2 samples with the simultaneous presence of thiacloprid (4 and 5.51 µg/kg) and acetamiprid (2 µg/kg) were found. One of these two samples also contain the three fungicides fenhexamid (23 µg/kg), myclobutanil (4 µg/kg) and penconazole (3 µg/kg). [67] Thus, little known but probably very dangerous synergistic effects on bee health are possible.

Below are some results on pesticide residues found in 60 beeswax samples. [67]

	Number of positive samples out of 60	Concentration ranges in µg/kg
Insecticides		
1. Acetamiprid	6	1 - 4
2. DMF + DMPF (Amitraz)	20	5 - 464
3. Carbaryl	1	1
4. Chlorantraniliprole	2	1-2
5. Diazinon	7	2 – 9.8 (average concentration of 5.11 µg/kg)
6. Phenpyroximate	3	1
7. Fipronil	1	1
8. Flufenoxuron	1	3
9. Imidacloprid	5	3 – 5.1
10. Indoxacarb	1	18.1
11. Phenthoate	1	4
12. Pirimiphos Methyl	1	3
13. Profenofos	1	2
14. Thiacloprid	3	4-10.4
Herbicides		
1. 2,4-D	4	3 – 18.9
2. Pendimethalin	2	1
3. Propyzamide	1	4
4. Terbuthylazine	6	3 - 12
Fungicides		
1. Azoxystrobin	2	1
2. Bupirimate	1	1
3. Diphenoconazole	2	1
4. Fenbuconazole	1	5.2
5. Fenhexamid	1	23
6. Kresoxim-Methyl	2	1 - 5
7. Myclobutanil	4	2 - 4
8. Penconazole	2	2 - 3
9. Spiroxamine	4	1 - 2

- A 4-year (2009-2012) monitoring of the presence of pesticides in beekeeping products from stationary apiaries in six states was conducted in North America. The apiaries were located in areas with different land uses: between 4% and 68% of the area devoted to agriculture and between 0% and 35% occupied by a residential area. [1203] A total of 168 pollen samples and 142 wax samples were taken, in which 82 pesticides, eight metabolites, and one synergistic action molecule were detected. Of the 91 molecules found, 38 (42%) were insecticides, 29 (32%) were fungicides, and 23 (25%) were herbicides. 78 pesticides were found in pollen (32 were found only in this matrix), 58 in wax (12 were found only in this matrix). In general, insecticides are the class of pesticides found with higher concentrations and greater frequency.
The most frequently detected fungicides in wax were azoxystrobin (39% of 142 samples) and pyraclostrobin (37%), while the highest concentration of a single fungicide was recorded for carbendazim (137 ppb).
In pollen samples, 20 different herbicides were found, while in wax 14 of them were found (9 were found only in pollen and three in wax). In pollen, atrazine was the most frequently found herbicide (in 50% of the 168 samples), while diuron had the highest concentration (275 ppb). Atrazine and diuron were also the herbicides found most frequently in wax (34% and 29% of 142 samples), while the highest concentration was recorded for halosulfuron-mythyl (386 ppb).
35 of the 38 insecticides detected were found in pollen and 24 in wax. 14 insecticides were present only in pollen and two only in wax. In pollen, the most frequently found insecticides were carbaryl (26% of 168 samples) and imidacloprid or its metabolites in 6% of the samples; the highest concentration was recorded for spinetoram (645 ppb).
In wax, the acaricide coumaphos was detected in 86% of the 142 samples and fluvalinate in 78% of the samples (amitraz metabolites were also found with a high frequency). In the years in which the monitoring was carried out, coumaphos was not authorized for agricultural use, but only for use in beekeeping. [1203]
Up to 13 residues were found in pollen at the same time while the average was 4 residues per sample (in pollen samples in some geographical areas up to 9 residues are found at the same time). Among the molecules found most frequently in pollen, there is the herbicide atrazine (inhibits photosynthesis), the insecticide carbaryl, the fungicide carbenzadim, and then the insecticides chlorpiriphos, pendimethalin and the fungicides azoxystrobin and propiconazole.
This research also shows the presence of multiple exposure with devastating effects: the death of the majority of colonies in many apiaries was recorded.

- In Egypt, in 2013, the concentrations of organochlorine pesticides and pyrethroids were investigated on 100 honey samples of different botanical origin (5 g samples were analysed by the gas chromatography technique with electron capture analyzer and mass spectrometry). [178] The most frequently found molecules were as follows: HCB or lindane or hexachlorobenzene[I] (banned by the European Union) was found in 68% of the samples,

[I] Exachlorobenzene (HCB) or lindane, first introduced in 1945, was long used in agriculture mainly as a fungicide in grain storage until 1981 when the European Community banned its use in agriculture. The chemical structure is relatively simple, as it consists of a hexa chloro substituted benzene ring. This molecule is also released into the environment as a by-product of some industrial processes, as an impurity during the production of some pesticides and during the incineration of municipal waste. The chemical-physical characteristics, such as semi-volatility, chemical stability and resistance to biodegradation, mean that HCB is classified as a persistent and bioaccumulative environmental pollutant. It is subject to long-range atmospheric transport and is one of the substances included in the Stockholm Convention on Persistent Organic Pollutants (POPs) and in the Protocol on POPs (LRTAP-POP) of the United Nations Economic Commission for Europe (UNECE) Convention on Long-permethrin (pyrethroid) was found in 22% of the samples, heptachlor-epoxide was found in 16% of the samples, endrin in 14% of the samples, aldrin in 13% of the samples. DDT and its metabolites were found in 16% of the samples. The organochlorine molecules are persistent and bioaccumulate easily in tissues. In this work the finding of DDT (and its metabolites DDD and DDE) is optimistically attributed to contamination that presumably occurred in the past. In this regard, it is useful to remember that DDT is metabolized by microorganisms into equally dangerous molecules and is used to produce another molecule: dicofol. [181, 183] In conclusion, some organochlorines have been officially banned also in Egypt since the '70s but they are found in honey: DDT and lindane.

- A study conducted in Spain, in 2014, examined the presence of pesticides used in orchards, mainly citrus but also peach, khaki and plum, in 4 different locations. [75] Bees that die inside the hive are removed outside; in this study, dead bees (*Apis mellifera*) were sampled and collected using special systems placed near the hive, which prevent access to birds. The natural mortality recorded in this study is about 20 bees per day per colony (there may be seasonal variations influenced by different factors). Peak mortalities have been as high as 500 bees per day and have been associated with insecticide use on citrus.

Thirty-four samples of dead bees were collected (between the months of January and June) and examined for the content of 58 pesticides (each sample consisted of 5 g of dead bees and was analysed by liquid chromatography and quadrupole mass spectrometer). On average, each sample of dead bees contained 4 pesticides and 8 of the 58 molecules sought were found (up to 7 active molecules were found at the same time). Coumaphos and organophosphates acaricides used by beekeepers to control *Varroa* mites were the most frequently found molecules (in 94% of samples). Coumaphos was found at an average concentration of about 50 ng/g (sampling began a few months after the removal of the acaricide application system).

Among the pesticides used in the field, found most frequently in dead bees, there were organophosphates, such as chlorpyrifos (79% of samples and at the maximum concentration of 751 ng/g) and dimethoate (in 68% of samples and at the maximum concentration of 403 ng/g), and neonicotinoids, such as imidacloprid (in 32% of samples and at the maximum concentration of 223 ng/g and the mean concentration of 53 ng/g). Chlorpyrifos and dimethoate were used in citrus groves. Omethoate is an equally toxic metabolite of dimethoate and is found with a similar frequency. Chlorpyrifos, dimethoate and imidacloprid were found simultaneously in 29% of samples from dead bees.

A total of 8 molecules were found. In addition to those already mentioned, carbendazim (fungicide) was found in 32% of the samples and at the maximum concentration of 616 ng/g, acetamiprid in 24% of the samples and at the maximum concentration of 44 ng/g, and fluvalinate in 3% of the samples and at a concentration of 91 ng/g.

The analysis of mortality peaks shows an association with the presence of coumaphos and chlorpyrifos in all apiaries. According to the Authors, coumaphos is not responsible for the increase in mortality because it is found at low concentrations. The simultaneous presence of chlorpyrifos and dimethoate was associated with an increased mortality (500 bees per day). This work confirms, although it was obvious, that the application of insecticides during citrus flowering has a significant effect on bee mortality. Dimethoate is very dangerous for bees,

range Transboundary Air Pollution. [180] Some human toxicity data come from the 1950s production in Turkey, following which there were approximately 600 cases (manifesting porphyria with skin ulcers, skin colour change, arthritis and liver, nervous system and stomach problems). HCB was classified by the International Agency for Research on Cancer (IARC) as a possible human carcinogen (Group 2B) and was a potential endocrine disruptor. In 1974, IARC had already reported that molecules such as aldrin, dieldrin and lindane had carcinogenic effects.

therefore it cannot be distributed during flowering and can only be used during sowing (EC Reg. 1107/2009). Unfortunately, the illegal use of dimethoate is confirmed. Also the high concentration of neonicotinoids shows the illegal use of these insecticides (e.g. during flowering). In conclusion, the mortality peaks are mainly associated with chlorpyrifos and dimethoate, and secondly with imidacloprid.
Below are the pesticides found in dead bees in this survey conducted in Spain, in 2014. [75]

Pesticides	***Number of positive samples and percentage of positive samples out of 34 samples***	***Minimum concentration in ng/g of dead bees*** *(wet weight)*	***Average concentration in ng/g of dead bees*** *(wet weight)*	***Maximum concentration in ng/g of dead bees*** *(wet weight)*
Coumaphos	32 (94%)	7	28	150
Chlorpyrifos	27 (79%)	3	100	751
Dimethoate	23 (68%)	13	102	403
Omethoate	21 (62%)	2	34	109
Imidacloprid	11 (32%)	12	53	223
Carbendazim	11 (32%)	3	141	616
Acetamiprid	8 (24%)	25	32	44
Fluvalinate	3 (9%)	10	52	91

Pesticides searched for in dead bees were as follows: acetamiprid, acetochlor, alachlor, atrazine, atrazine-desethyl, atrazine-desisopropyl, azinphos-ethyl, azinphos-methyl, buprofezin, carbendazim, carbofuran, carbofuran-3-hydroxy, chlorfenvinphos, chlorpyrifos, coumaphos, diazinon, dichlofenthion, dimethoate, diuron, DMA, DMF, DMPF, ethion, fenitrothion, fenoxon-sulfoxide, fenoxon-sulfonefenthion, fenthion-sulfone, fenthion-sulfoxide, fipronil, flumethrin, fluvalinate, hexythiazox, imazalil, imidacloprid, isoproturon, malathion, methiocarb, metolachlor, molinate, omethoate, parathion-ethyl, parathion-methyl, prochloraz, propanil, propazine, pyriproxyfen, simazine, tebuconazole, terbumeton, terbumeton-desethyl, terbuthylazine, terbuthylazine-desethyl, terbuthylazine-2-hydroxy, terbutryn, thiabendazole, thiamethoxam, and tolclofos-methyl.

- In Poland, in the spring of 2015, a study investigated the presence of 161 pesticides and other persistent organic pollutants such as PCBs (polychlorinated biphenyls) in 53 pollen samples collected from three hives in three apiaries located near rape fields (each sample was over 50 g and was taken, using special traps, by forager bees returning to the hive). [184] Of the 161 molecules sought, 29 were found in 60% of the 53 pollen samples examined (the analytical technique using gas chromatography with a quadrupole mass spectrometer analyser was used). 68% of the positive samples contained more than one molecule. The categories of molecules found most frequently were fungicides (triazoles such as tebuconazole which was found in 20.8% of the samples) and insecticides (found in 30% of the pollen samples), including neonicotinoids such as thiacloprid (found in 18.9% of the samples) followed by chlorpiryfos (in 13.2% of the samples). The pesticide found at the highest concentrations was prothioconazole (356 µg/kg), followed by thiacloprid (136 µg/kg), deltamethrin (69.8 µg/kg), and tebuconazole (64.6 µg/kg). Also in this research unauthorized molecules such as DDE were found in 5 of the 53 samples: DDE is associated with DDT which has been banned for over 20 years. 28% of the samples recorded concentrations of pesticide molecules in quantities higher than those provided for by the European legislation (Regulation EC 396/2005), such as: prothioconazole (3 samples), tetraconazole (1), thiacloprid (6), tebuconazole (2), deltamethrin (1), bifenthrin (1), metamitron (1). [185, 186]
Polychlorinated biphenyls (PCBs) were also sought and detected in all 53 samples at concentrations up to 413 pg/g. [184]

The Authors estimate that if a larva consumes 9.6 mg of pollen per day, it ingests 1.3 ng of the neonicotinoid thiacloprid per day, i.e. 235 ng along its lifetime (in this study the oral LD_{50} is considered 17.32 µg per bee: too high). The data from this research show that bee larvae can take 0.384 ng per day of chlorpyrifos, or 0.069 µg over their lifetime. This amount is approximately 57% of the LD_{50}. Under the worst conditions, i.e. when pollen has the highest concentrations of pesticides, significant adverse effects can easily be expected. Synergies may enhance the toxicity of neonicotinoids although these are not estimated in this work, and chronic effects have to be considered too. More significant adverse effects could be seen in queen bees as they live several years (3-5).
In this study it is shown that in the years 2005-2007, in Germany, 42 different residues were found in 70% of the examined pollen samples. [184]
Below are some of the pesticides found in the 53 pollen samples collected from bees in Poland in the spring of 2015. [184]

Pesticides in pollen	**Positive samples out of 53**	**Minimum concentration** (µg/kg)	**Average concentration** (µg/kg)	**Maximum concentration** (µg/kg)
Tebuconazole	11 (20.8%)	4	29.8	64.6
Thiacloprid	10 (18.9%)	3.3	61.3	136
Chlorpyrifos	7 (13.2%)	4.4	15.7	40.1
Propiconazole	6 (11.3%)	4.2	23.4	48.3
DDE	5 (9.4%)	5.5	7.4	9.9
Cyprodinil	5 (9.4%)	4.8	16.3	23.7
Pyrimethanil	3 (5.7%)	12.6	17.1	23.7
Boscalid	3 (5.7%)	7.6	19.2	26.6
Fenhexamid	3 (5.7%)	13.8	18.2	23.8
Tetraconazole	3 (5.7%)	14	17.7	21.8
Prothioconazole	3 (5.7%)	56.6	181.9	356.7
Tau-fluvalinate	2 (3.8%)	13.5	20.6	27.7
Deltamethrin	2 (3.8%)	23.7	46.7	69.8
Propyzamide	2 (3.8%)	12	17.7	23.3

Other active molecules found in 2 samples were: triadimenol, flusilazol, kresoxim methyl, propargit, azoxystrobin, cymoxanil; the following molecules were found in only one of the 53 pollen samples: bifenithrin, chlortalonil, metalaxyl, fludioxonil, bitertanol, imidacloprid, metamitron, clethodim, difenconazol.

- Research carried out in the USA (published in 2015) identified the presence of 53 pesticides of the 171 sought in eight crops: cotton, almonds, corn, apples, alfalfa, blueberries, melons, and zucchini. [765] The molecules are found in flowers, pollen and bees. The crops with the highest number of dead bees were cotton, zucchini, and alfalfa. Bees that died after visiting cotton fields contained 11 pesticides. Zucchini flowers contained 8 pesticides, pollen 11 and dead bees 14. Alfalfa pollen contained 5 active molecules, none of which were found in the flowers, and dead bees contained up to 10 different pesticides (individual pesticide molecules and their metabolites are found at concentrations of up to thousands of parts per billion). Bees are exposed to dozens of different pesticide molecules and their metabolites, and returning bees contain less pesticide than dead bees, which is to be expected. The hazard estimate of the exposure level, calculated from the acute toxicity of each active ingredient and the highest concentration recorded, is not related to the mortality recorded in the different crops. This result confirms the presence of effects other than acute toxicity and equally lethal.

- In Spain, research published in 2016 reports the assessment of the presence of organophosphates and azole compounds in bees. [46] Organophosphates and azole compounds may be found in water, soil and air. Organophosphorus compounds are voluntarily released into the environment because they are employed as additives in many materials and are used as flame retardants, plasticizers (in electronic devices, building materials, plastics, etc.) and have a similar structure to organophosphate insecticides. Among the organophosphorus pesticides, some are suspected of being neurotoxic[I], while others are carcinogenic[II]. Azole compounds are fungicides used in agriculture and in pharmaceutical products as anti-fungal agents: they are fluconazole, clotrimazole, propiconazole, and tebuconazole. These fungicides are considered endocrine disruptors as they disturb the synthesis of steroid hormones in humans and animals; in bees they inhibit enzymes such as aromatase (CYP19) and ergosterol synthesis.
The researchers investigated the presence of these two categories of molecules in bees. Five to eight forager bees per hive were sampled from 10 colonies located in different areas of Spain (2014). Among the azole fungicides, propiconazole (found in 7 out of 10 samples) followed by tebuconazole (5/10) were the ones recorded most frequently but at low concentrations (<7 ng/g). Fluconazole and clotrimazole were found in 2 samples.
Organophosphates were found in all foraging bee samples, which were probably contaminated by airborne molecules. [III] The organophosphate contamination was higher than the fungicide contamination. [46]

- In Spain, in 2016, pesticides contained in 35 samples of *Apis mellifera* wax were examined. [57] It is highlighted that Spain is the greatest European producer of honey (2016). The wax examined was either virgin (less than 7 days old) or old (bought from different recycled wax sellers or some beekeepers), and constitutes the most contaminated matrix of the hive. It should be noted that beekeepers have a habit of recycling beehive wax by heating it to over 70°C and centrifuging it at over 1,500 rpm. Contaminated wax can release substances to honey, pollen, royal jelly, and propolis (it may also receive substances from these matrices). Wax recycling operations, such as heat treatment (2 hours at 140°C), do not reduce the concentration of active molecules such as coumaphos (it has a half-life that may vary between 115 and 346 days).
Fifty-eight pesticides were searched for and each concentration was obtained from the average of two measurements made using liquid chromatography with mass spectrometry (triple quadrupole). Of the 58 active molecules searched for, 16 were found; 6 active molecules were found in virgin wax and 11 pesticides in old wax, with an average of 6.5 active molecules per sample. 86% of the samples were contaminated with 4 or more active molecules, 74% of the samples with 5 or more active molecules and 63% with 6 or more active molecules. Virgin wax revealed 18 times less contamination than recycled wax. The wax is contaminated mainly by acaricides used by beekeepers and to a lesser extent by insecticides and fungicides. Among the most frequently found acaricides, there is coumaphos (in 100% of the samples), fluvalinate

---

[I] Triphenyl phosphate (TPhP) and tributyl phosphate (TBP).

[II] Tris-(2-chloroetyl) phosphate (TCEP), tris-(1,3-dichloro isopropyl) phosphate (TDCPP), and tris-(2-chloro-isopropyl) phosphate (TCPP).

[III] Among the organophosphates, tributyl phosphate (TBP) and tris-(2-chloro-isopropyl) phosphate (TCPP) were found in all 10 samples in amounts between 3.3 and 113 ng/g. Tributyl phosphate (TBP) is also produced by wheeled transport (contained in hydraulic fluids). Tris-(2-butoxyethyl) phosphate (TBEP) is found in 8 out of 10 samples at concentrations below 13.1 ng/g. Tris-(2-chloroethyl) phosphate (TCEP) and triphenyl phosphate (TPhP) are also found in 9 out of 10 samples.

(86%), and amitraz (83%), at maximum concentrations of 26,858, 3,593, and 6,884 ng/g, respectively. Residues of DMF (a metabolite of amitraz) were found at the maximum concentration of 6,885 ng/g. Coumaphos and fluvalinate are widely used to control the *Varroa destructor* mite. In this research, it is shown that coumaphos and fluvalinate are the pesticides found most frequently also in wax samples examined in the USA (98.1% of samples with the highest mean concentrations of 3,300 and 7,474 ng/g, respectively). [57]
Other acaricides found in wax were chlorfenvinphos, acrinathrin and flumethrin in 77%, 71% and 54% of the samples, respectively.
Among the pesticides used in agriculture the organophosphate chlorpyrifos is present in 40% of the wax samples, dichlofenthion in 29% of the samples, malathion in 9%, fenthion-sulfoxide in 6%, and azinphos-methyl, carbendazim, ethion, hexythiazox, imazalil, and pyriproxyfen in 3% of the samples.
In recycled and/or old wax, the active molecules found most frequently were: coumaphos, fluvalinate and chlorfenvinphos in 100% of the samples; pyrethroids such as acrinathrin and flumethrin in 90% of the samples; for dichlofenthion, chlorpyrifos and DMF the frequencies were 30%, 40% and 70%, respectively.
The researchers in this publication conclude that wax, overall, is very toxic to bees. The average amount of pesticides in virgin wax was 2,726 ng/g, while the average amount of pesticides in recycled wax was 14,422 ng/g (5 times higher). The recycling of wax by beekeepers favours the accumulation of substances toxic for bees; the presence of acaricides in 100% of the samples and at high concentrations favours the appearance of resistant parasites such as the *Varroa destructor*. The high concentrations of the organophosphate chlorfenvinphos, the pyrethroid acrinathrin, and the insecticides azinphos-methyl, ethion, and carbendazim suggest the illegal use of these compounds by beekeepers.

- A study conducted in France, between 2012 and 2016, examined the presence of 13 pesticides and their metabolites inside hives. [662] A total of 488 samples of bees, wax and the mixture of pollen and honey (bee bread or larval food) were analysed. The latter matrix was the most contaminated, with 77% of the 276 samples containing at least one pesticide. An average of two active molecules per sample and a maximum of seven pesticides per sample were found in this food source for juvenile stages. In 61% of the 87 wax samples and 38% of the 125 bee samples, at least one pesticide was present. The active molecules most frequently found in all matrices (among the 13 active molecules tested) were neonicotinides (insecticides) and boscalid (a fungicide). Once again the dangerous exposure to which these insects are subjected from the earliest stages of development is demonstrated.

- A study published in 2017 estimated the exposure of bees to neonicotinoids (systemic insecticides) by monitoring honey. [373] 198 honey samples from around the World were analyzed for the presence of 5 neonicotinoids (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam). Of the 198 honey samples, 75% contained at least one insecticide (imidacloprid in 51% of the samples), 45% contained 2 or more of these molecules and 10% contained four or all five of them. The average concentration of these molecules was 1.8 billionths of a gram per gram (1.8 ng/g $\pm$ 0.56 ng/g) and the maximum concentration was 56 ng/g. In Europe, the maximum concentrations allowed in honey used for human consumption are 50 ng/g for acetamiprid, imidacloprid and thiacloprid, and 10 ng/g for clothianidin and thiamethoxam.
The amount of neonicotinoids found in honey is sufficient to generate noticeable changes in bee behaviour. This research shows how widespread the use of neonicotinoids is and that bees are exposed to mixtures with significant chronic effects. The research does not investigate the

presence of other pesticides and, therefore, is not able to estimate possible additive and synergistic effects.

- Pesticides are considered to be very dangerous for bees if they have a contact LD_{50} of less than 2 μg/bee, but not dangerous if the LD_{50} is greater than 100 μg/bee. Some acaricides can be very hazardous, such as tau-fluvalinate, which has a LD_{50} of 0.2 μg/bee. Acaricides can contaminate the wax secreted by the abdominal glands of worker bees between 12 and 18 days of age. Much of the wax is recycled by beekeepers to save bee fatigue, and to manage colony population development and honey production.

The presence of the parasitic mite *Varroa destructor* was first recorded in Belgium in 1984. Since then, beekeepers have used synthetic acaricides to control it. In Belgium, a monitoring of the level of wax contamination was conducted between 2015 and 2016: 97.3% of the 182 wax samples in which 294 pesticide residues were searched for were found to be contaminated with one or more of the 54 molecules detected. [1215] On average, 5 active molecules are found per sample with up to 12 molecules present at the same time.

Acaricides such as tau-fluvalinate and coumaphos were the most frequently found molecules (90% and 79% respectively), followed by propargite, chlorfenvinphos, bromopropylate (including the metabolite 4,4′-dibromo-benzophenone). It should be noted that the Authors of that publication point out that in Belgium the use of tau-fluvalinate and coumaphos in beekeeping is prohibited. [1215] Another acaricide banned (in agriculture and beekeeping), bromopropylate (and its metabolite dibromo-benzophenone), was also reported in 28% of the samples.

Other molecules detected with a high frequency were: the insecticide permethrin, the insect repellent DEET (diethyltoluamide; found in 36% of the samples), the fungicide pentachloroanisole (and its metabolite pentachlorophenol), as well as piperonyl butoxide (an additive, found in 29% of the samples and able to potentiate the toxic effects of other pesticides such as neonicotinoids, as it inhibits detoxification systems such as cytochrome P450); in one sample the neonicotinode thiamethoxam was found.

Recycled wax was found to be the most contaminated matrix: tau-fluvalinate up to a concentration of 8.7 mg/kg; coumaphos up to 7.4 mg/kg and chlorpyrifos-ethyl up to 4.4 mg/kg (one wax sample recorded amitraz at a concentration of 16.7 mg/kg). Among the very toxic molecules (LD_{50} less than 2 μg/bee) permethrin (in 27.1% of the samples), chlorpyrifos-ethyl (in 11.9% of the samples), p,p′-DDT, lindane, dianizon, cypermethrin, acrinathrin, deltamethrin, DDT, and tetramethrin were found. It should not be overlooked that other molecules banned and included in the Stockholm Convention because they are considered dangerous were found: DDT, p,p′-DDT and lindane (the latter in 3.3% of the samples).

Chlorfenvinphos, another insecticide not authorized for use in beekeeping, was found in 25% of the wax samples and in this study it was associated with an increased colony mortality. [1215] Other surveys also found chlorfenvinphos at high concentrations: in Germany 8.6% of the samples with concentrations up to 6.4 mg/kg, in Italy 34.5% of the samples with concentrations up to 0.63 mg/kg, in Spain 88.5% of wax samples with concentrations up to 10.6 mg/kg. These data support the suspicion that some banned molecules are used in beekeeping in several European countries. The information is very alarming and to worsen the situation some synergies are possible: even 12 pesticides are found at the same time, whose cumulative effects are largely unknown but certainly harmful. In addition to stopping the use of pesticides, wax recycling should be avoided with the aim of reducing the contamination of bee colonies and the intention of adopting less artificial breeding methods.

- Neonicotinoid insecticides may be distributed as sprays on leaves, on seeds or in the soil. These molecules are widely used in China in cotton plantations which are important nectariferous crops for bees. The use of cotton seeds treated with neonicotinoid insecticides (imidacloprid and thiamethoxam) may harm bees during flowering. It should be remembered that China grows cotton on extensive areas and is the World's greatest producer of honey (at least 400,000 t/year). [1210]
An insecticide is classified as very dangerous when the LD_{50} (dose that by contact kills at least 50% of the bees exposed in the laboratory in less than 48 hours) is less than 2 µg/bee: for imidacloprid an LD_{50} of 0.08 µg/bee and for thiamethoxam of 0.024 µg/bee is recorded; therefore they are very dangerous substances. Both active molecules may be distributed at label doses between 3.5 g and 5.3 g per kilogram of cotton seed.
A survey conducted in China in 2016 verified the presence of imidacloprid and thiamethoxam in 130 pollen samples, 130 nectar samples and 195 cotton leaf samples during flowering. [1210] Imidacloprid was found in all pollen samples, all leaf samples and 89% of nectar samples, while thiamethoxam was found in 90% of pollen samples, 56% of nectar samples and 54% of leaf samples.
Imidacloprid, distributed on cotton seed, was found in pollen at concentrations ranging from 1.6 to 64.6 ng/g and in nectar at concentrations up to 1.8 ng/g, while thiamethoxam is found in 90% of pollen samples at concentrations up to 14.5 ng/g and in nectar at concentrations up to 4.3 ng/g. [1210] In general, the concentration of imidacloprid was higher in pollen than in nectar (up to 4-fold). In leaves, imidacloprid was found up to concentrations of 45.1 ng/g, while thiamethoxam was found up to concentrations of 3.6 ng/g. The concentrations of imidacloprid are generally higher than those of thiamethoxam; it should be noted that imidacloprid is much more persistent than thiamethoxam: in the soil, imidacloprid has half-life times of more than one year, while thiamethoxam has half-life times of approximately one month.
The concentrations recorded are higher than those that generate detectable adverse effects in a few hours on bees (greater than 10 ng/g for imidacloprid), so these results confirm the presence of a high risk for pollinators, even if they are used at the doses indicated on the label (the LD_{50} for ingestion are 3.7 ng/bee for imidacloprid and 5 ng/bee for thiamethoxam).
Imidacloprid, distributed in sunflower seeds at a dose of 0,7 mg per seed, may be found in pollen at a mean concentration of 3,9 ng/g and in nectar at a mean concentration of 1,9 ng/g, while in maize pollen it may be detected at a concentration between 1 and 3 ng/g. [1210] Thiamethoxam distributed on rapeseed (at a dose of 3.2 g/kg seed) may be found in pollen at concentrations between 2 and 9 ng/g and in nectar between 3.2 and 12.9 ng/g.
Risk assessments often do not consider the chronic exposures (e.g. feeding on pollen and nectar stored in the hive), different developmental stages (larvae feed mainly on pollen) and sub-lethal and synergistic effects (e.g. metabolites with other pesticides) are underestimated. An increase in bee mortality and an irreversible decline in the abundance of wild pollinator species is reported in China too. The use of pesticides and the simplification of natural ecosystems to industrial agricultural fields are two fundamental causes.

- In the years 2014-2015, monitoring was conducted in North America (Lonoke County, Arkansas) in colonies located in two areas: an agricultural one mainly cultivated with soybeans, rice, corn and cotton (only 15% of the area was not cultivated and 4% was occupied by fish farming); and a second one where most of the area is occupied by forests and pastures. [1206]
Before monitoring began, the bee colonies recorded contamination by several pesticides: coumaphos (323 ppb in wax), fluvalinate (273 ppb in wax), chlorpyriphos (2.6 ppb in wax), and atrazine (97 ppb in bee packages, small colonies of honey bees without queens that die within a few weeks, used for pollination services).

During the two years of monitoring, 26 pesticides were detected among the 174 searched. In honey samples taken from the site occupied by woods and pastures, two active molecules are found: flubendiamide (2014) and DMPF (2,4-dimethylphenyl formamide; 2015); in bee bread samples (mixture of pollen and honey used to feed larvae) 4 pesticides were found in 2014 and 3 in 2015; in adult bee samples from 2014 no residues were found, while in 2015 only active molecules used by beekeepers were detected.
Wax taken from colonies in the agricultural area recorded 16 pesticides, while that from the forest area recorded 9 pesticides in 2014 and 7 in 2015. The molecules with the highest concentrations, at both sites, were the acaricides coumaphos (159 ppb) and fluvalinate (129 ppb) used by beekeepers. The colonies in the vicinity of the agricultural area recorded, as one would expect, the highest contamination.

- In the USA, 30 different pesticides may be used during the flowering of apple orchards, contaminating the food used by bees to feed their larvae: this is a mixture of pollen and honey called bee bread. Overall, 18 active molecules of the 25 searched were found (article published in 2017). [767] This figure highlights that bees are exposed to complex mixtures of hazardous substances from the earliest stages of development. Some of the active molecules that were found were not used in apple orchards. Bees can move within a radius of several kilometres and residues of molecules distributed in nearby fields and in previous years may be found, as is demonstrated in this research for thiamethoxam and cyfluthrin. The active molecules found most frequently were fungicides while those that probably posed the greatest risk to bees were insecticides. This study highlights the existence of a multiple exposure to mixtures of fungicides, insecticides and herbicides that may generate very dangerous synergistic and sub-lethal effects.
A review of the scientific literature shows that more than 150 different active molecules have been found in the food prepared by bees to feed the larvae.

- In Poland, the concentrations of 30 pesticides inside and outside the body of dead bees from colonies located in urban areas or rural areas were evaluated (article published in 2018). [47] Dead bees collected at the bottom of hives were used (5 dead bees per sample). Pesticides were found both outside and inside the bees' bodies. In bees collected from a rural area, the active ingredient oxamyl was found at concentrations 3 times higher than those determined in the urban area, while azinophosmethyl was more concentrated in bees from urban areas (5 ng/g). The herbicide alachlor was used in both urban and rural areas and is therefore found at similar concentrations in both areas. Azinophos-ethyl and coumaphos were only found in urban areas. Whereas thiamethoxam and methidathion were only found in rural areas. The bees collected in the rural area were also contaminated with methidathion (8 ng/g) which is an organophosphate banned in Europe.
Omethoate, qualiaphos, imidacloprid, and oxydemeton-methyl were also found outside the bee body. Hydrophobic compounds are more likely to be found inside the bee body and hydrophilic compounds outside the bee body. The highest concentrations of pesticides were found in rural areas and, in general, the molecules found at different sites were different, so bees can be used to get information about what is distributed in the environment.

- Bee colonies were monitored in the Czech Republic during the recording of increased mortality rates between 2015 and 2016. [1221] Pesticide concentrations were measured in live and dead bees taken near hives, in bee bread (a mixture of pollen, honey and bee secretion) and in

plant tissues of crops suspected of being the cause of poisoning. Bees were also sampled from three sites with no increase in mortality. Twenty-three pesticides and two metabolites were found in the 59 samples from sites with signs of intoxication. In the samples taken from areas where there were no recorded cases of bee poisoning, 16 pesticides and 1 metabolite were found at lower concentrations than at previous sites. The molecules most involved in mortality events were the organophosphate insecticide chlorpyrifos (measured in dead bees at a maximum concentration of 289 ng/g), the neonicotinoid imidacloprid (measured in dead bees at a maximum concentration of 5.3 ng/g), and the pyrethroids cypermethrin and deltamethrin. Other molecules distributed in the field in the same mixture and found in the samples were the fungicides prochloraz, azoxystrobin, and tebuconazole. An interesting observation that emerged in this study was that the weight of dead bees was significantly lower than that of live bees. [1221] Moreover, dead bees recorded the simultaneous presence of very toxic molecules (e.g. cypermethrin and prochloraz; chlorpyrifos and cypermethrin; chlorpyrifos, deltamethrin, prochloraz and thiacloprid). The results of the monitoring confirm that some cases of mortality were due to the exposure to pesticides, especially those used in the most common crop near the apiaries: rape.
A positive remark: the neonicotinoids clothianidin, thiamethoxan and imidacloprid have been banned in Europe since 2018 and thiacloprid since 2020 (barring exemptions, reconsiderations, and illegal uses). [1221]

- In India, 83 single-flower and 17 multi-flower honey samples were examined for information on the presence of 24 pesticides (paper published in 2018). [1218] Nine active molecules were found in 19% of the 100 samples: 6 organophosphates, 2 pyrethriodes, and 1 organochlorinated. The molecules found most frequently were: dichlorvos (in 6 samples at the maximum concentration of 226 ng/g), monocrotophos (in 5 samples and at the maximum concentration of 430 ng/g), profenophos (in 5 samples and at the maximum concentration of 43.2 ng/g), permethrin (in 4 samples and at the maximum concentration of 40 ng/g), ethion (in 3 samples and at the maximum concentration of 28 ng/g), and lindane (in 3 samples and at the maximum concentration of 99 ng/g; lindane has been banned in India since 2013). Phorate, chlorpyrifos, and cypermethrin were also found. The most contaminated honeys were found to be cotton and sunflower honeys. In this study, few molecules were searched for and in any case 18% of honeys record pesticide concentrations above the maximum limits allowed for export to Europe (10 ng/g; the Authors point out that in India there is no regulated maximum limit for pesticide residues in honey). [1218]

- Persistent organic pollutants are different substances such as organochlorine insecticides, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). In Argentina in the last 15 years the use of pesticides has increased by at least 300%, and chlorpyrifos and cypermethrin are the most used molecules. [1230] The main crops in Argentina include soybean, corn, wheat and sunflower. A study conducted in the Buenos Aires Province investigated the presence of persistent organic pollutants and the insecticide chlorpyrifos in several beekeeping products. The bee colonies were located in three types of sites: one in an area dedicated to fruit and horticulture, one mainly occupied by soybean and a third one near a municipal waste treatment centre (occupies 102 ha and receives 1,150 t of municipal solid waste per day). [1230] Beekeeping products were sampled in August and September 2016. Pesticides were found at high concentrations near the soybean fields and the site where the waste disposal takes place. Many toxic and banned molecules were found in the bee samples: DDT (and its metabolites up to 11.3 ng/g), although its use has also been banned in Argentina

since 1998; endosulfan, also banned since 2013; lindane, banned since 1998; heptachlor, banned since 1998; and dieldrin, banned since 1980. The organophosphate insecticide chlorpyrifos was found (at concentrations of up to 8.9 ng/g in bees) in colonies in the vicinity of soybean crops where it was used. PCBs were found at a maximum concentration of 47.7 ng/g in bees located in the vicinity of the waste disposal site. PBDEs were also found at high concentrations (up to 80 ng/g).
The pollen stored within the colony was also contaminated by pesticides (e.g. chlorpyrifos at a maximum concentration of 5.6 ng/g), PCBs (at a maximum concentration of 12.8 ng/g), and PBDEs (at a maximum concentration of 0.7 ng/g). PCB and PBDE concentrations in pollen were highest at the site with the waste disposal centre. [1230]
All honey samples contained DDT (up to 7.7 ng/g), lindane (up to 2 ng/g), endosulfan (up to 1.5 ng/g), and chlorpyrifos (up to 3.9 ng/g). PCBs and PBDEs were found in some honey samples.
The soil samples were also contaminated with these persistent substances, as one would expect: DDT up to a concentration of 2.8 ng/g (the half-life of DDT in the soil is about 25 years and it can be a derivative of dicofol), lindane (up to 0.5 ng/g), chlorpyrifos (up to 0.4 ng/g), PCBs (up to 2.5 ng/g), and PBDEs (up to 0.8 ng/g).
These persistent pollutants were found in flowers at concentrations overall higher than those recorded in the soil (lindane up to 1.4 ng/g; PCBs up to 10.8 ng/g).
In conclusion, the research shows the presence of toxic molecules, persistent and banned for years in all matrices examined: soil, flowers and beekeeping products; bees are the matrix with the highest concentrations.

- In Spain in June and July (2017), the pesticide content in live worker bees, pollen stored in the hive, and wax was examined in 45 apiaries located in 39 areas. The areas were classified as agricultural (with intensive agriculture) if more than 50% of the area was cultivated and were categorized into rural, urban or mountainous, and low or high agricultural intensity. [54] A total of 63 pesticides were tested in 133 samples taken from 5 randomly selected hives per apiary. Wax showed the highest concentrations of acaricides used by beekeepers, such as coumaphos, chlorfenvinphos, fluvalinate, and acrinathrin, which were present in 75% of the samples. Wax was contaminated with 8 pesticides with an average of 4.5 molecules per sample and up to 7 pesticides were found simultaneously (in 6 samples). Fluvalinate, coumaphos and acrinathrin were measured in 70% of the samples. The concentrations of fluvalinate, coumaphos, acrinathrin, and chlorfenvinphos in wax were 103, 2,252, 10,168 and 13,204 times higher than in live bees. Some molecules, such as coumaphos, do not degrade with the heat treatments carried out to recycle the wax and are stable (a concentration of up to 53,400 ng/g was measured in this study). The concentrations of acaricides measured in wax were also higher than those found in pollen (60-fold for acrinathrin and 132-fold for chlorfenvinphos): wax has a higher hazard level for bee health than pollen.
Pollen was sampled inside the hives (stored during the year) and was contaminated by both insecticides used in the field (chlorpyrifos and acetamiprid) and acaricides used by beekeepers. The highest number of pesticides was found in pollen. Pollen collected from beehives in agricultural areas was the most contaminated by insecticides. In pollen, 14 pesticides were found, 8 of which were derived from agricultural use and 6 from beekeepers (one sample contained 10 pesticides and only one was free of the residues searched). Chlorpyrifos was the most frequently found insecticide. The acaricides used by beekeepers were also the most frequently found molecules in pollen, such as coumaphos, fluvalinate and amitraz (and their degradation products). Pollen during storage in the hive may absorb contamination from the

wax and vice versa (e.g. fat-soluble pigments such as carotenoids in pollen colour the wax), whereas freshly collected pollen from bees does not contain this contamination.
In live bees, pesticides were found at lower concentrations and less frequently: 45 samples of bees (34%) were contaminated with 7 pesticides (maximum 4 molecules in the same sample of live insects). Acaricides used by beekeepers were the most frequently detected molecules: coumaphos, fluvalinate and amitraz.
Unauthorized products against *Varroa* such as chlorfenvinphos (an organophosphate) and acrinathrin (a pyrethroid) were found in all three matrices tested, at concentrations that could not be consequent to the use of these molecules in the field. Other molecules not authorized in Europe, such as dichlofenthion, carbendazim and fenitrothion, were also present in pollen and live bees.
In conclusion, if we consider the quantities, wax is the most contaminated matrix and is, for bees, a more dangerous matrix than pollen. [54] The latter contains a greater number of molecules: pollen is taken after being stored in wax cells, so it has also absorbed some pollutants contained in the wax and used by beekeepers. Samples from live bees are the least contaminated.

- Research conducted in Poland (published in 2018) examined mortality and behaviour induced by artificial feeding of groups of 160 female workers with pesticide-enriched feed. [485]
Worker bees aged 10 days (*Apis mellifera* of the Carniola race) were fed with a sugar syrup (at the concentration of 2 mol/dm^3). The sugar solutions contained different pesticides (a different one for each group of 160 bees): thiram (a fungicide administered at a dose of 0.6 g/100 cm^3 syrup), alpha-cypermethrin (an insecticide administered at a dose of 0.04 cm^3/100 cm^3), copper oxychloride (a fungicide administered at a dose of 0.35 g/100 cm^3), thiamethoxam (an insecticide administered at a dose of 0.04 g/100 cm^3), bentazone (an herbicide), and metamitron (an herbicide). The mortality rate in the control group was 1.92 bees per day, or 1.2% (the experiment lasted a maximum of 7 days). The highest mortality rate was recorded among bees fed with thiamethoxam, which was 57 times higher than in the control (over 110 dead bees per day out of 160, i.e. 69%): after 36 hours they were all dead. It should be noted that, while in the control, bees ingested 250.1 mm^3 of sugar solution per day, in the case of the solution containing thiamethoxam they ingested only 0.15 mm^3, i.e. 1,667 times less.
Mortality rates were also high for the group of bees fed with the fungicide copper oxychloride (35.15 dead bees per day, i.e. 22%): after 4.5 days they were all dead; in the case of bees fed with the insecticide alpha-cypermethrin (32.48 dead bees per day, i.e. 20%), they were all dead within 5 days. Also in these two cases, the amount of syrup ingested per day was much less than in the control: 52 times less for the inorganic fungicide (4.75 mm^3/bee/24 h) and 111 times less for the solution with the insecticide (2.25 mm^3/bee/24 h). [33]
The fungicide thiram (mortality of 5.2 bees per day out of 160), the herbicide bentazone (mortality of 3.2 bees per day out of 160) and the herbicide metamitron (mortality of 13 bees per day out of 160) show lower mortality rates (between 2% and 8% per day) but still higher than the control. These molecules also show a significant reduction in the intake of syrup contaminated with pesticides: between 18 and 15 times less than the control.
In the study, they tried to measure the variation of bee behaviour following the intake of the different molecules, compared to the control: aggressiveness, mobility, grooming (this term indicates the reciprocal cleaning that may have not only a hygienic function but also a social one of recognition and communication). The most conspicuous behavioural changes were recorded following the intake of thiamethoxam.
The intake of the copper fungicide resulted in a 75-fold increase in the concentration of this element in the bees' bodies compared to the control (maximum copper concentration of 2,103 mg/kg). Nickel was also found at 4.6 times higher concentrations than in the control

(concentration of 3.18 mg/kg). The group fed with thiamethoxam showed a similar increase in concentration (3.33 mg/kg). In conclusion, neonicotinoids generate in this experiment the highest mortality rate and the greatest behavioural change, and copper-based fungicides the highest accumulation of this element. [485] In the vineyards of some territories, copper has been the most widely used pesticide for more than a century. This metal can bioaccumulate in leaves, nectar and pollen.

- The honey bee (*Apis mellifera*) in the United States is the main pollinator for at least 0.8 million hectares but the number of colonies raised is decreasing: in Virginia the number of colonies decreased from 78,000 in the 1980s to 40,000 in 2018. In order to have more information on the causes of the decline in the number of colonies in Virginia, the presence of pesticides was investigated in 148 colonies distributed in 48 apiaries, between March 2014 and July 2016 (research published in 2019). [1193] In 288 samples of honey, 160 of pollen and 68 of wax, the following pesticides were searched for: pyrethroids (bifenthrin, cyhalothrin, permethrin, cyfluthrin, cypermethrin, and fluvalinate), organophosphates (chlorpyrifos, coumaphos, and coralox), organochlorines (chlorothalonil) and triazines (atrazine). The presence of 9 out of 11 pesticides was detected in at least one matrix: 3% of honey samples were contaminated, 8% of pollen samples and 19% of wax samples (cyfluthrin and permethrin were not found in any sample). The acaricides fluvalinate and coumaphos are the molecules found most frequently and at the highest concentrations, mainly in wax (at concentrations of 69,700 and 15,500 ng/g, respectively; the LD_{50} are 15,900 ng/g for fluvalinate and 46,300 ng/g for coumaphos). Therefore, bees are exposed to concentrations of acaricides used by beekeepers (to control *Varroa destructor*) that are certainly harmful: coumaphos was found in 90% of the wax samples, 26% of the pollen samples and 7% of the bee samples, while fluvalinate was found in 85% of the wax samples, 29% of the pollen samples and 4% of the bee samples. The Authors report that chlorothalonil records the highest concentrations in pollen.

- In Belgium, during 2016-2017, a study searched for glyphosate and its metabolite (aminomethylphosphonic acid or AMPA) in 379 apiaries reared by non-professional beekeepers (2,997 *Apis mellifera* colonies). This herbicide was introduced onto the market in 1974 and at least 471 tons were sold in Belgium in 2015 (the main application was as a spray on leaves). [1195] The degradation time of 50% of glyphosate in the soil is estimated between one and 67 days, while to record the degradation of 90% it is necessary to wait between 9 and 1,661 days (under conditions reproduced in the laboratory so in real conditions the times could be longer). In the environment, the AMPA metabolite is more persistent than the starting molecule: the degradation time of 50% in the soil is estimated between 30 and 330 days. The high variation of the interval highlights a large margin of uncertainty.
Glyphosate has toxic effects in humans at very low concentrations: it is teratogenic, carcinogenic and causes liver and kidney damage. Some of these effects may also be a consequence of other molecules present in mixtures of commercial products (they may enhance the toxicity of glyphosate).
Bees produce a matrix called bee bread, which consists mainly of pollen with which they feed their larvae. In this research the herbicide is sought in 179 samples of food used for the larvae, in 100 samples of wax from the larval cells and 10 samples of honey. [1195]
The 179 bee bread samples were taken in September and October 2016 from 193 apiaries (there were 865 colonies in total): 91% recorded glyphosate, 26% its metabolite AMPA, and 7% both molecules. The maximum concentration was 700 ng/g, which corresponds to the intake by the larvae, in 10 days, of about 84 ng of glyphosate (12 mg of pollen per day), which is below the

LD_{50} of 100 µg/bee. AMPA was found at the highest concentration of 250 ng/g (no LD_{50} was estimated for this molecule).
32% of the 100 wax samples contained glyphosate at a maximum concentration of 320 ng/g and 2% of the 10 honey samples contained the herbicide at a maximum concentration of 11 ng/g (the maximum concentration permitted for human consumption in Europe is 50 ng/g and concentrations as high as 163 ng/g have been measured in the literature). It should be noted that beekeepers also renew more than 50% of the wax each year.
Glyphosate concentrations reveal the possibility of recording sub-lethal effects such as reducing the amount of certain proteins in the insect body (e.g. beta-carotene), decreasing sensitivity to the presence of sugars (these effects are measured at exposures below 5 ng/bee), and altering the gut microbial composition (these effects are measured at exposures below 0.5 µg/bee). A limitation of these toxicology considerations is that synergistic effects with other molecules are not evaluated.

- In China, in the years 2016 and 2017, the presence of 66 pesticides was investigated in 189 pollen samples (taken by beekeepers) and 226 samples of bee bread (the mixture of pollen and honey prepared to feed the larvae). [1205] Thirty-two pesticides were detected in pollen, including 22 insecticides, 7 fungicides, 2 herbicides, and one insect growth regulator. Only 3.5% of pollen samples and 3.7% of bee bread samples recorded no pesticides (among those searched for). Samples with many residues at the same time were frequent, for example, more than 20% contained more than six active molecules (the simultaneous presence of carbendazim and chlorpiriphos is one of the most frequent combinations). The pesticides found in pollen samples with the highest frequencies were: carbendazim (76%), fluvalinate (53.4%), chlorpyrifos (51.3%), fenpropathrin (45%), bifenthrin (36%), deltamethrin (18%), chlorbenzuron (18%), thiamethoxam (18%), lambda-cyhalothrin (17%), fenvalerate (14%), triadimefon (14%), coumaphos (12%), and imidacloprid (11%). In bee bread samples, 31 pesticides were found, the most frequent ones being carbendazim (78%), fluvalinate (54%), chlorbenzuron (63%), fenpropathrin (49%), chlorpyrifos (35%), coumaphos (20%), triadimefon (16%), fenvalerate (15%), bifenthrin (13%), trichlorfon (13%), and imidacloprid (12%). Concentrations may be very high: carbenzadim in bee bread reached 14.5 µg/g. Some molecules were present in higher concentrations in pollen (thiamethoxam, fenpropathrin, bifenthrin, and chlorpyrifos) while coumaphos and fluvalinate (acaricides used by beekeepers) were recorded in higher amounts in bee bread. These results confirm that indispensable food sources are poisoned.

- In Brazil, in 2018, 43,000 tons of honey were produced and as in other countries of the World there is a decrease in the number of colonies: in Brazil, in the period 2013-2017, the number of colonies of bred bees (*Apis mellifera*) decreased by 50%; in the period 2017-2018 in the USA there was a decrease of 30% in the number of colonies; while in States such as Spain and Portugal it was of 32%. The main cause were pesticides: Brazil turns out to be the Nation with the largest global market for pesticides. [1194] The herbicide glyphosate is widely used in the cultivation of soybeans, coffee, sugar cane, corn, cotton, citrus (and other crops) therefore it is found in soils, water and food (in Brazil in 2017 at least 173,000 tons were distributed). It must be remembered that in Brazil, soybean cultivation promotes the deforestation of heritages such as the Amazonia forest. In Europe, a safety concentration for glyphosate in honey of 0.05 µg/g has been established, while in Brazil there are no indications in this regard. Some agencies (EFSA and EPA) have indicated a contact LD_{50} for glyphosate of over 100 µg/bee. However, some studies report sub-lethal effects at lower concentrations, such as memory impairment and impaired ability to return to the hive. Glyphosate exposure has been reported to have neurotoxic effects and impaired cognitive ability in rats and freshwater *zebrafish*. Glyphosate can be found in Brazilian honey: 38% of 40 honey samples from 23 different sites recorded the presence of this herbicide. The metabolite (AMPA) was found in only one sample and six samples showed glyphosate concentrations well above the safety threshold established in Europe (0.05 µg/g). The Authors point out that other studies confirm the presence of the herbicide in honey: glyphosate is detected in 59% of 69 honey samples in the USA, 98% of 200 honey samples in Canada, and 94% of 16 honey samples in Switzerland.

- In Greece, in the years 2014 - 2018, monitoring was conducted in apiaries where high mortality rates (greater than 50%) are recorded. [1204] Dead bees were taken in the vicinity of the hives. 70 pesticides were searched in 320 samples of dead bees which were found at the maximum concentration of 166.5 µg/g. The molecules found with the highest frequencies were clothianidin (20.1%), coumaphos (12.1%), and imidacloprid (9.1%). Other molecules recorded were neonicotinoids such as thiamethoxam (3.3%), acetamiprid (2.9%), and thiacloprid (2.4%), organophosphates such as dimethoate (4.1%), chlorpyrifos ethyl (3.3%), and pyrethoids such as tau-fluvalinate (2.3%), cypermethrin (2%), λ-cyhalothrin (1.3%), deltamethrin (1%), acrinathrin (1%), etofenprox (0.7%), and cylfuthrin (0.3%). Many metabolites such as imidacloprid-olefin, imidacloprid urea, 5-hydroxy imidacloprid, 6-chloronicotinic acid (6-CNA), chlopyrifos oxon, coumaphos oxon, acetamiprid-n-desmethyl, prothioconazole desthio, metabolites of fenthion, amitraz (DMF, DMPF, DMA) and fipronil were also found in bees. Some metabolites, such as those of neonicotinoids, were found at concentrations higher than the LD_{50} and it must be pointed out that some derivatives may have greater negative effects than the starting molecules, as happens for the action of inhibition of acetylcholinesterase by chlorpyrifos oxon. Seven molecules were also found in bees at the same time (difenoconazole, clothianidin, imidacloprid, carbendazim, trifloxystrobin, tebuconazole, pyrimethanil), which can generate very dangerous additive and synergistic effects (other mixtures in single samples were: coumaphos, imidacloprid, chlorpyrifos ethyl; clothianidin, DMF, DMPF). Among the molecules present at high concentrations in dead bees, there are cypermethrin (up to 260 times the LD_{50}, i.e. the dose that by ingestion or contact kills 50% of the exposed individuals in less than 48 or 72 hours), imidacloprid (up to 186 times the LD_{50}), a metabolite of imidacloprid (imidacloprid olefin), dimethoate (up to 123 times the LD_{50}; this molecule is used in olive cultivation and contaminates wild flowers), methomyl (up to 104 times the LD_{50}, deltamethrin (up to 55 times the LD_{50}), chlorpyrifos ethyl (up to 29 times the LD_{50}), malathion (up to 6 times the LD_{50}), clothianidin (up to 5 times the LD_{50}), thiamethoxam (up to 2.5 times the LD_{50}), λ-cyhalotrhin (up to 2.4 times the LD_{50}).
In 184 samples of dead bees (out of a total of 320), the presence of the parasitic fungus *Nosema* and the *Varroa* mite was investigated: 27% showed the presence of the fungus and 22.2% of the mite. 29.3% of dead bee samples were simultaneously exposed to pesticides and parasites (*Nosema* and/or *Varroa*). These synergies weaken the colonies further compromising their ability to survive. The results of this study confirm the main cause of bee death: pesticides.

- In Spain in the years 2016 - 2018 three apiaries were monitored of which two were located near intensively cultivated areas (mainly citrus), and a third in semi-natural areas with some olive plantations and other crops. [1219] During these two years, mortality was monitored and collections were made of 38 samples of live bees, 17 samples of dead bees (they were collected when there is an increase in the natural mortality rate, which is considered to be about 20 dead bees per day in the vicinity of the colony), 33 samples of bee bread (a mixture of pollen and honey prepared by worker bees for the larvae), and three wax samples taken at the beginning of the monitoring (June 2016). The wax was found to be highly contaminated with coumaphos (up to 5,085 ng/g) and chlorfenvinphos (up to 320 ng/g). These compounds were not used during the study therefore they are derived from recycled wax and suggest an illegal use. The acaricide amitraz was used during monitoring and its metabolite (DMF) was found only in the apiary in the semi-natural area at the maximum concentration of 190 ng/g.

In the 33 larval food samples, 17 pesticides with peaks of 8 molecules present at the same time were recorded, coming from the two apiaries located in the intensive agricultural area. Thus, bees are exposed to complex and dangerous mixtures from the earliest stages of development. In the samples taken from these two apiaries an average of 5 pesticides was found, while in the third one an average of 3 pesticides was detected (located in the semi-natural area). Overall, the bee bread collected from the colonies located in the two intensive agricultural areas is at least 6 or 7 times more toxic than that of the apiary in the semi-natural area. Some pesticides were recorded in bee bread at high concentrations: up to 34 ng/g of dimethoate (found in 25% of the samples), up to 28 ng/g of methiocarb (found in 9% of the samples), up to 167 ng/g of chlorpyrifos (found in 45% of the samples), up to 40 ng/g of acrinathrin. Amitraz was detected in 97% of the bee bread samples and coumaphos in 94% of them. [1219] Dangerous concentrations of acaricides used by beekeepers but also of pesticides used in agriculture (e.g. insecticides such as dimethoate, imidacloprid in 12% of the samples, or fungicides such as carbendazim found in 30% of the samples and tebuconazole found in 6% of the samples) are found in the food of bee larvae.

Apiaries located in intensive agricultural areas were more contaminated and in fact signs of bee poisoning were observed in these areas but not in the one located in the semi-natural area (three mortality peaks between 160 and 256 dead bees per day in one apiary and two peaks in the other). Dead bees were contaminated by dimethoate (76.5% of samples at concentrations above 338 ng/g) and its metabolite (omethoate), chlorpyrifos (in 41.2% of samples at concentrations above 2,700 ng/g), fluvalinate (35.3% of samples), imidacloprid (11.8% of samples, i.e. two samples with 22 and 476 ng/g, respectively), the metabolite of amitraz (5.9% of samples), hexythiazox (17.6%), coumaphos (5.9%), pyriproxifen (11.8%), and acetamiprid (11.8%).

Some molecules were found in dead bees but not in live ones: hexythiazox (maximum concentration of 266 ng/g of dead bees), pyriproxyfen (maximum concentration of 558 ng/g), imidacloprid (maximum concentration of 476 ng/g), acetamiprid (maximum concentration of 14 ng/g). The use of neonicotinoids is forbidden so the concentrations found suggest an illegal use in agriculture. It must be remembered that these molecules are able to show negative effects at doses of a few billionths of a gram per bee, i.e. at concentrations at least 100 times lower than those found in the dead bees.

The pesticides found in the apiaries are sufficient to explain the increase in mortality rates and, among the most incriminated molecules, the Authors highlight dimethoate and chlorpyrifos, two organophosphate insecticides (the first one in dead bees was present at a maximum concentration more than 10 times higher than in live bees, the second one 122 times), and neonicotinoids such as imidacloprid. In conclusion, this study confirms a high contamination of wax by beekeepers and the illegal use of pesticides by both farmers and beekeepers.

- In Croatia, organic honey production corresponds to about 0.4 percent of the total production (between 7 and 8 tons per year) despite allowing better earnings. A research compared the concentrations of different pollutants, such as metals, pesticides, polychlorinated biphenyls and antibiotics (sulphonamides streptomycin, and dihydrostreptomycin), in samples of organic and traditional honey with the same botanical origin. Between 2018 and 2019, 61 honey samples were examined, of which 16 were from organic farming. [1197] Some results can be summarized as follows

- A higher presence of chromium is detected in organic chestnut honey than in conventional honey. Samples of multifloral organic honey showed higher concentrations of manganese and nickel than conventional honey (although the differences were not significant).
- None of the antibiotics and polychlorinated biphenyls searched were found.
- Of the 121 pesticides sought, only amitraz (and its metabolites such as DMF), formamide and coumaphos were found. In Croatia, the use of the following acaricides by conventional beekeepers is permitted: amitraz (formamidine), coumaphos (organophosphate) or flumethrin (pyrethroid). Amitraz and/or coumaphos were found at concentrations below the maximum levels permitted in conventional honey (0.2 and 0.1 mg/kg, respectively) in 2 of the 16 organic honey samples and in 34 of the 45 conventional honey samples. Coumaphos is more easily found than amitraz because it has a longer persistence and because beekeepers probably use it more often, however it should not be present in organic honey.

In summary, honey of organic origin was found to be much less contaminated by acaricides than honey from traditional production: honey of organic origin registers active molecules less frequently and with lower concentrations, but it is still poisoned.

The use of pesticides and intensive agricultural production in general have harmed honeybees, but not only. In less than 60 years, chemical agriculture has favoured the extinction of animals and plants, altering the capacity of ecosystems to ensure essential services. The massive and constant use of poisons created by human ingenuity, such as insecticides, herbicides, and fungicides has favoured the reduction of biodiversity. The use of pesticides has increased the dependence of intensive agriculture on these poisons because it has reduced the capacity of ecosystems to provide a natural control of plant pathogens. In Europe, agricultural land occupies an area of at least 43%, making them the main refuge for wild species: at least 50% of bird biodiversity. In Germany and England 20-30% of the wild flora is found in agricultural areas.

Over time, industrial cultivations, like cereal cultivation, has increased yields per unit area but has worsened the chances of survival of wild plants and birds: there is a proven inverse correlation between cereal yields and the number of species nesting on the ground. [1198] On the other hand, as cereal yields have increased, the survivability of pests such as aphids has increased, as their natural enemies can no longer perform their biological control function. The presence of insectivorous birds decreases as the use of insecticides or fungicides increases. [433] Consequently, the frequency of predation on pathogenic insects such as aphids by insectivorous birds decreases with the use of pesticides. The application of the principles of organic farming, i.e. not using pesticides, may help increase the biodiversity useful to farmers such as that of wild plants and pollinators. Unfortunately, interventions such as the diffusion of pesticide-free agriculture do not generate consistent positive effects on the biodiversity of animals that move over long distances (e.g. birds, butterflies) as they are negatively affected by pollutants and alterations produced elsewhere. [1198]

INVESTIGATIONS IN ITALY INTO PESTICIDES AND OTHER MOLECULES SUCH AS POLYCHLORINATED BIPHENYLS (PCBS)

With the aim of underlining the dangers generated by the extensive and massive use of pesticides, we report the results of several monitoring studies conducted in Italy. These results also show a high and widespread contamination of beekeeping products. Bees are systematically exposed by beekeepers and farmers to hundreds of dangerous molecules.

- It is interesting to summarize some results of a survey carried out in 34 Italian Provinces using 400 monitoring stations. [17] Between 1983 and 1986, 581 samples of dead bees were analysed. 76% (442 out of 581) of the dead bee samples were positive for the following active molecules:

- Dithiocarbamates (mancozeb, maneb, metiram, zineb, ziram): 70.8%.
- Dimethoate: 15.3%.
- Parathion: 14.7%.
- Azinphos-methyl: 11.9%.
- Carbaryl: 11%.
- Methyl parathion: 10.4%.
- Endosulfan: 7.2%.
- Omethoate: 7.2%.
- Methamidophos: 2.4%.

More than thirty years ago, the signs of bees being exposed to very dangerous substances were already evident.

- A monitoring (lasting two years: 1987-1988) carried out in the Province of Ferrara (northeastern Italy) confirmed that the molecules most frequently used by farmers were those found most frequently in dead bees. [17] This study confirms the usefulness and effectiveness of bees as bioindicators.

- A survey conducted in Emilia Romagna in 1998, using 13 biomonitoring stations (each of which consisted of two hives), recorded 47 events of abnormal increase in mortality and, of the 47 bee samples analysed, 38 (80.9%) recorded the presence of at least one pesticide. [17] Active molecules used against aphids such as dimethoate and omethoate were found, as well as fenitrothion, methyl parathion, methamidophos, methidathion and fenoxycarb (whose use has been prohibited in Italy since 1995). The pollen found in the dead bees made it possible to trace the crops visited and to know whether the treatments had been carried out during flowering.

- A three-year biomonitoring conducted in Italy in 2008-2010 investigated pesticide contamination in 10 nature reserves in the Marche Region (central Italy). [33] Twenty-two bee colonies were used in 11 stations from which honey, live bees (100 bees per each sample) and dead bees were sampled in the months from May to October. Dead bees were collected with special traps placed in the hive. The samples did not contain the pesticides tested (30 organophosphates, 13 pyrethroids, and 16 triazoles).

- In Italy, a monitoring was carried out using 130 apiaries (with more than 10 hives each) distributed throughout the national territory (stationary apiaries were used). [163] The monitoring was conducted in 2009 and 2010. Each colony was sampled four times a year: at the end of winter, between spring and summer, at the end of summer and in autumn. Samples consisting of 50 live insects, the mixture of pollen and honey used to feed the larvae, and pieces of wax (5 cm x 5 cm) were examined: a total of 1,500 samples were taken per year and 128 chemical compounds were searched for (by means of liquid chromatography with mass spectrometry and gas chromatography with electron capture detectors). [163]

Wax was the most contaminated matrix: 40% of the samples had pesticides and 12.7% had more than one residue (at most 4 pesticide residues were found). Pesticides were found in 12% of the honey samples.

A survey conducted later (in 2012) on Italian honeys detected neonicotinoids in 15% of the analysed honey samples. [35]

Returning to the national monitoring (2009/2010), 25 different molecules were found in the mixture prepared by bees to feed the larvae (it consists of pollen and honey), 21 active molecules in honey, and 17 in wax. [163] The most frequently found pesticides were organophosphates and pyrethroids; coumaphos and tau-fluvalinate were found in wax. These molecules are used by beekeepers against *Varroa* (parasitic mite). Coumaphos was the molecule found with the highest concentration in wax: 12,779 ng/g. Other molecules found inside the hive and at high concentrations were propamocarb (fungicide) and flumethrin (452 ng/g in honey). Prohibited molecules were also found (chlorfenvinphos and rotenone; the latter was found in 35% of honey samples in 2009).

The risk analysis reveals that the molecules with the highest concentrations of concern (exceeding the LD_{50}) were the neonicotinoids (imidacloprid, thiamethoxam, clothianidin) and fipronil. Imidacloprid and clothianidin were found in high concentrations in live bees and thiamethoxam in larval food. Some insecticides were also found in honey.

In 2009/2010, bee colony mortality was 19% (in 2007 it was 30-40% in Northern Italy and 10-30% in Central-Southern Italy). Mortality rates in the various regions of Italy were variable, ranging from 0% to 34%. A positive relationship between pesticide residues found in adult insects and mortality is evident, as one would expect. This study confirms the correlation between the mortality of bee colonies and the percentage of agricultural land near the hives.

- The examination of the presence of 61 organic contaminants in 47 samples of Sicilian and Calabrian honey, collected in the years 2012-2013, recorded organophosphate pesticides in 100% of the samples and organochlorine pesticides, pyrethroids, and other hazardous molecules (e.g. insect growth regulators) in 53.2% of the samples. [29] Polycyclic aromatic hydrocarbons (PAH) were found in 46.8% of honey samples.

The honey samples were taken directly from the beekeepers, who gave assurances that they had not carried out any treatments in the six months prior to harvesting the honey. Each sample consisted of 500 g of honey from different floral combinations: 21 samples from wildflowers, 6 chestnut honeys, 6 citrus honeys, 3 from sulla, 3 from wildflower honeydew, 3 from acacia, 2 from eucalyptus, 1 from thyme, 1 from chestnut and eucalyptus, and 1 from wildflower.

All samples were contaminated with one or more of the molecules searched. 46.8% of the samples (22 of 47, including 21 samples of Sicilian honey and one from Calabria) showed concentrations higher than the maximum limits allowed in Europe for chlorfenvinphos (however, it was found in all 47 samples), tebuconazole, coumaphos, t-fluvalinate, triphenyl phosphate (or TPP), lindane (or γ-hexachlorocyclohexane or gamma-HCH). In detail: 22.7% of the samples showed concentrations above the maximum limits allowed by the regulations for triphenyl phosphate (or TPP), 4.5% for tebuconazole, coumaphos, t-fluvalinate, and lindane. Other results are summarized as follows:

- Among the organophosphates, coumaphos was found in 98% of the samples, triphenyl phosphate (or TPP) in 85% of the samples, diazinon in 30% of the samples, phosmet in 23% of the samples, chlorpyriphos-ethyl in 2% of the samples.
- Among organochlorine pesticides, 4,4'-DDT was found in 23% of the samples, lindane in 19% of the samples, 2,4'-DDT in 13% of the samples, endosulfan II in 13% of the samples.
- Among polycyclic aromatic hydrocarbons, phenanthrene was found in 34% of honey samples, fluorene in 26% of the samples, pyrene in 11% of the samples, acenaphtalene in 2% of the samples.

Piperonyl butoxide, in 28% of the samples, tebuconazole in 11% of the samples, flusilazole and pyripoxyfen in 6% of the samples were also found.
The Authors of the publication point out that chlorfenvinphos, which was found in all 47 samples, could be accumulated in the wax, which is recycled and can transfer part of its active molecules to the honey.
This research demonstrates the use of pesticides in high quantities and the use of banned active molecules that are found in honey at concentrations dangerous to bees but also to consumers.

- A three-year research conducted in Italy (in 11 Regions), in the years 2012-2014, investigated the presence of pesticides in pollen in 53 apiaries, each formed by 5 colonies, of which 65% produced honey certified as organic (samples were collected between March and September). [32] Pollen was collected from insects returning to the hive using special mechanical systems. Pollen is a very important food for the juvenile stages (it is a source of proteins), so its contamination risks dangerously to poison the new generation (they eat more than 9.5 mg/bee/day). Among the 66 molecules searched for, 10 fungicides and 8 insecticides were found. A total of 554 pollen samples were analysed and 62% contained at least one pesticide. In 38% of the samples, more than one residue was detected: in 1% of the samples, up to 7 molecules were detected at the same time; 24% detected only one molecule among those searched for.
13% of the pollen samples showed concentrations of pesticides considered dangerous for bees and 39% of the samples showed concentrations considered dangerous for human health by the European legislation (*acceptable daily intake* or *maximum residue limit*). Among the molecules found at concentrations higher than those considered non-hazardous for humans, there are: azoxystrobin, benalaxyl, boscalid, chlorfenvinphos, chlorpyrifos, dimethoate, mandipropamid, metalaxyl, phenthoate, and tebuconazole. The active molecules found most frequently were:

- the insecticide chlorpyrifos (in 30% of the samples and with the highest concentration of 179 µg/kg recorded in Piedmont, northwestern Italy);
- the fungicide mandipropramid (in 19% of the samples, with the highest concentration of 261 µg/kg);
- the fungicide metalaxyl (in 16% of the samples and with the highest concentration of 2,463 µg/kg);
- the fungicide spiroxamine (15%);
- the neonicotinoid insecticide imidacloprid (12%).

17% of the pollen samples contained neonicotinoids, which are very dangerous to bee health. These molecules are persistent in the soil and may also be found in water. From the

concentrations of pesticides measured in pollen, it is possible to predict a 50% increase in mortality in 10 days.
The insecticide chlorpyrifos was also found more frequently because it is marketed in large quantities in more than 100 countries and, in Italy, it was sold in at least 115 products. Other monitoring studies found it in 100% of pollen samples in Val d'Aosta (northwestern Italy) and Sicily. [32] In Puglia (southeastern Italy) chlorpyrifos has been associated, together with dimethoate, with the death of colonies due to treatments carried out in vineyards, while in the Province of Bolzano (northern Italy) chlorpyrifos has been associated with the death of colonies near apple orchards. Unfortunately, it is also easily found in honeys certified as organic.
Eight systemic pesticides (6 fungicides and 2 not allowed neonicotinoids: imidacloprid and thiamethoxam) were found in 36% of the samples. Pesticides banned in Italy were also found, such as phentoate, which has been banned since 2003 (17 positive samples in Piedmont, Veneto, Emilia-Romagna and Tuscany), carbaryl, which is a carbamate insecticide banned since 2012 (one positive sample in Piedmont), chlorfenvinphos, which is an organophosphate insecticide banned since 2006 (25 positive samples in Piedmont, Lombardy and Veneto). It is interesting to note that no pesticides were found in pollen collected in September.
In conclusion, even beekeeping systems that should be more careful about chemical contamination, such as those applying organic certification specifications, are polluted by dangerous and prohibited substances. The Authors highlight a critical point: hives used in the production of organically certified honey can be placed near crops that use hazardous products, when the plants are not flowering or if they are not considered relevant from the pollination point of view.

- In Italy, the presence of acaricides in 1,319 wax samples was monitored during 10 years (2005-2014). [1208] The active molecules found most frequently were: coumaphos (49%), fluvalinate (38%), chlorphenvinphos (25%), and 2,4-dimethylphenylformamide (DMPF, a metabolite of amitraz; 6%). This contamination highlights the widespread use by beekeepers of pesticides to control the *Varroa destructor* mite. Acaricides are lipophilic and do not degrade easily so they accumulate in wax. The use of acaricides has been widespread for at least 30 years, so much that even in organic farming it is difficult to find recycled wax without these substances. For this reason, a compromise was accepted to tolerate high concentrations in wax for organic beekeeping: 0.2 mg/kg for coumaphos, 0.1 mg/kg for fluvalinate, 0.01 mg/kg for chlorfenvinphos; for the metabolite of amitraz (DMPF) and cymiazole the maximum permissible concentrations are included in the general tolerance limit for all pesticide residues of 0.3 mg/kg. In this study cymiazole was never found and it should be noted that like coumaphos it has been banned since 2009.
As shown in the following table, 64% of the 1,319 wax samples recorded the presence of at least one of the acaricides sought and, in 2014, 91% of the samples were found to be contaminated. In some years, the average concentrations were higher than the tolerance limit established by the regulations for organic beekeeping in the years 2006, 2010, and 2014; and with concentration peaks, in 2014, of 8.9 mg/kg for coumaphos (a molecule banned but found in 75% of the 2014 samples), 0.4 mg/kg for fluvalinate, and 3.9 mg/kg for chlorfenvinphos (this active ingredient has never been authorized in beekeeping: it is 50 times more toxic than coumaphos). This research supports the suspicion of an illegal behaviour on the part of some beekeepers, and the concentrations of the acaricides searched are high and therefore very dangerous.

Analysis results

Year -	**Positive samples** (%) -	**Number of samples** [1208]
2005	69	217
2006	70	217
2007	55	186
2008	56	162
2009	32	59
2010	47	139
2011	54	97
2012	37	87
2013	56	50
2014	91	105
Total	**64**	**1,319**

- Research conducted between 2013 and 2015 examined the contamination of wax by pesticides. [55, 70] It is customary among beekeepers to recycle beeswax; this practice favours the accumulation of hydrophobic molecules, resistant to melting, high temperatures and persistent, such as some acaricides. Little information is available on the presence of pesticides in wax in Italy. A total of 247 pesticides were searched for in 178 beeswax samples. The samples consisted of 10 g of beeswax taken randomly from 60 apiaries located in different Italian regions over three years: 40% of the samples were collected in Tuscany, 14% in Piedmont, 9.5% in Lombardy, and 9.5% in Emilia Romagna. 73.6% of the samples were found to be contaminated with at least one of the molecules sought and 15.7% of these positive samples contained pesticides at concentrations greater than 0.2 mg/kg, which is the limit for beeswax from organic beekeeping. Only 26.4% of the wax samples were free of the substances sought. Of the 247 molecules analysed, 41 were found: up to 14 molecules were detected at the same time, and molecules banned in Italy and Europe, such as DDT and its metabolites, and coumaphos, were also recorded. The latter is an organophosphate insecticide/acaricide found in 60.7% of the samples (up to 0.99 mg/kg) and is used in veterinary medicine to control ectoparasites, also by adding it to cattle and chicken feed to reduce the growth of fly larvae in faeces; in Italy the use of this insecticide in veterinary medicine has been prohibited since 2009. Lindane and heptachlor, which have been banned for years, were also found. Other molecules found were piperonyl butoxide, tau-fluvalinate (found in 50% of samples) and rotenone (a compound banned in organic farming and beekeeping because of its toxicity to bees).

The molecules found most frequently were acaricides such as coumaphos (as already mentioned, recorded in 60.7% of the samples), tau-fluvalinate (a pyrethroid recorded in 50% of the samples), and chlorfenvinphos (an organophosphate insecticide recorded in 35.4% of the samples). Tau-fluvalinate, in 16 samples, was detected at concentrations higher than 0.1 mg/kg which is the maximum tolerable residue in beeswax from organic farming (it was recorded at the maximum concentration of 1.07 mg/kg). Chlorfenvinphos was detected at concentrations up to 0.61 mg/kg, despite the fact that the maximum permissible limit in organic farming is 0.01 mg/kg. The highest concentrations were recorded for pyrethrins (up to 4.42 mg/kg) and piperonyl butoxide (up to 2.3 mg/kg). Amitraz is a molecule authorized in Italy for anti-varroa treatments (the maximum limit allowed in honey is 200 ng/g) and, within a few hours, it is transformed into other molecules (metabolites) that are persistent and resist the thermal process applied to recycle the wax. In this regard, it is useful to remember that in Italy *Varroa* strains resistant to amitraz have been registered. [55]

In conclusion, molecules that have been banned for decades were also found, such as DDT in 24 samples (and its metabolites with a maximum concentration of 0.43 mg/kg), lindane and heptachlor: the concentrations recorded suggest an illegal use.

- In Italy, in 2015, the presence of pesticides, PCBs (polychlorinated biphenyls), polybrominated diphenyl ethers and PBDEs was monitored in 59 organic honey samples. [58]
Honey samples were taken from apiaries located in three regions: Calabria (14 samples from citrus), Trentino Alto Adige (18 samples from apple cultivation), Lombardy (27 samples of multifloral honey from industrial area). Honey samples (2 g each) were analysed using a triple-quadrupole mass spectrometer and a gas chromatograph as instruments. The molecules most frequently found in samples of organic honey obtained from apple or citrus cultivation were: diazinon, mevinphos, coumaphos, chlorpyrifos, and quinoxyfen. Samples originating from different agricultural areas contain different pesticides, confirming the possibility of using honey to get information on the molecules used by farmers.
Organochlorinated pesticides and organophosphates (they block acetylcholinesterase) are persistent, stable, low volatile, and lipophilic, so they may bioaccumulate easily in the food chain. [58]
Fifty percent of organic citrus honey samples from Calabria showed the presence of eldrin, at concentrations between 1.95 and 18.9 ng/g. In one sample the concentration was higher than the maximum allowed limit (EC Reg. n. 396/2005). [55] Diazinon was found in 64% of the samples at a maximum concentration of 1.14 ng/g, mevinphos was found in 86% of the samples. [58]
In organic honey obtained near apple orchards in Trentino, aldrin was found in 5% of the samples at a maximum concentration of 1,174 ng/g, and eldrin in 44% of the samples at a maximum concentration of 13,343 ng/g (dieldrin and aldrin metabolites are present). Heptachlor, DDT and its metabolites (ppDDE) were also found in 17% of the samples at the maximum concentration of 0.09 ng/g for DDT and 1.47 ng/g for ppDDE. In honey from apple orchards, 12 different organophosphates were present such as quinoxyfen, found in 100% of the samples at concentrations between 3.09 and 4.23 ng/g. Also diazinon was found in 100% of the samples, at concentrations between 1.13 and 1.15 ng/g, while mevinphos was found in 67% of the samples.
In organic honey produced in Lombardy, ppDDT and its metabolites ppDD and ppDDE were found at concentrations up to 1.99 ng/g and at frequencies of 41%, 22% and 33%, respectively. Heptachlor was detected in 11% of the samples, at the maximum concentration of 1.19 ng/g, and dieldrin was found in 41% of the samples at the maximum concentration of 2.93 ng/g. The presence of the fungicide captan was also recorded in 37% of the samples with the maximum concentration of 20.56 ng/g.
Chlorpyrifos was found in samples from all three regions with the highest frequency of occurrence in organic honey from Calabria (29%) and the highest concentration (389.5 ng/g).
Although these honeys are of organic origin, in honey samples from Calabria the acaricide coumaphos is found in 78% of the samples and in those from Trentino in 79% of the samples, at the maximum concentration of 2.06 ng/g. [58]
Brominated flame retardants (such as polybrominated diphenyl ethers) are very dangerous molecules that persist in the environment to the extent that they may be found in food. They are suspected of being endocrine disruptors and of generating negative effects on the nervous system which are reflected in behaviour. Brominated flame retardants are not found in any organic honey samples.
The six PCBs searched were found in all samples from the three Italian geographical areas, at concentrations between 0.27 and 0.92 ng/g. No significant differences in the concentrations of these molecules were recorded in the samples.

- A research conducted in Italy, in 2016, examined the presence of 250 pesticides in 80 wax samples from 40 conventional and 40 organic apiaries. [70] Analyses were performed by means of gas chromatography and liquid chromatography with mass spectrometer. Samples consisting of 5 g of wax were analysed within 48 hours of sampling. The results showed that no wax samples from traditional beekeeping were free of pesticides and that only 11 samples (27.5%) from organic beekeeping were free of pesticides. Among the 250 molecules sought, 30 were found in samples from traditional beekeeping and 24 in organic samples. The average positivity was 5 molecules per wax sample from traditional beekeeping and 3 for organic samples. In general, the concentrations in the traditional samples were higher, but in the organic sample molecules like pyrethroids were found at higher concentrations. In both groups, positivity was found for some molecules that have been banned in Europe for several years. This emerges in the group of conventional wax samples for the molecules acrinathrin (authorized for agricultural treatments in 2011), chlorphenvinphos (withdrawn from the market in 2004), and in the case of organic wax for the molecules rotenone (banned from the European market in 2008) and cypermethrin (authorized for agricultural treatments in 2009). Although the illegal sale of pesticides cannot be excluded, the positive findings for substances banned on the Italian and European market, according to the Authors, are more likely related to the reuse of wax and the high persistence that characterizes these substances. The reuse of wax for the production of wax sheets determines the recirculation of chemicals, whose concentrations remain constant for very long periods and which are added to other chemical compounds over the years. A statement on acaricides widely used in beekeeping in Switzerland shows that residues of bromopropylate, coumaphos, fluvalinate, flumethrin, and thymol are found in wax treated at high temperatures. [70, 1280]
The lack of controls facilitates the circulation of beeswax from non-European countries, which are subject to different regulations and different limits for the various active molecules. A striking example is the presence of paradichlorodiphenyltrichloroethane (DDT) and its metabolites in several samples. The relationship between the "mother" molecule pp-DDT and its metabolites suggests the recent use of DDT, which has been banned in Europe for about 40 years but is still used in many African and Asian countries.

The qualitative and quantitative results of the chemical analyses carried out on the "conventional" beeswax samples are reported below, indicated in sequence with: *the name of the molecule*; *the number of positive samples* (*out of a total of 40 samples*)*; the maximum concentration* (*mg/kg*)*; the average concentration* (*mg/kg*). [70]

1. acrinathrin **11** - 0.063 - 0.026
2. amitraz **7** - 0.016 - 0.012
3. bromopropylate 4 - 0.021 - 0.018
4. chlordane 1 - 0.011 - 0.011
5. chlorfenvinphos **28 - 0.52** - 0.145
6. chlorobenzilate 1 - 0.02 - 0.020
7. chloropropylat 3 - 0.021 - 0.018
8. coumaphos **31 - 0.76** - 0.160
9. cymiazole 1 - 0.028 - 0.028
10. cypermethrin 2 - 0.67 - 0.064
11. cyprodinil 1 - 0.012 - 0.012
12. DDD-pp **6** - 0.086 - 0.056
13. DDE-pp **1** - 0.013 - 0.013
14. DDT-pp **5 - 0.131** - 0.089
15. fludioxonil 1 - 0.011 - 0.011
16. flumethrin 3 - 0.079 - 0.050
17. fluvalinate-tau **19 - 1.1** - 0.351
18. heptachlor 1 - 0.016 - 0.016
19. iprodione 1 - 0.011 - 0.011
20. lindane 1 - 0.01 - 0.01
21. penconazole 1 - 0.056 - 0.056
22. permethrin 1 - 0.111 - 0.111
23. pyrethrin 1 - 0.65 - 0.650
24. piperonyl butoxide **14 - 2.3** - 0.524
25. pyrimethanil 1 - 0.011 - 0.011
26. rotenone **10** - 0.03 - 0.017
27. spirodiclofen 1 - 0.013 - 0.013
28. spiroxamine 1 - 0.011 - 0.011
29. tebuconazole 1 - 0.01 - 0.010
30. tetramethrin 2 - 0.37 - 0.192

The qualitative and quantitative results of the chemical analyses carried out on the "organic" beeswax samples are given hereafter, in the following sequence: *the name of the molecule; the number of positive samples (out of a total of 40 samples); the maximum concentration (mg/kg); the average concentration (mg/kg).* [70]

1. α-HCH 1 - 0.038 - 0.038
2. amitraz **7** - 0.017 - 0.012
3. bromopropylate 2 - 0.018 - 0.014
4. chlordane 1 - 0.013 - 0.013
5. chlorfenvinphos **18** - 0.029 - 0.018
6. chlorpyriphos ethyl 2 - 0.045 - 0.043
7. coumaphos **29** - 0.024 - 0.016
8. cypermethrin 2 - 0.104 - 0.071
9. DDD-op **2** - 0.044 - 0.028
10. DDD-pp **3** - 0.07 - 0.040
11. DDT-op **1 - 0.**017 - 0.017
12. DDT-pp **8 - 0.1** - 0.059
13. diazinon 1 - 0.027 - 0.027
14. flumethrin 5 - 0.066 - 0.034
15. fluvalinate-tau **26** - 0.082 - 0.042
16. heptachlor 2 - 0.016 - 0.013
17. pendimethalin 2 - 0.018 - 0.014
18. pyrethrin 3 – **2.08** - 1.051
19. piperonyl butoxide **16** - 0.08 - 0.033
20. rotenone **8** - 0.062 - 0.04
21. tebuconazole 1 - 0.011 - 0.011
22. terbuthylazine 1 - 0.011 - 0.011
23. tetrachonazole 3 - 0.016 - 0.012
24. tolifluanid 2 - 0.015 - 0.014

Producers of organic honey should place their hives at least 3 km far from sites that may favour the contamination of beekeeping products. [55] As you can guess, the application of this preventive measure is almost impossible.

In Italy, the following pesticides are allowed in traditional beekeeping (2019): amitraz, tau-fluvalinate and flumethrin, all of which may be used without a veterinary prescription. [219] Tau-fluvalinate (a pyrethroid whose maximum tolerated concentration in honey is 10 ppb) cannot be used during honey production and, if so, must not be intended for human consumption; furthermore, nest wax cannot be used before 180 days have elapsed since the suspension of treatment. [219, 220]

In organic beekeeping, the following molecules are permitted to control the parasitic mite *Varroa desctructor*: thymol, formic acid, acetic acid, and oxalic acid. Formic acid and oxalic acid may be found in honey at very low concentrations. [107] When used at high concentrations, formic acid may reduce the survival of larvae, worker bees and queen bees and ultimately decrease colony size. Oxalic acid is distributed in a sugar solution in the form of crystals which, on the cuticle of the insects, stimulate mutual grooming. This technique induces mutual removal of the parasite, the *Varroa* mite. Then oxalic acid may be ingested generating negative effects such as reduced activity and longevity. [107]

Thymol, a volatile terpene produced by certain flowers and distributed by beekeepers in the hive by fumigation, may also be used against *Varroa.* This molecule may remain in honey and wax and, at high concentrations, may cause damage to the hive and modify the genetic

expression (e.g. it reduces the expression of the gene that produces the protein vitellogenin; the expression of this protein depends on pollen consumption). [13, 107]
The regulations (2018 update) provide for a maximum permissible concentration of pesticides in honey, propolis and royal jelly of less than 0.01 mg/kg. [77] In the case of wax, a maximum concentration of the sum of 6 active molecules (coumaphos, fluvalinate, chlorfenvinphos, cymiazole, amitraz, flumethrin) of 0.3 mg/kg is regulated, which is much higher than the generic limit of 0.01 mg/kg.
In organic wax, the maximum permissible concentrations are: coumaphos ≤ 0.2 mg/kg, flumethrin ≤ 0.2 mg/kg, fluvalinate ≤ 0.1 mg/kg, and chlorfenvinphos ≤ 0.1 mg/kg. [77] In beeswax from organic beekeeping, concentrations of acaricides above the limit of 0.01 mg/kg are tolerated. Voluntary organic regulations tolerate wax contamination capable of generating adverse effects to the colony, so it is programmed to meet economic rather than health and environmental criteria.

- Honey bees can be contaminated by persistent pollutants such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBDEs) which are ubiquitous in the environment. In Italy, 95 samples of organic honey from Piedmont, Lombardy, Emilia Romagna, and purchased in the market (18/95 are mixtures of European honeys) were tested for the presence of several persistent pollutants. [1196] PCBs are identified in three of the five areas with a frequency of occurrence between 7% and 33% of the samples, and with concentrations up to 234 ng/g. These pollutants are ubiquitous and, in fact, PCB molecules are not correlated with the geographical area of origin of the honey. Flame retardants are also found with the same trend indicating independence from the geographical area of origin as they are persistent pollutants. Polycyclic aromatic hydrocarbons and molecules banned since the 1970s, such as metabolites of the organochlorine insecticide DDT (pp'DDD up to 24 ng/g) were also detected. Lindane was found in a sample from Hungary at a concentration of 16.9 ng/g and in one from Ukraine at a concentration of 116 ng/g. Another unauthorized molecule (mevinphos) was also found at dangerous concentrations in honeys from four of the five areas examined. Organic honey from Ukraine and Hungary recorded disulfoton at an alarming concentration of 65 ng/g.
In conclusion, this work confirms the presence, also in organic honey, of ubiquitous organic contaminants: PCBs, PBDEs, flame retardants, polycyclic aromatic hydrocarbons are found in urban, industrial and agricultural contexts, although honey from industrial areas is more contaminated. In organic honey, some pesticides are registered at dangerous concentrations: banned molecules are found at concentrations higher than the maximum allowed limits. Finally, good news: neither antibiotics nor neonicotinoids were found in this study.

- In Apulia (Italy), in the years 2019 and 2020, a monitoring was carried out on 98 samples of organic honey produced in different areas. [1201] Different categories of persistent pollutants were searched for, such as PCBs (polychlorinated biphenyls), PBDEs (polybrominated diphenyls), PAHs (polycyclic aromatic hydrocarbons), and some pesticides such as organochlorines (alpha-HCH, beta-BHC, lindane or hexachlorobenzene, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, endrin aldehyde, endosulphan I, endosulphan II, endosulphan sulphate, trans chlordane, 4,4'-DDE, 4,4'-DDT, 2,4'-DDT, 4,4'-DDD, and methoxychlor), the organophosphates (anziphos methyl, boscalid, bupiramate, captan, chlorantraniliprol, chlorpyriphos, coumaphos, diazinon, disulphoton, ethoprophos, fenchlorphos, fenthion, fluazinam, iprodion, methyl parathion, mevinphos, penconazol, phorate, prothiophos, pyraclostrobin sulprophos, quinoxyfen, spirodiclofen, tetrachlorpyrophos, tribuphos, trifloxystrobin, florisil, and 4-nonylphenol), and other molecules (glyphosate, glufosinate and AMPA). The results of the analysis of 98 samples of Apulian organic honey showed that: [1201]

- All samples record traces of polycyclic aromatic hydrocarbons (benzofluoroanthene). Some molecules are found only in some samples (chrysene).
- Traces of the six PCBs tested are found in all samples. The presence of PCBs is not correlated to geographical areas highlighting that they are ubiquitous.
- Traces of PDBE are detected in all samples. This finding also confirms that these are persistent and ubiquitous pollutants.
- Traces of organochlorine pesticides are found in all samples, including DDT and its derivatives. These are very persistent molecules, such as DDT and its metabolites, which have a half-life in soil of 25 years. The most frequently found molecule was exachlorobenzene (or lindane). These molecules have been banned for years.
- Organophosphate pesticides were detected at low concentrations in all the geographical areas examined. In two samples from an area with orchards, azinphos methyl was found at high concentrations (0.33 and 1.32 ng/g). The maximum concentration of coumaphos acaricide was 2.13 ng/g, although its use is forbidden (organic honey).
- Glyphosate, glufosinate and AMPA were not detected.

Honey certified as organic contains several persistent pollutants, all of anthropogenic origin, and the illegal use of some molecules (e.g. acaricides by beekeepers) cannot be excluded. Environmental contamination is so widespread that very dangerous molecules (persistent, bioaccumulative and toxic) are found everywhere, both in agricultural and in urban and industrial areas. The synergistic effects between these different categories of pollutants are largely unknown but predictably very harmful.

CONTAMINATION OF BEES DURING SOWING

The use of seeds treated with systemic pesticides such as neonicotinoid insecticides exposes bees to toxic concentrations. Insects come into contact with these pesticides if they fly into the sowing area or, indirectly, through drift: flowers in the vicinity of the sowing fields will contain dangerous concentrations. This is an undesirable but unavoidable contamination.

- In Italy (in 2009 and 2010), the risk of contamination of bees (*Apis mellifera*) by particulate matter and insecticides during maize sowing was assessed. [73] Four active molecules used in maize for seed treatment were examined: imidacloprid, clothianidin, thiamethoxam, and fipronil. The first three are neonicotinoids and have a contact toxicity (LD_{50} or lethal dose by contact able to kill 50% of exposed insects in a few hours) of 18 ng/bee, 22 ng/bee, and 30 ng/bee, respectively; fipronil has a LD_{50} by contact of 4 ng/g. During maize planting, the concentration of these 4 molecules in dust and air was measured. Contamination may occur when flying near sowing and when bees feed on the guttation droplets of maize (*Zea mays*) leaves. [359, 400] Guttation consists in the elimination of water in the liquid state from the leaves and occurs when, due to excessive atmospheric humidity, transpiration cannot take place regularly or when the quantity of water absorbed by the roots is greater than that transpired by the leaves (the phenomenon may occur through the stomata, the openings on the leaves). [234]

The molecules sought are found at concentrations above those that generate very dangerous sub-lethal effects, in particular: [73]

- Very low doses of fipronil (0.5 ng/bee) applied by contact damage the olfactory ability. Sub-lethal effects for fipronil have been measured at doses of 1/80 and 1/40 of the LD_{50}.

- Very low doses of clothianidin (a neonicotinoid) applied once (0.7 ng/bee) impair the ability of bees to return to the hive. Even lower doses (0.47 ng/bee) allow bees to return although they are unable to resume their foraging activity for several hours.

The corn seeds in this study contained 1 mg/seed of imidacloprid, 1.25 mg/seed of clothianidin, 0.6 mg/seed of thiamethoxam, and 0.5 mg/seed of fipronil. Seeds were sold in bags of 25,000 seeds which therefore contain 71 g of neonicotinoids and 12.5 g of fipronil. Seeds are planted at a density of 75,000 seeds per hectare corresponding to the distribution of about 214 g of neonicotinoids per hectare and 38 g of fipronil per hectare. The dust produced during sowing was estimated at about 3 g per 100 kg of seeds. The following concentrations of active molecules were found in the dust produced during sowing (at a distance between 5 and 20 m from the sowing machine and in $\mu g/m^3$):

- clothianidin 0.1-0.5;
- fipronil 0.01-0.04;
- imidacloprid 0.01-0.06;
- thiamethoxam 0.02-0.03.

These concentrations represent the exposure that bees theoretically undergo when flying near the sowing machine. In this work, the exposure was estimated considering that seeding lasts 80 minutes per hectare and that bees fly 10 times for 50 m around the seeding area. According to the Authors, by applying the best possible seeding system, bees are exposed by contact to concentrations of these 4 molecules, during flight, between 0.3% and 0.5% of the LD_{50} (the most widespread seeding methods could generate a higher exposure). These doses could be sufficient to generate sub-lethal effects. A previous research (2001) had also detected this possible contamination pathway. [73]

- Dust produced during the sowing of imidacloprid-treated maize contaminates the grass and flowers in nearby fields. In flowers in the vicinity of maize fields, imidacloprid was found at concentrations between 22 and 54 µg/kg. [376] Considering that the dose that may kill bees in 48 hours has been estimated to be between 5 and 500 billionths of a gram per bee (ng/bee), this means that the concentrations found in flowers may be 1,000 times higher. Therefore, bees may be seriously harmed by flowers in the vicinity of crops. The Authors in the conclusions suggest as a solution to add a system of gluing the pesticide to the seed: it would be more far-sighted to suggest the non-use of these molecules. [376]

- In later work it was found that as bees fly during the sowing of seeds coated with neonicotinoids, they pick up about 100 billionths of a gram per bee (ng/bee) of insecticide on their bodies. [399] In this case, neonicotinoid insecticides such as clothianidin, at a concentration of 1.25 mg per maize seed, and imidacloprid, at a concentration of 0.5 mg per maize seed, were used; sowing took place at a density between 73,000 and 74,000 seeds per hectare (this quantity corresponds to approximately 90 g of clothianidin per hectare). During their flight near the sowing area, bees collect doses of insecticides that may kill them. High amounts have been recorded: up to 674 ng of clothianidin per bee or up to 3,661 ng per bee of imidacloprid (in insects found dead or dying a few hours after sowing). [399] The dose that by contact kills 50% of exposed insects (LD_{50}) is 21.8 ng per bee in the case of clothianidin and 17.9 ng per bee in the case of imidacloprid. By examining the concentrations, it follows that insects flying during seeding are exposed to doses of neonicotinoids much higher than those considered toxic and lethal within a few hours. In this study it is highlighted that the increase of air humidity potentiates the toxic effects of neonicotinoids distributed during seeding. It is surprising that the Authors, at the end of the article, propose as a possible solution the use, in the future, of seeding machines that could reduce the dispersion of insecticides in the air. This proposal will not avoid the dispersion of very toxic and persistent molecules in the environment, so it is not a solution.

On the other hand, the manufacturers of neonicotinoids such as imidacloprid claim that there is no risk of contamination of bees during seeding. [700] However, there are several reports in the scientific literature and in the news of increased bee mortality in connection with the sowing of seeds coated with neonicotinoids, such as maize and sunflowers (e.g. in France and Canada). [701, 702] Wild bees are also adversely affected by the use of neonicotinoids in seeds. [411]

CONCLUSIONS

In conclusion, the numerous results just summarised show that voluntary chemical contamination of apiaries is, by itself, sufficient to explain the increased mortality of bees, pollinators and others. Probably if a group of researchers uninformed about the real situation tried to estimate the mortality levels expected from the recorded concentrations they would be surprised to record such low mortality levels in honey bees.

Another negative aspect to highlight is the use of public economic resources to obtain information on the level of contamination of bee colonies without having practically any information on the molecules used in the field, doses, distribution methods, etc. In practice, the agricultural and beekeeping sectors seem to be absent from these monitoring projects. In industrialised countries such as Italy, in theory all treatments carried out, both in agriculture and in beekeeping, must be recorded. It is truly disarming and discouraging to measure in these publications the total absence of collaboration between those who profit from poisoning the Planet and those who try to give indisputable and useful confirmations. Yet the agricultural sector, at least in Europe as in North America, is supported by very generous public economic resources. Probably the time has come to demand a different kind of collaboration in exchange for the huge public economic resources. Valuable resources are being wasted looking for molecules that are not used and there is probably not enough information on other molecules that are widely used. Moreover, no guarantee system can stand up if it is mainly based on a final analytical control or on the possible ability of public research, retrospectively, to confirm the dangerousness of choices made almost exclusively to benefit entrepreneurs (according to selfish and unsustainable rules).

METAL CONTAMINATION

METAL POLLUTION IN SOIL

Metals are an environmental contaminant continuously emitted by a multitude of sources and are characterised by their non-degradation. They can enter biochemical and physical cycles, and are able to remain in circulation in the food chain for long periods. Anthropogenic sources of metals are numerous: mining, industrial waste management, wastewater discharges, vehicle and incinerator emissions, coal combustion, agricultural activities such as fertilization, use of fungicides (e.g. copper-based fungicides), and others. [71, 176]

Some metals present in the atmosphere, such as cadmium, mercury, nickel, and lead are indicators of anthropogenic activities and may be very dangerous to health. [38] For example, cadmium (Cd), which is used to produce chemical fertilizers and polyvinyl chloride (PVC), is classified as carcinogenic to humans (group 1 of the classification made by the IARC or International Agency for Research on Cancer).

Cadmium and mercury (Hg) may be used as environmental indicators of emissions from incineration plants, while the other metals may derive from more diffuse sources such as vehicle traffic. Chromium (Cr) and copper (Cu) are associated with urban traffic and cadmium (Cd) with industrial emissions. [64]

Metals emitted into the atmosphere are deposited in soils, which can also be contaminated by the use of fertilizers (e.g. sewage sludge, compost), pesticides or polluted water.

The following ranges of concentrations of certain metals may be found in soils: [768]

- Cadmium between 0.005 mg/kg and 750 mg/kg (soils contaminated by zinc and leadworks).
- Copper between 0.3 and 3,215 mg/kg (vineyards are among the soils containing the highest amounts, where copper-based fungicides are used).
- Lead between 0.015 mg/kg and 400 mg/kg in soils close to busy roads.

Metals in the soil may be absorbed by plants, which can also take them up from the atmosphere and contaminated water. Therefore, metals, once released into the atmosphere or water, may easily enter the food chain.

The following table shows the values of metal deposition on soil measured in different types of European areas (special bottle-shaped deposition meters[I] with a cylindrical funnel were used). [38, 48, 50]

	Rural areas	**Urban areas**	**Industrial areas**
Arsenic (µg/m^2 per day)	0.082-0.43	0.22-3.4	2-4.3
Cadmium (µg/m^2 per day)	0.011-0.14	0.16-0.9	0.12-4.6
Nickel (µg/m^2 per day)	0.03-4.3	5-11	2.3-22

At different sites, anthropogenic impacts may vary considerably: in industrial areas where, for example, metals are processed, deposition values between 126 and 243 µg/m^2 per day may be

[I] The deposimeter may consist of a funnel and a container, with a capacity of 10 litres, connected together; both are usually made of high-density polyethylene (HDPE). The deposimeter is installed on a metal tripod support structure equipped with a black cylindrical jacket, to protect the sample from solar radiation. The height of the funnel mouth of the deposimeter from ground level is usually between 1.5 and 2 metres.

reached for arsenic, between 11.3 and 40.7 µg/m^2 per day for cadmium, and between 53 and 76 µg/m^2 per day for nickel. It follows that the characterization of the site is an important preliminary step to finalize the type of research to be carried out.
In some European countries, such as Germany, Switzerland, Belgium, and Croatia, national limit values are set for the assessment of the levels of metals that may be deposited in the soil from the atmosphere (in µg/m^2 per day). [38, 48, 50]

Ground deposition value from the atmosphere in µg/m^2 per day	**Arsenic**	**Cadmium**	**Mercury**	**Nickel**	**Lead**	**Titanium**	**Zinc**
Belgium	-	20	-	-	250	10	-
Croatia	4	2	1	15	100	2	-
Germany	4	2	1	15	100	2	-
Switzerland	-	2	-	-	100	2	400

So soil is a good indicator of metal pollution, as well as plants that accumulate them from both soil, air and water. Metals may bioconcentrate in plant tissues. Copper, for example, may accumulate in the flowers and leaves of the common radish (*Raphanus sativus*) which is an edible plant, belonging to the Brassicaceae family (this family, together with the Asteraceae, is one of the most numerous of the Angiosperms with about 350 genera and 3,000 species). [768, 769]
Plants in the Brassicaceae family may also accumulate selenium in pollen and nectar. It follows that both herbivorous insects and pollinators may be exposed to these metals.
Copper is an essential micronutrient as it is important for the functioning of enzymes such as those for detoxification. It may be bioaccumulated in leaves at concentrations up to 25 mg/kg and may generate toxic effects, such as inhibition of photosynthesis, damage to metabolism (e.g. of carbohydrates), and may cause a reduction in the root system. One of the primary sources of copper are fungicides, which are used in large quantities in crops such as vineyards. The constant and repeated application of copper-based fungicides generates a dangerous increase in the soil concentration (in vineyard soils in Brazil, a concentration of 3,216 mg/kg of copper has been recorded). [768]
Cadmium and lead are not useful to the plant, but cadmium is easily accumulated as it is mistaken for zinc which is instead a micronutrient. Because of this mistake, cadmium may easily bioaccumulate in plants and generate several negative effects, such as reduced photosynthesis and transpiration.
Lead reaches the soil mainly from air pollution (e.g. road transport) and may bioaccumulate in plants. Lead may also be found in the bodies of bees, in pollen, honey and wax.

Plants are able to bioconcentrate metals such as cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), lead (Pb), and zinc (Zn). The ability to bioaccumulate (BCF or bioconcentration factor) usually decreases as the concentration of metals in the soil increases, except for some cases such as cadmium in wheat; however, the absolute amounts of metal accumulated increase as the concentration in the soil increases. Cadmium and copper may accumulate in wheat and maize seeds. [933] The ability to bioaccumulate metals is so pronounced that some researchers suggest that corn cultivation may be used to reduce the concentration of certain metals in the soil (remediation). Some concentrations are reported: [933]

- Maize bioaccumulates manganese up to 332 mg/kg, while rape and wheat up to 180 mg/kg.
- Corn accumulates cadmium up to 65 mg/kg and wheat even more, especially in the seeds.
- Wheat accumulates lead up to 380 mg/kg, field pea up to 260 mg/kg.

- Wheat and fodder vetch accumulate chromium up to 4,500 mg/kg, rape up to 7,046 mg/kg.
- Fodder vetch accumulates zinc up to 1,200 mg/kg, corn and wheat over 1,000 mg/kg.

This investigation shows that wheat accumulates large amounts of cadmium and copper, mainly in the edible part, while maize in the part not edible to humans. Some metals are more concentrated in the straw (e.g. cadmium and copper in maize stalks, leaves and roots), while others are in the seeds (e.g. cadmium and copper in wheat seeds). In general, wheat, field pea and vetch were less resistant to high metal concentrations than maize and rapeseed. Unfortunately, the monitoring of hazardous substances in plants used as feed does not receive the same attention as the plants intended for direct human consumption (e.g. pesticides and metals), partly because it is wrongly and superficially considered unavoidable.

Some plants have a high capacity to bioconcentrate metals to the extent that they may potentially be used to reduce the contamination in soils. This practice is sometimes referred to as bioremediation. For example, the common radish (*Raphanus sativus*) is able to accumulate lead, cadmium and copper in its roots and flowers. This is an annual plant that requires entomophilous pollination, so it is a useful indicator of soil pollution and risks to phytophagous and pollinating insects. It has been revealed that the presence of aphids, which are phytophagous, is reduced as the concentration of cadmium in plants increases (200 mg/kg). [768] Plants may concentrate the metals to the point where they become less attractive to phytophagous insects: they become poisonous. This insecticidal effect is also detected in pollen and nectar (e.g. 13 mg/kg cadmium in flowers). [768] An interesting aspect is that the bioconcentration decreases as the concentration of these metals in the soil increases, indicating that the phytotoxic effects, above certain values, become predominant. Thus, the damage to insects will be greater when soil concentrations are lower, because they will be exposed to higher doses in plant tissues. Conversely, by increasing concentrations in the soil, adverse effects on plant growth and toxic effects will become predominant. Undesirable effects of metal contamination in soils include reduced biodiversity and number of wild pollinating insects. The presence of solitary bees, which usually have a range of a few hundred metres and nest in the soil, is reduced near metal-contaminated soils (e.g. from industries such as foundries). [770] An important exposure route for this type of insect is pollen, which accumulates metals such as cadmium and lead.

METALS IN BEEKEEPING PRODUCTS

Metals in the atmosphere can be deposited on the bees' bodies or absorbed through pollen, nectar, honeydew and water (but also from the smoke used by beekeepers). Foraging bees, making 12-15 flights per day when temperatures are between 20 and 25°C, are potential biological samplers of metals.

In live bees, from colonies within the urban area, the highest concentrations of zinc, copper, strontium, nickel, and chromium (Zn, Cu, Sr, Ni, and Cr) may be recorded. The areas most polluted by lead, such as industrial and urban areas, generate a higher exposure (by ingestion and inhalation), so it is easier to find high concentrations of metals both inside the body and on the surface. Nickel and chromium may also be found both inside the body and on the surface of bees. [17]

Honey may be used to obtain information on the presence of these pollutants, as it is nothing more than concentrated nectar: it reflects the pollution present in the plants from which it was collected. Metals such as lead may be found in honey produced by hives located in contaminated areas, such as industrial and urban areas, at concentrations 2.7 times higher than in hives located in control stations (natural parks). In urban and industrial areas, nickel may also

be recorded at concentrations up to 3.6 times higher, and chromium up to 4 times higher. This confirms the possibility of using honey as an indicator of metal contamination in the atmosphere. To give another example, fluorine monitoring may be conducted through both honey and propolis.
Pollen and, secondly, wax are considered more suitable matrices than honey for detecting environmental pollutants such as metals. [17] Bees too register more metals than honey (they represent the pollution accumulated over a few days).
Metal biomonitoring with bees should be conducted in colonies that have not been exposed to smoking systems to inspect the hive, as they may release metals that alter the results. Operators should also be prohibited from smoking during hive inspections, as smoking is another source of metals. The sample container too should not release metals.

Determination of the concentration of metals in honey and flowers may provide information on soil contamination. Nectar honey has a very low mineral content, between 0.1 and 0.2%, and the composition depends on plants and soil too. The mineral composition of honey may be used to determine its botanical and geographical origin. [44] The mineral composition of honey may also be used to get information about anthropogenic activities in the area such as smelters, mines, fossil fuel combustion, and use of fertilizers. In the vicinity of smelters, high concentrations of cadmium, lead and zinc have been associated with a reduction in the diversity and abundance of wild solitary bees. [768, 770]
Some metals such as arsenic (As) are an industrial pollutant and have been used as an insecticide. [107] Arsenic is capable of generating acute toxic effects (mortality) at doses of 400-500 µg/bee and may generate oxidative stress.

Metals, at different stages of the colony, may cause very different negative effects. Selenium is an industrial and agricultural pollutant, and may accumulate in some plants. In pollen collected by bees it has been found at concentrations above 2,830 mg/kg. [107] Selenium is toxic because it replaces sulphur in amino acids and changes the conformation of the structure of proteins, altering their functions. Bee larvae, compared to adult insects, are 30 times more sensitive to the exposure by ingestion of amino acid-bound selenium and 50 times more sensitive to inorganic selenium. Selenium at concentrations below 0.72 mg/kg, in the laboratory, generated increased mortality and developmental problems in larvae (sub-lethal effects). [768] Selenium may be bioaccumulated in the nectar and pollen of Brassicaceae plants.

The negative effects of metals on bee colonies are demonstrated by several studies. The artificial addition of metals such as cadmium (Cd), copper (Cu), lead (Pb), and selenium (Se) to the diet of bees, at concentrations that may easily be found in honey and pollen, has shown negative effects on the colony (in this case the insects were fed with sugar syrup containing added metals and a pollen replacement feed that is usually based on amino acids and vitamins, and also contains fats). [177] The results reported in this study show that the concentrations of metals in flowers are higher than those recorded in adult bees, and the latter have higher concentrations than those present in honey. [176] Cadmium, copper and selenium cause a higher mortality in juvenile bees, and selenium decreases the total weight of worker bees in the colony. The concentrations of all four metals in the adult bees were always higher than those recorded in the larval stages, thus showing that, in the short life of these insects, there is a sort of bioaccumulation. Also for other pollinating insects there is evidence of negative effects of metal exposure (e.g. *Osmia rufa*). [176]

The following table shows the concentrations of cadmium, copper, lead and selenium recorded in some plants, honey and bees. [176]

Metals	**Matrices** (*Apis mellifera* or plants)	**Areas**	**Concentrations** (mg/kg)
Cadmium	Worker bees	Urban and industrial area in Belgium	0.06-0.1
	Forager bees	Urban centre close to a motorway in Italy	2.87 - 4.23
	Forager bees	Industrial site in Finland	0.05 - 1.2
	Forager bees	Industrial site in the Czech Republic	0.74 - 1.75
	Forager bees	Polluted site in Italy	0.05 - 0.06
	Forager bees	Industrial site in Poland	0.39 - 0.81
	Honey	Urban area in Turkey	0.32
	Honey	Industrial area in Romania	0.017
	Honey	Agricultural and industrial area in Egypt	0.1 - 0.41
	Clover flowers	Agricultural and industrial area in Egypt	0.41
	Raphanus sativus flowers	Greenhouse	13
Copper	Forager bees	Industrial site in Finland	14 - 27
	Forager bees	Industrial site in the Czech Republic	31.9 - 37.7
	Forager bees	Industrial site in Poland	20.2 - 25.5
	Honey	Turkey	0.2
	Honey	Agricultural and industrial area in Egypt	2.3 - 11
	Honey	Industrial site in Poland	0.01 - 23.5
	Clover flowers	Agricultural and industrial area in Egypt	51
	Raphanus sativus flowers	Greenhouses	32
Lead	Worker bees	Urban and industrial area in Belgium	0.33 - 0.41
	Forager bees	Industrial site in Poland	1.46 - 2.32
	Honey	Industrial area in Romania	0.19 - 0.2
	Honey	Agricultural and industrial area in Egypt	1
	Clover flowers	Agricultural and industrial area in Egypt	2.9
	Raphanus sativus flowers	Greenhouses	1.16
	Pollen	Urban area in Italy	0.27
Selenium	Forager bees	Industrial site in Poland	1.7-11
	Honey	Industrial site in Poland	0.11 - 0.83
	Raphanus sativus flowers	Fields	25
	Raphanus sativus pollen	Fields	5.6 – 2,830
	Brassica juncea nectar	Greenhouse	110
	Nectar of *Stanleya pinnata*	Greenhouse	150

INTERNATIONAL SURVEYS ON METALS IN BEEKEEPING PRODUCTS

The results of some metal monitorings in beekeeping products carried out abroad (outside of Italy) are reported.

- In the USA, researchers were able to map the distribution of dangerous metals such as cadmium, fluorine, and arsenic in an area by examining concentrations in pollen and bees. [31] The research was carried out, between 1982 and 1983, in the Washington region during the months of July to September. 64 beekeepers collected samples from 72 sites covering an area of approximately 7,500 square kilometers. By measuring the variation of the concentration of these elements in the bees, it was possible to construct a map of the constant concentration curves and identify the most contaminated areas. It was practically possible to isolate the areas where the substances in question were released.

- In France (May-June 1999), a study examined the concentrations of several metals in 150 samples of acacia honey (*Robinia pseudoacacia*) taken from contaminated and uncontaminated areas. Several elements were searched for: Ag, Ca, Cr, Co, Cu, Fe, Li, Mg, Mn, Mo, P, S, Zn, Al, Cd, Hg, Ni, and Pb. [17, 33] Silver (Ag), copper (Cu), aluminium (Al), zinc (Zn), and sulphur (S) were found in honey samples from hives located near industrial areas. Calcium (Ca), magnesium (Mg) and phosphorus (P) were detected in all analysed samples. Aluminium (Al), molybdenum (Mo) and sulphur (S) were found in more than 50% of the samples, cobalt (Co) in 30% and chromium (Cr) in 20%. The minimum and maximum concentrations measured for some metals were as follows:

Metals in acacia *(Robinia pseudoacacia)* **honey samples**	**Positive samples out of 150**	**Percentage of positive samples**	**Minimum and maximum concentration, in ppm**
Aluminium	99	66	0.05-1.4
Silver	10	**6.7**	0.08-0.16
Calcium	150	100	2.9-108.5
Chrome	33	**22**	0.05-0.52
Cobalt	46	**30.7**	0.03-0.25
Copper	72	48	0.03-2.3
Iron	107	71.3	1.4-109.5
Lithium	5	3.3	0.02-0.2
Magnesium	150	100	1.4-109.5
Manganese	141	94	0.06-10.3
Molybdenum	86	57.3	0.07-0.81
Phosphorus	150	66	32.1-397.5
Sulphur	84	6.7	1.6-67.7
Zinc	67	100	0.04-5.9

It is important to note that in this monitoring nickel, mercury, cadmium and lead were not found in any of the 150 acacia honey samples. Silver and chromium signal the presence of a contaminated industrial area. Sulphur is a contaminant that could be an indicator of the presence of heavily trafficked roads (e.g. highways) or particular industries (e.g. textiles). Metals highlighted in bold such as silver, chromium and cobalt indicate pollution by human activities.
Other results reported by the same publication are the following: [17]

- Another monitoring conducted in France, between 1986 and 1996, reported that 10% of 97 honey samples contained lead;
- In 1994, 3% of 122 samples of French honey contained cadmium.

- A monitoring conducted in Poland (2009) examined the concentrations of metals such as cadmium in an industrial and an agricultural area. [40] Nickel, lead, iron, zinc, and magnesium were searched for in samples of multifloral honey, propolis, wax (of the year) and pollen (taken from bees with a special mechanical system). Samples were randomly selected in the 10 hives of each of the 8 apiaries used. During 2009, 80 samples were taken for each type of matrix (a total of 320 samples of 2 g each). The lowest concentration of the metals sought was recorded in honey, while wax recorded the highest values.
Honey contained the lowest concentrations of cadmium, nickel, lead, zinc, and iron, while wax contained the highest concentrations of cadmium (0.099 µg/g), iron (334 µg/g), and lead (3.13 µg/g), and propolis contained nickel (9.81 µg/g). Lead could also be found at high

concentrations in pollen and propolis. Pollen contained the highest concentrations of magnesium (2,580 µg/g) and zinc (159 µg/g); these metals were also contained in high concentrations in wax and propolis. Iron, zinc and magnesium are essential metals, so they are found in high concentrations in pollen. In this study, wax was found to be contaminated with lead and cadmium. It must be remembered that wax is secreted by glands located in the abdomen of bees and can be recycled by beekeepers (in this study, year's wax was used, therefore not recycled), while honey is derived from flower nectar and feed that can be supplied by beekeepers.

- A study carried out at several sites in Finland (urban, industrial and control; published in 2000) found that pollen and honey are not suitable bio-indicators of the presence of metals such as cadmium, lead, copper, iron, manganese, and zinc in the environment. [708] On the other hand, bees may be used to monitor some metals, as the following mean concentrations were recorded: cadmium (Cd) 0.423 g/g; copper (Cu) 19 g/g; zinc (Zn) 76.25 g/g; iron (Fe) 172.5 g/g. In this study, bees are more contaminated with metals than pollen and pollen is more contaminated than honey.

- A research in Poland examined the concentration of the metals zinc, copper, lead, arsenic, and cadmium in propolis samples (a plasma spectrometer was used). [74] Ninety samples were taken from 30 apiaries. Each sample (30 g) was obtained by mixing propolis taken from 3 colonies of the same apiary (the laboratory used 2 g). The samples were taken in Poland in 2010 between the months of May and August. Metals were found in the following decreasing order of concentration: zinc (Zn) >> copper (Cu) > lead (Pb) > arsenic (As) > cadmium (Cd), at the following average concentrations, in mg/kg, of: 55.79, 8.94, 6.54, 0.698, 0.203, respectively; and at the maximum concentrations, in mg/kg, of: 115.22 for zinc, 18.32 for copper, 18.29 for lead, 1.81 for arsenic, and 0.811 for cadmium (in another study, the presence of a cement factory provided a maximum cadmium concentration of 0.795 mg/kg, i.e. a similar concentration). [74] All metals tested were found at average concentrations above the regulated limits for propolis in Poland, except for copper for which the limit is 10 mg/kg (*Polish Standard PN 1998*). Concentrations of lead and arsenic were found to be high. The concentrations of the latter are influenced by the presence of metallurgical and chemical industries in the area where the hives were located: in this area the concentration of arsenic in the soil was 2,500 mg/kg. The Authors suggest to submit propolis to analytical controls before using it for food or pharmacological purposes.

- A monitoring conducted in Turkey in 2010 examined six honeydew samples (from *Pinus brutia*) and eleven bee samples with the aim of detecting the effects generated on bee products by a thermoelectric plant burning coal and lignite. [30] The hives were located between 11 and 22 km from the plant. Bee samples consisted of 20 to 50 live bees taken during the return to the hive. This study searched for different metals and concluded that bees are a better indicator of the presence of metals than honeydew honey and that the insects record the highest concentrations of lead (up to 24.1 µg/kg) and cadmium (up to 7.7 µg/kg). [30]

- It may be useful, in order to save time and resources, to try to predict how metals are distributed in the environment so that the matrices where they concentrate may be identified more easily. Metals (e.g. lead) released into the atmosphere may accumulate in the soil, from

there in plants and subsequently in bee products. A study, carried out in 2009 in Romania, shows that lead in the hive is distributed, in descending order, as follows: forager bees (mean of 50.7 mg/kg; higher concentrations and up to 607 mg/kg may be measured in dead bees) > drones (mean of 32.7 mg/kg) > propolis (mean of 22.2 mg/kg) > wax (mean of 21 mg/kg) > larvae (mean of 17 mg/kg) > honey (mean of 14.6 mg/kg) > royal jelly (mean of 1 mg/kg). [732]
The mean concentration of lead in soil was 1,840 mg/kg (much higher than that measured in the most contaminated hive matrix), the maximum concentration was 12,134 mg/kg and the minimum concentration was 369 mg/kg. Only a fraction of this lead is easily absorbed by plants: between 1.4% and 13.7%. The concentrations measured in pollen vary: 0.12 mg/kg in *Rubus fructicosus*, 0.14 mg/kg in *Trifolium repens*, and 3.13 mg/kg in *Lamium album*. Analyses carried out on 13 plants visited by bees show that the concentrations in pollen or flowers are lower than in soil. Therefore, the concentrations compared to the soil, in the different plants, are lower: in white clover pollen (*Trifolium repens*) concentrations of 0.2% of those recorded in the soil were measured, in chestnut pollen (*Castanea sativa*) 3%, in white nettle (*Lamium album*) 4%, in bird's foot trefoil (*Lotus corniculatus*) 9%, in the garden loosestrife (*Lysimachia vulgaris*) 11%. In some cases, pollen measures higher concentrations than those measured in flowers (e.g. *Trifolium medium*, *Achillea millefolium*). The roots register the highest concentrations, which may reach, in the white nettle, 43% of those measured in the soil. This research shows that the soil is the most contaminated matrix, followed by the roots of the plants and then the foraging bees. Therefore, the hive product that is the best indicator of lead contamination is foraging bees, but plant roots and soil are more likely to provide information as they contain much higher amounts of lead.

- The results of a research that investigated the presence in two areas of Brazil of 11 metals (Al, Ca, Cd, Cr, Cu, K, Mg, Mn, Na, Pb and Zn) in 42 propolis samples (from *Apis mellifera*, by means of atomic flame absorption spectrometry and electro thermal atomization; each of the 42 samples weighed 0.5 g and was measured three times) are summarised as follows. [62] Samples were taken in the months of January to March 2011. None of the hives contained metallic parts. The average concentrations recorded for some metals were as follows, in mg/g: aluminium (Al) 0.68; calcium (Ca) 1.66; potassium (K) 7.59; magnesium (Mg) 1.27; manganese (Mn) 0.08; sodium (Na) 0.58, and zinc (Zn) 0.02.
The highest concentrations were measured for the metals Al, Ca, K, Mg, and Na in all propolis samples. Mn and Zn were detected at lower concentrations.
The concentrations of Al, Ca and Mg in propolis from different regions were statistically different so they may potentially be used to determine the geographical origin. The mean concentrations of Cd, Cr and Pb, which are indicators of the presence of contamination, were 0.13, 5.53 and 9.85 µg/g, respectively.
The concentration of lead, according to the United Kingdom standard for foodstuffs, must be below 1 mg/kg; this reference is lower than the limit proposed by the *Codex Alimentarius* of 2 mg/kg. In a research conducted in Spain the highest concentration of lead recorded was 3.8 mg/kg, in Poland up to 3.28 mg/kg of lead and 10.96 mg/kg of Cu; in China Pb was found up to 19.92 mg/kg and Cd up to 0.6 mg/kg. [62] Chromium was found up to 3.5 mg/kg in a study conducted in Argentina on propolis. Cadmium and lead may be derived from manure, sewage, industrial effluents, and fossil fuels through atmospheric deposition. The tolerable weekly intake that a 60 kg adult can ingest has been proposed to be 25 µg per kg body weight for lead and 7 µg per kg body weight for cadmium. Ingesting 1 g of propolis per day can result, at the concentrations measured in this work, in up to 5% of the safe dose of lead for a 60 kg adult. [62]
Thus, propolis is highly contaminated with metals.

The Authors conclude that the metal content of propolis may be used to identify its geographic origin because the concentrations reflect those of the soil and the plants.

- A study examined the concentration of 14 elements in the soil, in flowers and honey samples of sunflower (*Heliantus annus*) and black locust (*Robinia pseudoacacia*) in five regions of Hungary without industrial areas and busy roads (work carried out in 2015). [44] Honey samples were chosen randomly by selecting 5 hives per site (samples consisted of 100 g of honey). A high correlation between the concentration measured in the soil and that detected in honey was shown, with the following decreasing order of probability: copper (Cu) > barium (Ba) > strontium (Sr) = nickel (Ni) > zinc (Zn) > manganese (Mn) = lead (Pb) > arsenic (As). In the two monitored plants (sunflowers and black locusts), the bioconcentration of metals in honey was similar. Sunflower honey had higher concentrations of boron (B), barium (Ba), copper (Cu), iron (Fe), lead (Pb), strontium (Sr), zinc (Zn), and lower concentration of nickel than acacia honey. Micro-nutrients were found in acacia honey in the following order of increasing concentration: molybdenum (Mo) < lead (Pb) < arsenic (As) < barium (Ba) < nickel (Ni) < strontium (Sr) < copper (Cu) < manganese (Mn) < iron (Fe) < zinc (Zn) < boron (B); while those measured in sunflower honey were: Mo<Cd<Co<Ni<Pb<As<Ba<Sr<Cu<Mn<Fe<Zn<B. [44] Note that the order of the last 5 elements is identical.
For the macro-elements, the correlation between the concentration measured in the soil and that measured in the honey was most evident for potassium, sulphur and phosphorus. In acacia honey, the macro-elements recorded the following increasing order of concentration: magnesium (Mg) < sodium (Na) < sulphur (S) < phosphorus (P) < potassium (K); while for sunflower honey the following results were obtained Na<Mg<S<P<K. This study concludes that honey may be used to obtain information on soil concentrations of copper and barium, and to obtain information on the presence of strontium, nickel, zinc, manganese, lead, and arsenic. Barium is the only element more concentrated in honey than in soil.
Acacia flowers bioconcentrate over 14 times more potassium, 11 times more phosphorus, 8 times more sulphur, 5 times more boron, and 3 times more molybdenum than the recorded soil concentrations so they can be used to get information about these metals in the soil. Sunflower flowers bioconcentrate boron also more than 22 times, sulphur more than 8 times, potassium more than 6 times, phosphorus and molybdenum more than 3 times than the concentrations recorded in the soil. This study reveals that the concentration of certain metals measured in black locust and sunflower flowers is a better indicator than honey. The search for these metals in flowers may easily provide information about the level of soil contamination because of their bioconcentration.

- A research examined the concentrations of some metals (As, Al, Cd, Co, Cr, Cu, Mn, Pb, and Zn) inside and outside the body of dead bees from colonies located in urban and rural areas in Poland (article published in 2018). [47] Dead bees collected at the bottom of the hives were used (5 dead bees per sample). Some metals such as zinc and chromium are mainly found on the outer surface of the bees' bodies, while lead was found inside (e.g. in bees in the vicinity of industrial areas). Cadmium may be recorded in the hemolymph of the insect. Lead and cadmium may be used as markers of the presence of industrial activities and urbanization. Elements such as copper (Cu), manganese (Mn), arsenic (As), and aluminium (Al) are also constituents of pesticides such as insecticides or fungicides, so this may be another source of contamination.

The research shows that the highest concentrations of arsenic (As), aluminium (Al), lead (Pb), and cadmium (Cd) may be found in colonies located in urban areas. Aluminium (Al), arsenic (As), and chromium (Cr) may be found more easily on the external surface of the body of dead bees and cadmium inside the body (cadmium is also found on the external surface).

SOME ITALIAN RESULTS ON THE MONITORING OF METALS IN BEEKEEPING PRODUCTS

The results of some monitoring, conducted in Italy, on the presence of metals in beekeeping products are summarized hereafter.

- In Italy, samples of honey, foraging bees, larvae, and pollen were examined for concentrations of lead, chromium, nickel, and cadmium. [69] Colonies were placed in five sites in the Modena area and the research was carried out from 1987 to 1989. Each sampling station consisted of 2 hives, located in urban areas characterized by intense vehicular traffic. Samples were taken once a month, between April and September. Pollen was collected from the bees when they returned to the hive. The ratios obtained by dividing the measurements made in the honey samples (median concentration in µg/kg) with the concentrations detected by the air monitoring stations (median concentration in µg/m^3) were as follows: [69]

- 1,000 - 2,000 for lead and nickel;
- 2,000 - 4,000 for chrome;
- 3,000 - 5,000 for cadmium.

The Authors of this paper conclude by stating that honey can be used to get information about air pollution.

- Measurements of lead in hives located in a polluted area in the Municipality of Portogruaro (in the Province of Venice) reveal concentrations of 1.84 ppm in honey, 13.7 ppm in propolis, and 13.1 ppm in royal jelly. [17]

- A study conducted in Italy examined the concentrations of metals (mercury or Hg, chromium or Cr, cadmium or Cd and lead or Pb) in bees (*Apis mellifera*) sampled when they were alive. [65] The research was carried out from May to October 2007 at two sites, one in Lazio and one in Abruzzo. Four apiaries were located in the Riserva dei Calanchi di Atri in Abruzzo, two were located near a busy road, one apiary was located near an airport (Ciampino airport in Rome) and one apiary was located in an urban area with the presence of an incinerator. Therefore, the colonies were subjected to different sources of contamination. Each sampling station consisted of three hives with 10 combs each. The 24 hives used were wooden and no fumigation was carried out. Live bees were sampled during the day and only pollen-cleaned worker bees were used (bee samples were 2 g each). Some results are summarized as follows: [65]

- None of the samples contained mercury.
- Lead (average concentration of 0.52 mg/kg) and cadmium were with higher concentrations near the airport.
- Cadmium was found at low concentrations with no significant differences between the sites, so it was also present in natural reserves. In this respect, it is important to note that 85%-90% of cadmium in the atmosphere is of anthropogenic origin, as it may result from the combustion of fossil fuels, refineries, and waste incineration.
- Chromium is the element found at the highest average (0.97 mg/kg in August) and absolute concentrations (of 5.07 mg/kg).

This study concludes that it is not possible to have information on the bioaccumulation of metals by examining their content in bees.

- A research conducted in Sardinia investigated the presence of metals (cadmium or Cd, chromium or Cr and lead or Pb) in beekeeping products (each analysis was carried out in triplicate, using the technique of atomic absorption spectrometry). [72] There is a mine at the site that supplied lead and zinc for over 130 years (from 1848 to 1980). At the time of the research, the mine was decommissioned and abandoned, and zinc is not investigated in this study. Cadmium, chromium and lead were searched for in soil, running water, foraging bees, honey, and pollen in samples collected during three years (2007-2009). Three beehives were used at each monitoring station; one monitoring station was located near the mine and one was located 50 km away (at the University of Sassari). Bee samples consisted of 50 bees taken from each colony, thus 150 bees per station. Honey was taken from cells with unripe honey (4 g per hive and, therefore, 12 g per monitoring station). Pollen was collected from forager bees using special mechanical systems, which were placed one week before sampling. Samples were taken during the months of March, May, July, and November.

Analyses of cadmium and lead in the water at the control site recorded very low concentrations, below the quantification limit, while high concentrations were recorded in the mine area. In detail, the limits for drinking water in Italy, 5 µg/L for cadmium and 10 µg/L for lead, were exceeded at the two sites monitored in the mine area (by 268 times for cadmium and 137 times for lead). Metal concentrations, in the soil at the mine site and in bees that were kept near the mine, were always higher (significantly) than those in the control samples. The use of bees to monitor metals, in this case, works given the results of the comparison between control hives and hives placed at the contaminated site. Measuring metals in the soil provides better information as the concentrations are much higher: [72]

- 2,000 times higher for lead measured in contaminated soil than in pollen, which contains the highest amount of lead among bee products;
- 7 times more for chromium in the soil than in bees, which have the highest concentration among beekeeping products;
- 3 times more for cadmium in the soil than for cadmium in bees, which have the highest concentration among bee products.

Honey is always the least contaminated matrix and therefore is not a good indicator of the presence of metals as it does not accumulate them. No beekeeping sample bioaccumulates the metals examined, because the concentrations are always lower than the environmental ones (in this research they did not evaluate the concentrations of metals in propolis and wax). The Authors point out that there are no regulated maximum concentrations for these metals in honey, and they also report some maximum allowed limits, proposed in Europe, for honey: 1 mg/kg for lead and 0.1 mg/kg for cadmium. The honey samples examined in this study do not exceed these limits. The Authors conclude that for the examination of the contamination of these three metals, live bees are a better indicator than pollen and honey, as the concentrations correlate better with those measured in the soil. Bees located at the contaminated site record significantly higher metal concentrations than those located at the comparison site. In conclusion, it is better to look for these contaminants directly in the soil unless one wants to assess a risk to food safety or bee health.

During this work, the biodiversity of the ants present in the mine site and in a nearby control site was measured. The ants were captured every 15 days starting in May, using special traps that were placed at the vertices of a square of 12 m side. [72] Altogether 37 species of ants were classified.

- In 2008-2010 (three years), biomonitoring was carried out in 10 nature reserves in the Marche Region. [33] Twenty-two colonies were used at 11 sites, from which honey, live bees (100 bees for each sample), and dead bees were sampled in the months from May to October. Dead bees, which are the individuals that are removed by the insects themselves in the hive, were collected with special traps placed in the hive.
The highest concentrations were recorded for chromium followed by cadmium and lead; metals released into the atmosphere were the most likely source of contamination although 24% of groundwater was contaminated with chromium, cadmium and lead. Lichens were used to monitor air pollution in the same areas and revealed the presence of cadmium, chromium, lead and nickel contamination. Chromium concentrations were increasing in October, suggesting an anthropogenic source.
Some pesticides that are not found (30 organophosphates, 13 pyrethroids and 16 triazoles) were also searched. [33]
In this study, honey does not turn out to be a good indicator of metal or even pesticide contamination.

- The examination of the lead content, in Italian honeys, recorded the exceeding of the maximum allowed concentration (0.1 mg/kg) in 4.2% of the samples taken in the year 2012 and in 1% of the samples in 2015. [35]

- A study conducted in Italy, in Trieste, used colonies of *Apis mellifera ligustica* to monitor the presence of metals at two sites, an urban and a suburban one, where there is an iron foundry and an incinerator. [64] Apiaries of volunteer beekeepers were used and samples were taken in June of the year 2015. The samples consisted of live bees taken from 10-frame wooden hives. 11 metals were identified in two different orders of decreasing concentration:

- in foraging bees from the suburban area: Zn (zinc) > Cu (copper) > Sr (strontium) > Bi (bismuth) > Ni (nickel) > Cr (chromium) > Pb (lead) = Co (cobalt) > V (vanadium) > Cd (cadmium) > As (arsenic);
- in bees from the urban area: Zn > Cu > Sr > Cr > Ni > Bi > Co = V > Pb > As > Cd.

In all samples, the concentrations of zinc, copper, strontium, nickel, chromium, and bismuth were found to be the highest. The highest concentrations of zinc, copper, strontium, nickel and chromium were recorded in the urban area. Some concentrations recorded in the urban area were as follows: As 0.027 µg/g, Bi 0.416 µg/g, Cd 0.024 µg/g, Co 0.135 µg/g, Cr 0.465 µg/g, Cu 17.93 µg/g, Ni 0.425 µg/g, Pb 0.113 µg/g, Sr 1.9 µg/g, V 0.13 µg/g, and Zn 58.11 µg/g.
This study reveals that bees located in the urban area contain higher concentrations of chromium and copper, and lower concentrations of cadmium, thus chromium and copper were associated with the exposure to urban traffic while cadmium with industrial emissions.

- Metals in the atmosphere such as lead may be found in bees at high concentrations. A study conducted in Milan (presented in 2017) reveals atmospheric concentrations of lead of 4 ng/m^3 and, simultaneously, concentrations of 0.7 mg/kg of lead in bees; atmospheric concentrations of lead of 15 ng/m^3 gave a result of 0.9 mg/kg in bees. [196]

- A work conducted in Italy investigated the presence of fine particulate matter (PM) in live bees collected from hives located near a cement factory and busy roads (article published in 2018). [63] Fine particles are very dangerous for human health and the ultra-fine ones may directly enter the brain through the olfactory bulb. Cement production is one of those industrial activities that produces fine dust in almost all processing steps (crushing, homogenization, heating to about 1,450°C etc.). The cement plant under study occupies 61,000 m^2 and produces

900,000 t/year of unburnt residue. Many hazardous substances such as metals may be present in the dust. To monitor the presence of fine dust, live bees in the Province of Piacenza were used, which were reared at a distance of 800 m from the cement plant. An apiary located 7 km from the cement plant, which was exposed to nearby busy roads, was also used. In this study, dust was examined with a scanning electron microscope (SEM) on the external surface of the foraging bees' bodies. The bees were collected at noon, during sunny days, when the temperature was around 19°C. In the publication, several electron microscope photographs are included showing the presence of substances in different parts of the outer surface of the forager bees' bodies (wings, head, hairs). Among the metals detected in the inorganic substance present on the external surface of the insects' bodies there are: Pb, Cu, Ti, Bi, Cr, Mn, Sn, and Zn as well as Si, Al, Na, K, Fe, Ca, Mg, Ba, Zr, and S. [63]
Calcium carbonate and aggregates of Bi, Cr, Cu, Mn, Sn, Zn, and Pb were observed on the surface of the bees placed near the cement factory, and these certainly came from this activity.
Some metals such as Cr, Cu, Zn, Pb, and barium sulphate ($BaSO_4$), which are generated by road transport (e.g. tyres and brakes), were found on the surface of bees located near roads and far from the cement factory.
Under the microscope, calcium oxalate crystals are also detected as a result of the anti-*Varroa* treatments carried out by beekeepers.

THE DETECTION OF RADIOACTIVITY

Bees have long been used to monitor the presence of radioactive substances produced by the invention of atomic energy, as they are a good natural sampler of various isotopes. In order to measure the increase in radioactivity generated by nuclear experiments (in particular the strontium isotope: ^{90}Sr), bees were probably first used in the ’50s. [17]
It has been shown that honey may bioaccumulate cobalt (^{60}Co) and sodium (^{22}Na) from contaminated water present at the site visited by bees rather than from flowers. The concentrations measured in honey are higher than those found in the contaminated water: about 50 times for cobalt (^{60}Co) and 30 times for sodium (^{22}Na). Forager bees, i.e. those that go out to patrol and look for water and food, are the ones that get contaminated first.
Throughout Europe, bees have been used to monitor the spread of pollutants produced as a result of the accident at Chernobyl (Ukraine) on 26 April 1986. [118] The explosion of the nuclear reactor released various isotopes (such as xenon, iodine, caesium, tellurium, strontium, barium, zirconium, molybdenum, ruthenium, neptunium, plutonium and curium). Among the most dangerous, there are the isotopes of iodine, caesium and strontium. Iodine is fixed in the thyroid gland, increasing the risk of cancer, while caesium and strontium are incorporated into tissues as homologues of potassium and calcium respectively. The explosion carried these dangerous substances more than a kilometre into the atmosphere, allowing them to spread throughout the northern hemisphere. Increased radioactivity was recorded in Europe, the USA, Canada and Japan.
The half-life of caesium, isotope 134, is about 2 years (2.06), while that of isotope 137 is 30.17 years. The presence of these two isotopes is a consequence of nuclear tests (bombs) or accidents such as Chernobyl. In France, these two isotopes of caesium (^{134}Cs and ^{137}Cs) have been used to measure the variation of radioactivity. [17] Honey recorded the presence of these isotopes indicating an increase in radioactivity from far away and at low concentrations. Honeydew honey showed the highest levels of radioactivity, as is to be expected, as the food chain bioaccumulated the isotopes first in plants, then in aphids and later in bees, increasing their concentration. Thus, honeydew honey is a possible bioindicator and can be used to monitor the variation in radioactive contamination levels over vast areas. In multi-flower honeys the

concentration may be very variable depending on several factors such as differences between plants (e.g. chestnut honey seems to bioaccumulate more than acacia honey).

Also in Croatia the presence of caesium (isotope ^{137}Cs) recorded after the Chernobyl accident was monitored. [17] Radioactivity is not uniformly distributed in the environment, in fact in Croatia the difference of radioactivity between the most contaminated soils and the less contaminated ones was up to 50 times. These differences may be due to the climate (intensity of rainfall) and the physical and chemical characteristics of the soil. In Croatia too, an increase in radioactivity was recorded following the 1986 accident, both in plant tissue samples and in honey. The radioactivity (^{134}Cs) measured in honey, after 10 years, had become much lower and frequently not measurable. In this study honey proved to be a good biological matrix to detect the presence and variation over time of radioactive isotopes from far away and at low concentrations. Furthermore, it is confirmed that honeydew honey is a better indicator than nectar honeys, as the recorded radioactivity is higher (^{137}Cs). Heather honey[I] was found to be a good indicator of radioactive caesium (^{137}Cs) contamination. The measurement of this isotope in honey makes it possible to detect what happened a long time ago, as its half-life is 30 years, so the concentration in the environment remains constant for a long time. This work concludes that honeydew honey from hives located near fir and coniferous forests may be used to monitor caesium, chromium, rubidium, copper, lead, and nickel. [17] Also in Italy the conclusions of the results of biomonitoring of radioactive isotopes confirm the possibility to use honeydew honey to determine the presence of radioactive isotopes (^{137}Cs and ^{134}Cs) and also identify pollen as a possible indicator of the variation of the presence of radioactivity in the environment. [17]

MONITORING METAL CONTAMINATION WITH ANTS

Other insects such as ants may be used to monitor metal contamination. A study conducted in Prato, Italy, and published in 2013 examined the ability to bioconcentrate six metals (copper, cadmium, nickel, manganese, lead, and zinc) in an ant widespread in the Mediterranean area. [734] *Crematogaster scutellaris* is an ant belonging to the subfamily Myrmicinae that has an ovoid head of a reddish colour, contrasting with the rest of the body which is instead blackish. [735] It has a stinger that can release a repellent venom. These ants preferably nest in wood, under the bark of trees, with a preference for olive, pine (*Pinus pinea*, *Pinus nigra*), and oak trees. It is a voracious predator of small invertebrates, but can also feed on dead insects; it is also often found associated with colonies of aphids, whose honeydew is a source of sugary substances. Because of its predatory activity, it has been studied as a possible tool for biological control of the pine processionary moth (*Thaumetopoea pityocampa*). The life expectancy of the colony, made up of thousands of insects, may be more than 10 years, thus exceeding that of many other invertebrates.

The search for metals in soil and insect bodies generated the following results:

- Metals accumulate in the abdomen of the insect rather than in the head or thorax. This also occurs in bees. [750]
- The concentrations of metals found in ants reflect the levels of environmental contamination recorded at the different sites.
- In ants, zinc and cadmium concentrations were higher than those recorded in the soil. Thus, these two metals are bioconcentrated and allow to propose ants as bioindicators.
- Copper, nickel, manganese, and lead were more concentrated in the soil than in ants.

[I] The heather plant (*Calluna vulgaris*) is an herbaceous, perennial, evergreen, and needle-like leaf plant. [105]

By surveying the presence and numerosity of ants, it is possible to obtain information on the state of health of the agricultural ecosystem, since usually agronomic practices do not permit their survival. Soil movement (e.g. ploughing), the absence of semi-natural areas where they can take refuge and the absence of food like other insects (e.g. due to insecticides) are devastating for ants.

MONITORING OTHER POLLUTANTS, MICROORGANISMS AND DISEASES

BIOMONITORING OF POLYCYCLIC AROMATIC HYDROCARBONS IN PLANTS

Polycyclic Aromatic Hydrocarbons (PAHs) are organic contaminants found widely in the environment. [36] They are formed by the incomplete combustion of organic materials, in particular wood and fossil fuels, such as coal and oil, and are components of particulate matter. They are also produced by refineries, waste incinerators and are present in tobacco smoke; they are also generated by volcanic eruptions and forest fires. These substances may easily move through the air over long distances. Living beings may take them in by breathing, contact, and ingestion. Fine particles can also result from photochemical reactions and oxidation processes; in this case we speak of secondary particulate matter.

The legislation provides for the measurement of total suspended particulate matter called PM_{10}. The acronym PM comes from Particulate Matter and the number on the side indicates the (aerodynamic) diameter, in thousandths of a millimetre (micro-metres or millionths of a metre: μm). The smaller the diameter of these particles, the greater the danger. Particles with a diameter of less than 2.5 μm easily reach the deepest parts of the lungs and enter the bloodstream. The smallest fraction is, therefore, the most dangerous and may contain carcinogenic substances, such as some polycyclic aromatic hydrocarbons generated by diesel engines.

The PAH family includes several hundred compounds that are very heterogeneous and consist of two or more benzene rings. [59] Some of these compounds consist only of hydrogen and carbon, others also contain atoms such as nitrogen and sulphur. PAHs are generally toxic, mutagenic and carcinogenic. [66] 16 types of PAHs have been identified as very hazardous by the EPA (US environmental agency). [59] In Europe 15 molecules belonging to PAHs are considered most important. The Scientific Committee on Food concluded in its opinion of 4 December 2002 that several PAHs are carcinogenic, genotoxic, persistent, lipophilic, and bioaccumulative. [60, 849] The most toxic substances are probably the molecules that have four to seven rings. The most studied component is benzo(a)pyrene (BaP), a five-ring compound with high toxicity, so much so that it is used to represent the environmental pollution of the whole group of PAHs. In Europe, benzo(a)pyrene can be used as a marker for the presence and effect in food of carcinogenic PAHs [including benzo(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, chrysene, cyclopenta(c,d)pyrene, dibenz(a,h)anthracene, dibenzo(a,e)pyrene, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene, dibenzo(a,l)pyrene, indeno(1,2,3-cd)pyrene, and 5-methylchrysene]. [849] Other important molecules, due to their mutagenicity and carcinogenicity, are anthracene, fluoranthene, pyrene, and their derivatives.

On foggy days the highest concentrations may be recorded. They can bioaccumulate in plants and enter the food chain. In urban areas the highest values are found in the winter months (values between 1.5 and 11 ng/m^3), while the lowest values are found in the summer period (0.15-0.68 ng/m^3). In urban soils PAHs may reach very high concentrations (20,060 ng/g). [38]

The main health risk associated with polycyclic aromatic hydrocarbons is their ability to induce cancer. The International Agency for Research on Cancer (IARC) classifies some of the PAHs as possible carcinogens and others as probable carcinogens. Among these, benzo(a)pyrene

(BaP) is the most studied component because of its wide diffusion in the environment and its high toxicity. Each 1 ng/m^3 increase of benzo(a)pyrene in the atmosphere may result in a risk of 9 new cases of cancer per 100,000 people. Benzo(a)pyrene may also contaminate water from coal tar used to seal storage tanks. Most PAHs are capable of causing cancer in experimental animals (e.g. by inhalation). In addition to respiration, an important route of exposure for humans is the ingestion of agricultural and livestock products that have bioconcentrated these molecules.

Many organisms have been used as bioindicators of PAH contamination, including fish and molluscs, for the aquatic environment, and mosses, lichens and conifers for the terrestrial environment. [59] Conifers may absorb PAHs from air, soil and water. Young pine needles have been used as bioindicators of the presence of these substances.

Mosses are particularly suitable for monitoring the deposition of pollutants from the atmosphere for several reasons. In mosses (e.g. *Hylocomium splendens*) the uptake of nutrients and water occurs through atmospheric precipitation and not from the soil. The advantage of using mosses to detect metals or polycyclic aromatic hydrocarbons is that they accumulate them, facilitating analytical determination. Conifer needles, deciduous plant leaves and lichens have also been used to monitor environmental contamination by polycyclic aromatic hydrocarbons (and metals). By examining the different concentrations of different polycyclic aromatic hydrocarbons, it is possible to determine what are the main sources.

Work conducted in Spain monitored the environmental contamination by polycyclic aromatic hydrocarbons using mosses. [759] The average concentration of 13 polycyclic aromatic hydrocarbons measured in mosses (*Hylocomium splendens*) was 133 ng/g. Concentrations increase in winter, as it is logical to expect, as combustion increases and photochemical degradation is less intense than in summer. The average deposition rate of 6 polycyclic aromatic hydrocarbons was about 166 ng/m^2/day, which is equivalent to about 606 g/ha/year. This is a characteristic level of contamination for rural areas.

A study conducted in Italy monitored polycyclic aromatic hydrocarbons in the atmosphere by measuring concentrations in a species of lichen, which is a symbiotic association between fungi and algae (*Pseudevernia furfuracea*). The concentrations recorded in winter were much higher than the summer ones; in detail during summer the concentrations ranged from 48 to 272 ng/g (dry matter), while during winter they ranged from 289 to 1,575 ng/g (dry matter). [852] This study confirms that lichens bioaccumulate polycyclic aromatic hydrocarbons and that they have a retrospective memory of about 45-60 days.

BIOMONITORING OF POLYCYCLIC AROMATIC HYDROCARBONS IN BEEKEEPING PRODUCTS

The following are the results of some investigations of environmental PAH contamination through beekeeping products.

- Dead bees and honey were sampled for PAH monitoring in Italy (2003-2004). [38] The concentrations of benzo(a)pyrene (a carcinogenic PAH) measured in an industrial area were as follows: between 0.14 and 0.18 µg/kg in dead bees and between 0.27 and 1.54 µg/kg in fresh honey.

- A research conducted in Turkey measured naphthalene in honey and reported that 80.4% of 100 samples were contaminated with this substance at an average concentration of 1 µg/g (article published in 2012). [41] In this publication it is highlighted that wax is a useful matrix to investigate this and other molecules, such as polychlorinated biphenyls (PCBs), originating from refrigerants, motor oils and lubricants.

- A study carried out in Turkey, in April-May 2014, monitored polycyclic aromatic hydrocarbons in samples of live worker bees, propolis and pine needles taken from 5 sites located in an industrial area, a rural area, and a site with a highway. [59] Samples were also obtained in an area characterized by the presence of a petrochemical industry, factories processing steel and iron, companies demolishing boats, a gas and coal power plant and an oil deposit. A gas chromatograph with a FID (flame ionization) detector was used for the measurement. During the live bee sampling period, no smoke was used to inspect the hives and no treatments with pesticides or other chemicals were allowed. Fifty grams of live bees were taken from each hive. Bees were cleaned of pollen before use. Needles were taken from 25-30 trees for each individual conifer sample.

The recorded concentrations of PAHs varied between 261.18 μg/kg and 553.33 μg/kg of dry matter in live bee samples, between 138.57 and 853.67 μg/kg of dry matter in pine leaf samples and between 798.61 and 2,905.53 μg/kg of dry matter in propolis samples. So propolis had higher concentrations, followed by pine leaves and then live bees. In bees, the PAHs with different ring number recorded the following concentration gradient 5>3>6>4>2, in pine leaves: 5>3>4>6>2, and in propolis: 5>4>6>3>2; the distribution of the PAHs with a different ring number in the three matrices varies. The PAHs searched in this work included the 16 PAHs considered most hazardous by the Environmental Protection Agency in the USA (EPA): [59]

- acenaphtene (3 rings),
- acenaphtylene (3 rings),
- anthracene (3 rings),
- benzo(a)anthracene (4 rings) which is found up to 56.62 mg/kg in propolis,
- benzo(a)pyrene (5 rings),
- benzo(b)fluoranthene (5 rings),
- benzo(k)fluoranthene (5 rings),
- benzo(g,h,i)perylene (6 rings),
- chrysene (4 rings),
- dibenzo(a,b)anthracene (5 rings),
- fluoranthene (4 rings),
- fluorene (3 rings),
- indeno(1,2,3-c,d)pyrene (6 rings),
- naphtalene (2 rings),
- phenanthrene (3 rings),
- pyrene (4 rings).

Acenaphtylene, acenaphtene and phenanthrene are characteristic for the emission profile of coal and creosote. Anthracene is characteristic of wood combustion, and chrysene is considered a marker for diesel fuel. Fluorene, phenanthrene, fluoranthene, and pyrene allow to distinguish between pollution from road transport and pollution from incinerators.

In this work, the analysis of the profile of the different PAHs made it possible to determine whether the samples came from an area where the main contamination comes from the combustion of coal or wood rather than petroleum derivatives such as diesel. Thus, from the profile of PAHs measured in the colonies it is possible to know what are the prevalent forms of combustion. The concentration of PAHs in propolis may be correlated with that in the soil (soil, plant, propolis). Surprisingly, we read in this work that PAHs are found in propolis because bees, instead of taking resin from plants (such as conifers), collect hydrocarbons and gummy substances from asphalt and tar. Bees, in the absence of plant resins such as those produced by pine needles, have learned to use asphalt and tar which contain various PAHs. At this point it is

useful to reflect on the attribution of anti-bacterial, anti-viral and anti-fungal properties to propolis, which is also used in pharmaceutical products.
In conclusion, the Authors point out that by studying the profile of PAHs in live bees and propolis it is possible to predict the major sources of these compounds in a given area. By using more hives and examining the concentration profile it could be possible to locate the sites of the most important sources.

- A study conducted in France in 2008 and 2009 investigated the presence of PAHs (Polycyclic Aromatic Hydrocarbons) in six apiaries. [60] PAHs enter the food chain and represent a danger as they are carcinogenic (e.g. benzo(a)pyrene). As already written, in Europe, at least 15 molecules belonging to PAHs are considered more important (priority according to EC Reg. n. 108/2005) because they are carcinogenic and genotoxic.
In this research carried out in France, the following four molecules were searched for: benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, and chrysene, which are considered by the European Food Safety Authority (EFSA) as markers of carcinogenic PAHs in the food chain. These substances were searched for in honey, pollen and bee samples, collected by beekeepers who voluntarily participated in the biomonitoring test. Six apiaries were used and samples of pollen freshly collected from the bees were analysed (pollen was taken with special traps placed three days before sampling); samples of live bees and honey, obtained from 8 hives per apiary, were also taken. Four samplings per year were carried out in two years (April-October). A total of 144 bee samples (of 10 g each), 144 honey samples (of 1 g each) and 144 pollen samples (of 1 g each) were taken: 432 samples in total (these were analysed by means of gas chromatograph and triple-quadrupole mass spectrometer).
Honey samples showed the lowest contamination (minimum = 0.03 µg/kg; maximum = 5.80 µg/kg; mean = 0.82 µg/kg). Bee samples showed a higher concentration than honey (minimum = 0.32 µg/kg; maximum = 73.83 µg/kg; mean = 7.03 µg/kg). Pollen samples recorded the highest concentrations: (minimum = 0.33 µg/kg; maximum = 129.41 µg/kg; mean = 7.10 µg/kg). Pollen was the matrix with the highest concentrations, followed by bees and then honey. [60]
Plants are affected by PAH contamination in soil and by atmospheric deposition (highways, airports, industrial areas). Some types of pollen may have up to 25.4 % lipids (e.g. *Brassica napus* plant), so PAHs may be easily accumulated there as they are lipophilic. Bees may collect PAHs through contact with airborne dust (on body hairs) and pollen. Also these Authors point out that, to make propolis, bees can collect road tar instead of plant resin, in the absence of the latter. Bees could be used to detect peaks in the concentration of PAHs in the atmosphere and measure the differences between different sites. Honey is derived from nectar which is a sugary substance so it has little affinity for PAHs and also because of this it does not bioaccumulate them. In this work there is no evidence of transfer of PAHs from pollen or bees to honey. PAHs could also contaminate the hive due to the smoke used by beekeepers when inspecting the hive. This voluntary use could be responsible for the contamination found in honey (between 0.03 µg/kg and 5.80 µg/kg).

- A study conducted in Italy examined the concentration of PAHs in live bees and honey taken from colonies kept in an urban area and in a nature reserve. [66] Samples were collected between May and October 2007 in two different areas in Lazio (urban areas) and Abruzzo (a nature reserve). Four sites were located in the nature reserve, one near Ciampino airport in Rome and one near roads and an incinerator. Each monitoring station consisted of three colonies of 10 combs. The bees (2 g per sample) were cleaned of pollen before use, the hive contained no metals and fumigation was not used (by beekeepers during inspections). Honey was separated from wax and 5 g samples were taken. The analytical instrumentation was a high pressure liquid chromatography (HPLC with fluorescence detector). All determinations were repeated at least three times and all samples contained PAHs. Benzo(a)pyrene was never detected, while fluorene, phenanthrene, anthracene, fluoranthene, benz(a)anthracene, benzo(b)fluoranthene, and benzo-(k)fluoranthene were recorded in bees; honey contained phenanthrene, anthracene and chrysene. [66] Phenanthrene was the molecule with the highest average concentration in both honey and bees and was found in 87% of bee samples and 49% of honey samples. Honey was less contaminated than bees. In bees the maximum concentration of PAHs was 10 ng/g of fresh weight, while in honey it was 3 ng/g of fresh weight. The PAHs present with the highest frequency were those with 3 rings (93%), followed by those with 4 rings (6%) and then those with 5 rings (1%). The Authors conclude that honey is not a good bioindicator of PAH contamination. In fact, honey contains about 18% of water and is rich in sugars so that hydrophobic substances such as PAHs do not dissolve in such a polar matrix. The highest concentrations, which were found in samples taken during the month of May, were probably generated by the use of fumigation, used by beekeepers to stun bees during inspections. No statistically significant differences were found between PAHs found in urban areas and those found in nature reserves.

MICROORGANISMS IN HONEY

Several microorganisms may be found in the digestive tract of bees: 1% yeast, 27% Gram-positive bacteria (*Bacillus*, *Bacteridium*, *Streptococcus*, and *Clostridium* spp.) and 70% Gram-negative bacteria (*Achromobacter*, *Citrobacter*, *Enterobacter*, *Erwinia*, *Escherichia coli*, *Flavobacterium*, *Klebsiella*, *Proteus*, and *Pseudomonas*). [41] Most of the intestinal microorganisms are not found in honey, which is produced and transported in the digestive tract, and is exchanged between different bees (during trophallaxis it is regurgitated and delivered to the sisters several times). Some spore-forming microorganisms can survive in honey, such as: *Bacillus cereus*, *Clostridium perfringens*, and *Clostridium botulinum*. *Clostridium botulinum* can be very dangerous and some monitoring have found it in honey: in Argentina (2/177 samples were positive), in Brazil (6/85), in California (9/90); in this last case six of the nine positive samples were given to newborns who developed infant botulism. Seven cases of infant botulism have been recorded in France, between 1991 and 2009, due to honey, and at least one case has also been recorded in Italy. In Japan, 3 out of 56 samples of sugar sold for beekeeping contained *Clostridium botulinum* spores. Up to 25% of honey samples produced in the USA contain *Clostridium botulinum* spores. [41] Spores of these microorganisms, in honey, may be inactivated with gamma radiation. The Authors of this scientific review conclude that honey that has not undergone analysis or sanitization processes should not be fed to infants (those under one year of age).

The *Aspergillus* fungus can grow on pollen stored in the hive and can produce ochratoxins and aflatoxins. [107] The honey bee seems to be adapted to the presence of mycotoxins as it possesses enzymes (cytochrome P450) capable of detoxifying them (e.g. aflatoxin B1 and ochratoxin). The fungus *Aspergillus flavus* may cause bee disease (damage to the brood). This is probably a micro-organism that could be transmitted from bees to humans generating a very rare disease (affects the lungs). [230]

ANTIBIOTICS IN HONEY

It is well known that good pollen feeding, under natural conditions, is sufficient to ensure the colony's ability to fight bacterial diseases. [243] The veterinary practice imposed on beekeepers to limit the spread of bacterial diseases is the destruction of infected colonies. In the state of Pennsylvania (USA), more than 32,000 colonies were burned between 1930 and 1965. [484] Bees are able to make food choices; for example, larvae in colonies affected by the parasitic fungus *Nosema*, if given the choice, prefer honey with higher antimicrobial activity. [718]

In the course of time, different antimicrobial molecules such as oxytetracyclines, tylosin, and sulfathiazole have been used in different parts of the World against foulbrood bacteria, while fumagillin isolated from *Aspergillus fumigatus* has been used to control *Nosema apis*.

In Europe, the use of antibiotics is banned, while they may be used by beekeepers in the USA, for example, to treat American foulbrood. [77] In Italy too, the use of antibiotics in beekeeping is banned, and it is important to remember that bees are capable of producing proteins with antibiotic action (e.g. *apidaecin*). Unfortunately, however, even where the use of antibiotics is forbidden they are detected (e.g. in Italy). [35] Therefore, antibiotics are banned and, in general, there is little information on the monitoring of the illegal use by beekeepers. In fact, antibiotics are the only weapon available to beekeepers to fight some microbial diseases, in addition to the alternative of destroying infected colonies. [17] Antibiotics may be effective against certain bacteria such as the agents of American foulbrood and European foulbrood (*Paenbacillus larvae* and *Melissococcus plutonius;* these bacteria are excreted in bee faeces). [107] Antibiotics do not act on spores.

The molecules used to control these bacteria include sulphonamides, sulphatiazole (which is used illegally in Italy and has already led to the emergence of resistant European plague bacteria), tetracycline, oxytetracycline, streptomycin, sulphonamides, erythromycin, lincomycin, monensin, enrofloxacin, tylosin, and chloramphenicol. [35, 41] In honey, oxytetracycline, chloramphenicol, and erythromycin have been measured at dangerous concentrations (erythromycin between 50 and 1,776 ng/kg). Antibiotics may be found in honey even 290 days (e.g. lincomycin) after their administration. [41]

Several strains of the American foulbrood bacteria (*Paenibacillus larvae*) have long been reported to have acquired the ability to resist certain antibiotics such as, in descending order of susceptibility, tetracyclines, oxytetracyclines (the only one authorized in the USA in beekeeping), tylosin, and lincomycin. [484, 744] The finding of drug-resistant pathogenic strains reinforces the suspicion that antibiotics are used by beekeepers, as the presence of the genetic characteristics of resistance are selected by the use of these molecules. The different susceptibility to antibiotics of different strains of American foulbrood may be used to study the characteristics of bacterial populations in bees.

A survey conducted in China records the following molecules in honey: tetracycline, oxytetracycline, doxycycline, chlortetracycline and chloramphenicol. [41]

In 2006, the monitoring of honey samples imported from India to Europe or the USA showed 14% of samples contaminated with tetracyclines; in 2007-2008, 28% of honey samples were contaminated with antibiotics at dangerous concentrations; in 2009-2010, 29.2% (of 362 samples). [41]

In Europe, the presence of antibiotics in honey is forbidden and there are no maximum concentrations allowed except in a few countries that have set them between 0.01 and 0.05 mg/kg (e.g. Belgium, United Kingdom, and Switzerland). Several studies confirm the use of antibiotics by beekeepers: [41]

- A survey conducted in Switzerland found chloramphenicol in 17% of honey samples (13/75).

- A research in Greece found that 29% of 251 honey samples contained tetracyclines.
- In Germany, 21% of 183 honey samples were contaminated with streptomycin.
- In Europe, 3,855 honey samples registered 1.7% non-compliance for the following antibiotics: streptomycin, sulfonamides, tetracycline, chloramphenicol, nitrofurans, tylosin, and quinolones.

In Italy, surveys conducted between 2002 and 2007 recorded antibiotics in 2.7% to 7% of the honey samples analysed (e.g. tetracyclines and oxytetracyclines, sulphonamides and tylosin). [35]
Tylosin may be found in honey even eight months after its administration to the colony.
An interesting note is that the herbicide asulam [*Methyl N-(4-aminophenylsulfonyl)carbamate*] converts to an antibiotic, sulfanilamide, which may be found in honey. [41, 79]
It is important to highlight that bacteria that have become resistant may spread the genetic factors that confer this dangerous ability to bacteria of different species, amplifying the negative effects and related costs.
A microorganism (an actinomycete of the *Nocardiopsis* genus) may be found in the intestine of bees, and is capable of producing molecules (*phenazines*) that have an antibiotic action against certain bacteria (*Bacillus* spp). Some bacterial symbionts with bees, among those that can be found in the intestine, are able to inhibit the growth of the fungus that causes calcified brood (*Ascosphaera apis*) and that of the bacterium that generates American foulbrood (*Paenibacillus larvae*). [35] The use of molecules with antibiotic properties, in addition to generating the well-known phenomenon of the selection of resistant microorganisms, alters the existing balance in the microbiome (in this case we refer to the set of microorganisms present in bees). Thus, the use of antibiotics may favour pathogenic bacteria because it damages their natural competitors, whose relationships with the host (bees) and pathogenic microorganisms have evolved and been regulated for a very long period of time; these relationships are largely unknown.
It is also interesting to describe another inhibiting molecule which is not an antibiotic because it is directed against fungi: *fumagillin* which has been extracted from certain fungi (*Aspergillus fumigatus*) and is used by beekeepers to control unicellular fungi which are intestinal parasites of bees (*Nosema ceranae* and *Nosema apis*). These pathogenic fungi spread through the environment via bee feces. The ingestion of a spore by bees leads to the production in the digestive tract, in about 2 weeks, of 30-50 million spores (the LD_{50} of *Nosema apis is* about 20-50 spores). [35] Therefore, the ingestion of very few spores is sufficient to cause the death of a bee and contaminate the whole colony. The ingested spore can germinate within 30 minutes inside the stomach and a bee may contain more than 200 million spores. [601] This fungus can also be spread by ants, wax moths, and the bee-eater (an insectivorous bird that likes to eat bees).
Antibiotics are found in bee products even when they are not administered by beekeepers, due to the contamination of the environment by livestock manure. Bees may obtain their water supply from animal dung, which may be contaminated with antibiotics. Some researchers have found that bees, even in the presence of clean water sources, prefer livestock manure. [13] Thus, antibiotics enter the hive and may contaminate beekeeping products when not used by beekeepers. Some pesticides used in animal husbandry to fight diseases such as parasites may also be found in the manure used by bees. In one French region (in 2009), an increased bee mortality was associated with treatments against ticks and intestinal worms in sheep. [230]
The use of insecticides (e.g. pyrethroids such as permethrin) to disinfest livestock establishments has led to bee deaths (in 2009 and 2014 in France). In addition, some pesticides have long been registered as antibiotics (e.g. glyphosate which is used as an herbicide). [500]
Therefore, exposure to some pesticides with an antibiotic action may promote the emergence of resistant strains and alter the balance between the symbiotic microorganisms normally present in the bees' body.

There is also another possible negative mechanism that should push us not to use antibiotics: the synergy between antibiotics and some pesticides. For example, a certain synergy between the acaricides used by beekeepers (e.g. coumaphos and fluvalinate) and some antibiotics (e.g. oxytetracycline) is known to increase bee mortality (between 7% and 51% in the case of the simultaneous exposure to coumaphos and oxytetracycline; between 5.6% and 39% in the case of the simultaneous exposure to fluvalinate and oxytetracycline). [601]
The abuse of antibiotics in the livestock, health, and food sectors generates enormous social costs such as, for example, in addition to the development of antibiotic-resistant microorganisms, the increase in the incidence of certain diseases such as obesity (due to the alteration of the microbial composition in the digestive tract). [752, 756, 757]

MONITORING OF PLANT DISEASES WITH BEES

The bees of a colony, moving around the territory and collecting water, pollen, nectar, honeydew, and resins from thousands of different points every day, are able to transport and spread phytopathogens. Assuming that an apiary is made up of 10 hives placed in the same site, that at least 10,000 bees fly daily from any hive in search of nectar and pollen, and that each one visits at least 800 flowers (making 10 flights a day), it is easy to imagine the possibility of distributing plant parasites among different plants such as certain bacteria: theoretically, an apiary of 10 colonies can visit over 80,000,000 flowers a day. In this regard it is useful to report the example of a bacterium (*Erwinia amylovora*) that may damage more than 200 crops (e.g. apples and pears) and that is certainly transported by bees. This disease is very serious because it is almost impossible to stop its spread and it may be distributed by many vectors, so that it has forced farmers to abandon the affected crops several times. Probably the nomadism of beekeeping favours the spread of these pathogens.
Erwinia amylovora is a gram-negative bacterium (of the Enterobacteriacea family), the agent of the plant disease known as *fire blight of pome fruit*. This disease affects more than 200 species belonging to 40 genera of the Rosaceae family, among which many are of great importance for human economy (apple, pear, loquat, quince, rowan) and some ornamental species (hawthorn) or other species such as black locust. [17, 18] In Italy, the first outbreaks were detected in 1990 in pear fields in Apulia and later in 1994 also in Emilia Romagna. [17, 34] In the European Union, due to the necessity of contrasting its spread, the bacterium has been included in the European list of quarantine pathogens (Directive 2000/29/EC) and, in Italy, it is subject to compulsory control measures (Ministerial Decree of 10 September 1999, n. 356). This disease is a bacterial disease that may spread through vector insects. Flowers are the first organs to be affected. The earliest symptoms are manifested on the receptacles, as spots that blacken and spread on the peduncles. The affected flowers dry up but often remain attached to the branch that generated them, thus becoming dangerous sources of inoculum for secondary infections. Primary infections occur on the flowers, which wither and emit exudates that become sources of inoculum for secondary infections. Scalar flowering of the various host species guarantees a prolongation of the period of appearance of primary infections, contributing to the spread of the disease. After having infected the various floral organs, the bacterium, passing through the intercellular spaces, crosses the floral peduncle reaching the base of the inflorescence and the twig. Continuing to grow in the cortical layers, the bacterium reaches the other shoots and the stem, where it gives rise to new cancers. Fruits show susceptibility in the ripening stages from fruit set to veraison. The bacterium also affects other plant tissues such as leaves and shoots. *Erwinia amylovora* overwinters on the margins of the branch cankers formed during the previous season.

Shoots may be infected by insect vectors and by rainfall that dilutes and distributes exudates on new shoots, within which the bacteria penetrate through wounds and lesions. Bacteria may also spread directly from cankers where bacterial cells move internally into the plant via the xylem and reach neighbouring shoots.

More than 70 genera of insects have been described as vectors of *Erwinia amylovora*, the most important of which are bees and wasps. Their activity leads them to visit flower organs and they are attracted by sugary substances such as bacterial exudates. In this way, the disease spreads rapidly within the orchard. The bacterium can remain viable for at least 48 hours on the insect's body or in the intestinal tract. However, if the bacterium is incorporated into nectar, pollen or honey, it can remain active for weeks, which explains why beehives may be dangerous sources of inoculum. However, the infectious potential is drastically reduced with time and contaminated swarms will probably no longer be able to infect new plants in the following growing season. Through the analysis of pollen contained in combs or honey of infected colonies, the species visited by bees can be monitored. By carrying out continuous monitoring (e.g. weekly or bi-weekly) it is possible to know through the bees if the bacterium is present and with the analysis of the pollen in which species (traps may be used that capture the pollen from the bees when they arrive at the hive; they are installed at the entrance for a few hours; the colony of bees in which the mechanical pollen collection system is placed responds to this reduction in the availability of proteins by increasing the number of foragers that collect the pollen, so it cannot be used for long). [31] Probably monitoring allows to know if the bacterium is present before the appearance of obvious symptoms in the orchards. [17, 34] Bee colonies may be used to monitor in what direction and how quickly phytopathogens move from one infected area to another. Conversely, insects such as bees may be vectors, moving from contaminated areas into pristine areas.

To monitor the presence of this parasite, groups of 2-3 beehives may be placed near crops that do not show signs of the disease. For the determination of the microorganism there are rapid analytical systems (immuno-enzymatic ones). [34] Phytopathogenic microorganisms such as *Erwinia amylora* or the *fire blight of pome fruit* can be looked for in pollen and honey. In this case it may be useful also to carry out pollen analyses to identify the species that have been visited by bees, i.e. those most likely hosting the phytopathogen.

Insects may promote the transmission of plant pathogenic fungi, which may be harmful to humans and livestock too. Ergotism is a disease that may occur in humans with severe symptoms such as insomnia, intestinal pain, joint pain, and seizures. This disease is caused by intoxication with *ergot*, a fungus (*Claviceps purpurea*) that infects the seeds of wild herbs and grasses, particularly rye (*Secale cereale*). Between 591 and 1789, at least one hundred and thirty-two epidemics of ergotism were recorded in Europe.[704] The fungus disperses its spores by wind and infects the flowers. The infected flower is filled with honeydew taken from the sap of the host and added with *ergot* spores. Insects in search of nectar help transmit the fungus to other flowers. The *ergot* contains toxic molecules that act on the nervous system such as lysergic acid from which the famous drug called LSD is obtained. Thus, this fungus may be spread from the soil and wild plants to cultivated plants by wind and insects.

In conclusion, bee nomadism may promote the spread of diseases to insects but also to plants and other animals, and for these reasons too it should be restricted.

REFLECTIONS ON BIOMONITORING

EXPOSURE TO PESTICIDES: THE DEADLY FACE OF PROGRESS

Pesticides are used in large quantities and are therefore found in insects, human bodies, flowers, soil, water, and food. The collection of monitoring results examined in the previous chapters confirms a high contamination of farmed bee colonies. This study focuses on beekeeping products, but contamination affects the entire biosphere, including humans.
Pesticides not only kill insects indiscriminately, including wild insects considered useful, but also kill birds, poison water, kill fish, amphibians and cause the death of organisms useful to the delicate and indispensable ecosystem of agricultural soil such as worms or the microbiome such as the intestinal one. [416] Negative effects can also occur indirectly and in a less obvious way; for example, organochlorine insecticides (e.g. DDT) promote the decline of birds due to their negative effects on fertility. [418]
The effects recorded in conjunction with the use of some molecules (e.g. in maize, cotton, rape, sunflower) have led to non-resolving decisions such as that of banning, on some crops and in some countries, specific substances (e.g. three neonicotinoids and fipronil). In Italy, thanks to some suspensions, monitoring networks have observed a reduction in bee mortality from 19% to 10%, between 2010 and 2013. [196] These actions are weak positive signals, not decisive, which give hope for an increase in sensitivity. The incriminated molecules, such as neonicotinoid insecticides, are still among the most widely used molecules in the World. A survey conducted on 198 honey samples from around the World (published in 2017) found that 75% were contaminated with at least one of five neonicotinoid molecules, 45% recorded 2 of them, and about 10% four to five. [196] The concentrations detected, very often are low, to the point that it is not easy to determine them instrumentally, but the toxicity of these molecules is very high: one gram of neonicotinoids has the same toxicity for bees equivalent to that of more than 7 kilos of the insecticide DDT. Another sign that gives hope for better times is the attribution, by the Italian judiciary, of the crime of environmental disaster for the use of neonicotinoids (2018).

As we have seen, bees (*Apis mellifera*) are used to study the effects of pesticides on insects, but they are not the best alarm bell or reference point for a number of reasons:

- They are bred in artificial nests and no longer have to build their own honeycombs, as the beekeeper saves the bees a lot of work by recycling wax. So they do not have to build their own hives anymore.
- They are feed fed and therefore are more resistant to environmental factors such as nutrient deficiency. Ultimately, they no longer need the flowers.
- When things go wrong (e.g. cold weather, lack of flowers, pesticide treatments) the beekeeper moves the bees even thousands of kilometres. This recent symbiosis has provided bees with the service of transport on wheels.
- If the queen bee has some problems or dies, it is replaced by buying it from centres which can do the artificial fecundation (there is a trade of seminal liquid). [670] Therefore, the queen bees may travel by plane between Continents, very quickly.
- The beekeeper uses chemicals to treat bee diseases.
- The beekeeper carries out various practices such as the inspection of the hives, which is usually preceded using smoke, with the aim of making them tame (the

smoke can set off a fire alarm: the bees could prepare for an emergency escape). Another curious practice is to open the colony and sprinkle the bees with powdered sugar. Beekeepers, in order to favour the detachment of the *Varroa* mite, have learned to sprinkle the bees' bodies with powders such as icing sugar, which induces a more intense reciprocal cleaning behaviour.

For these reasons, monitoring the health status of bees does not provide reliable information on other animals such as wild pollinators. Monitoring with bees, the effects of oilseed rape seeds coated with neonicotinoids and pyrethroids (clothianidin and beta-cyfluthrin), is not sufficient to assess the damage to wild pollinators. [596] This research shows that insecticides significantly and worryingly harm bumblebees, solitary bees and other insects. Using domestic bees to get information about risks, underestimates some effects such as reduced biodiversity of wild pollinators. In general, wild insects may be more sensitive to the negative effects of pesticides (e.g. insects that lay their eggs on leaves or those that do not benefit from colony protection such as the queen bee, that are called solitary bees). [596]

Some pollutants are known to alter fertility and reproduction in animals such as insects, humans or fish, but the effects on plant gametes are less well known. The measurement of pollen viability following exposure to pollutants is an aspect that could be further investigated: for example, *Pinus nigra* pollen is altered and its viability is reduced as the concentration of nitrogen dioxide in the atmosphere (NO_2 produced by combustion) increases. [870]

The results presented by several studies highlight several critical issues. The methodologies used to establish the toxicity of pesticides to pollinators and non-target species before marketing are not sufficient to establish the real danger. Our knowledge shows that there are sub-lethal effects that occur at lower doses than those used in acute toxicity tests and are equally lethal. Instead of occurring in a single individual in less than 72 hours, they occur after a few days or weeks and affect the whole colony (in the case of bees). Very low doses of some pesticides, their metabolites or other molecules present in commercial formulations affect behaviour, reproduction, memory, orientation ability, communication through dance, ability to search for food, and return to the hive. [1227] There is also another major problem, that of synergistic and additive effects. Doses that fail in the laboratory to register negative effects under real conditions are just as lethal but in a slightly longer time. This scientific knowledge should be used to review the procedure adopted to establish the dangerousness of molecules (doses capable of killing more than 50% of exposed insects in a few hours by contact or ingestion are used). [1227] Furthermore, the results obtained on honey bees cannot be transferred to other insects. The current risk assessment cannot provide guarantees for both bees and non-target species such as other pollinators or birds. However, there is a considerable body of scientific work that should lead us to preventive measures and increased precaution.

SOME PERSONAL CONSIDERATIONS

It should be pointed out that in some of the publications examined, the Authors, after presenting a very alarming picture (such as the finding of several pesticides in the same sample of pollen or wax, and at dangerous concentrations), tend to minimize the problem in the conclusions, in a superficial and unconvincing way, or to shift the attention to unproven hypotheses. [188] It is important to remember that it is possible to find, within bee colonies, 121 different pesticide molecules at concentrations exceeding 200 ppm, with the simultaneous presence of up to 39 molecules in wax and others in pollen and bees. [597]

The research results presented on biomonitoring pesticides, metals and PAHs with beekeeping products have several limitations, for example:

- They use different survey methodologies so it is not easy to make comparisons (e.g. methods of analysis, sampling methods).
- The methodology of presentation of the analytical results is often different or incomplete (e.g. the concentrations do not consider the water content of the different matrices and, therefore, in some cases it is not known if they are referred to the dry weight or to the whole weight).
- Different molecules are sought, and in different matrices.
- Sites around hives, within bee range, are rarely described in detail.
- In the studies examined, the botanical origin of the honey is often not indicated. The absence of this information is another major limitation.
- The concentrations found in beekeeping products are rarely compared with those found in other matrices such as plants (e.g. fruit and vegetables; flowers; leaves), soil, air and water (information from air monitoring stations used by health authorities could be used and hives could be placed near these stations).
- There is no information on the phytosanitary treatments carried out in the field (e.g. field notebooks). There is no information on how toxic are the molecules used in the field, on the mixtures, on the method of distribution, on the quantities used, and the timing of treatments. Neither do we have information on the formulations, i.e. the molecules that are distributed together with the active molecules of pesticides and that often make up most of the weight of the commercial product.
- Little information is available on metabolites originating from pesticides, which are often the most important molecules from a toxicological point of view.
- No information is available on the use of pesticides by beekeepers (e.g. acaricides-insecticides, fungicides, fumigation).
- There is no information on the quality of soil, water and air which also affects the quantity of flowers, pollen and nectar.
- There is no information on emission inventories (e.g. metals) and waste generated and/or released on sites, such as those classified as contaminated or industrial.
- Other bioindicators such as fish, wild plants, wild insects, lichens, and birds are not evaluated.
- In samples from live bees it is more difficult to measure the pesticides sought for several reasons: only a small fraction of the molecules is absorbed, bees that have been contaminated die and do not return to the hive.

Therefore, the information presented in the various publications consulted has many limitations, but in spite of that it shows a very dangerous situation for the health of bees and not only.

Estimating the bioconcentration factors of pesticides that are particularly dangerous to bees could be very interesting, as little information is currently available. By analysing concentrations in soil, water, air, flowering plants (e.g. pollen and flowers), living bees, dead bees, larvae, honey, propolis, and wax, it is possible to make useful comparisons and assessments for beekeepers. The same could be done for the most dangerous metals such as cadmium, mercury, arsenic, chromium, lead, and some polycyclic aromatic hydrocarbons. A review of the scientific literature suggests that bees and beekeeping products are not the best matrix for obtaining information on environmental contamination by pesticides, metals or polycyclic aromatic hydrocarbons. In the future, different comparisons could be made, such as those suggested below:

- For metals and radionuclides, it might be interesting to compare concentrations in soil, flowers (which bioconcentrate), water, and air with those in propolis, pollen, and wax (preferably from hives producing honeydew honey).

- For pesticides it might be interesting to compare concentrations in plants (e.g. in flowers, leaves and other plant tissues such as fruits and seeds we eat), pollen, soil, and water with those in wax, propolis and honey.
- For polycyclic aromatic hydrocarbons (PAHs) it might be interesting to compare concentrations in soil, air, leaves (and other plant tissues), and pollen with those in wax and propolis (preferably from hives producing honeydew honey). Honey is derived from nectar which is a sugary substance so it has little affinity with PAHs and also for this reason it does not bioaccumulate them. [60]
- For plochlorobiphenyls (PCBs) it might be interesting to compare concentrations in soil and plants with those in wax and propolis.

Another aspect to be commented on is that there are considerable differences in the definition of the limits of the permitted concentrations of hazardous substances in products such as honey (even more than 100 times) between different countries. In some cases, this factor makes it more difficult to compare the results of studies conducted in different countries. Some substances are allowed in some countries and, on the contrary, are not tolerated in others and, in many cases, there are no regulations (in the latter case, the interpretation of the precautionary principle should suggest to prescribe the absence of potentially dangerous substances). The resulting risk analyses are therefore not always easy to compare as they start from completely different health assumptions. [178]
Another limitation is the heterogeneity of the sampling, extraction and analysis methods used. The lack of the standardization of procedures and analytical methods implies a variability that may not allow easy comparisons. [179] Information is sought from a few grams of sample (e.g. 2-5 g of pollen) that will be treated with solvents to extract the molecules sought in millionths of a litre solutions. This very small quantity is inserted into the instrument that will have to detect the quantities of hundreds of different molecules. As you can understand, the representation of the seriousness of the pollution, through the only analytical determination, is insufficient and incomplete.

ENVIRONMENTAL EDUCATION IN THE FIELD

It might be interesting to plan some activities related to the biomonitoring activity, with the intention of involving the population and carrying out environmental education. Below are proposed activities that could be complementary and supportive to biomonitoring.

1) Citizen Science or science open to all

The aim is for the public to carry out scientific activities under the direction of professionals and in collaboration with scientific institutions. Involving the public may provide valuable support for environmental protection, including cultural improvement. Many types of projects may have significant benefits from public involvement, generating scientifically interesting data and information. [1187] Among the most widespread applications, there is the implementation of censuses of species of insects, birds or plants. In a cheap and widespread way on the territory, as volunteers are involved, it is possible to monitor the presence of species (e.g. invasive ones), collect data on the health status of plants, have information on migrations or save resources for the search of fossils. [1187] These are opportunities for positive collaboration and confrontation between professionals and the population that is the main custodian and beneficiary of local environmental resources. Positive synergies may arise from this interaction that may influence decision makers in the direction of a greater attention towards the environment.
Examples of projects already carried out are given. The internet pages could include information and photographs of the most important species to be monitored (e.g. flowers,

insects and birds), and the forms to be filled in to report their presence. With the help of volunteers it could be possible to carry out a census, also a photographic one, of the pollinators and flowers present in a given area (bees, wasps, bumblebees and butterflies) using online resources (atlases, registration forms, etc.).[2] Monitoring of flowers in trees or meadows (for a few minutes, during the hours and conditions when it is easier to observe bees in flight) could be promoted at synchronized times.
To monitor the presence of bees and other pollinating insects, feeders may be installed, containing a solution consisting of one part honey, three parts sugar and three parts water. [764] In this way it is possible to attract honeybees and other insects. Using two distant feeders it is possible, through triangulation, i.e. measuring the prevailing flight angles, to determine in which direction the nest is located. Furthermore, if it were possible to mark individual bees by calculating flight times, it would be possible to have an indication of the distance. By photographing the insects present in the baited feeder, positioned at different points, it is possible to have a measure of the density of pollinating insects such as solitary bees and other wild insects (e.g. butterflies). Photographs could be taken at predetermined times and in predetermined ways (e.g. during the hottest hours in spring, in the absence of wind and rain). This census could easily be done by students and volunteers, and photographs with geographic locations, area descriptions, and other information could be collected and archived by the researchers.

2) Decline of bees and loss of floral biodiversity

About 70 of the more than 100 most important plant species we eat are pollinated by bees (e.g. apples, almonds, pears, strawberries, carrots, broccoli, olives, onions, peanuts, avocados). [2, 17, 45]
For several years, all over the Planet, the death of beehives has been recorded, caused by different factors such as the use of pesticides in agriculture and the reduction of biodiversity, with a consequent food monotony and lack of food. Optimistically, it is possible to highlight that without bees the price of food is bound to increase and the possibility of choice is reduced. An effective project for science dissemination and environmental education could draw attention towards the importance of bees for pollination. The messages that could be communicated may be summarized in the following phrases: "*Save the bees to save the World*" or "*We need the bees, the opposite is not true*" or "*What is not useful to the swarm is not useful to the bee* (French philosopher Montesquieu". Together with the communication of the importance of floral biodiversity, activities could be planned with the objective of increasing the area occupied by wild species useful for the survival of pollinators. [232, 233, 862] The installation of green areas, corridors of spontaneous vegetation and the planting of attractive plants could be planned. Environmental education could be accompanied by the restoration of urban and agricultural ecosystems. Ecological education may be conveyed by planting plants and making abandoned and degraded areas greener. Getting active in the places where one lives makes the process of eco-education much more effective.
Bees may be used to monitor the quality and quantity of pollen and, therefore, they may be an indicator of the health of the floral biodiversity of the territory. A complementary activity could be to measure floral diversity through pollens collected by bees or in those contained in honey, with the help of experts.

3) Urban beekeeping

The installation of beehives for educational use, using hives built for this purpose, is a strategy that has already been used for some time. As a confirmation of how interesting urban greenery can be for beekeepers, it has been estimated that the city of Turin has a melliferous potential of 440,000 kg. [35]

Bee society, like human society, is made up of individuals who need a complex organization in order to survive. The interest of single individuals is sacrificed for the collective wealth. Bees have inspired poetry, literature, art and music, and in this regard the metaphors "*to be busy as a bee*" and "*bees in industrial society like canaries to miners*" are recalled. Information on several crucial aspects of industrial and sustainable agriculture (pesticides, biodiversity, fertilizers, soil fertility reduction, climate change) could be promoted.

In conclusion, among the organizations that have used bees to promote a better environmental culture and carry out biomonitoring, it is worth mentioning:

- Universities and Research Centres;
- Public administrations (e.g. local governments);
- Health Service;
- Environmental organizations;
- Foundations operating in the environmental sector;
- Trade associations (e.g. agricultural associations);
- Private individuals (e.g. in waste management, food production and renewable energy).

There are many examples of biomonitoring applications carried out by the subjects just listed, also in Italy.

PESTICIDES: THE IGNORED KNOWLEDGE ON LONG KNOWN DANGERS

IF EVERYTHING HAS A PRICE, THE ENVIRONMENT BECOMES A LUXURY

It is estimated that in the World the biological enemies of plants cause a loss of 45% of food. On a planetary level, insects, other plant pests and weeds alone generate food losses of about 30%. Other 10% or 20% may be added to these losses due to living beings that cause damage after harvesting, such as rodents, microorganisms (e.g. moulds) and insects. [580] Another negative note: in Italy 30% of the food ends up in the waste when it is still edible. To counter and try to reduce these losses, pesticides have been used for over 60 years, but they are the cause of serious problems: [537]

- Damage to human health (in the USA, in 2005, these costs were estimated at over $1.1 billion per year).
- Damage caused by the emergence of pesticide-resistant plants and pests (in the USA these costs were estimated at over $1.5 billion per year).
- Crop losses due to pesticides (in the USA, this cost was estimated at about $1.4 billion per year).
- Damage to natural biodiversity and in particular to birds (in the USA pesticide damage to birds generates costs of $2.2 billion per year).
- Water contamination (in the USA this generates costs of over $2 billion per year).
- Destruction of natural plant enemies.
- Reduction in pollinators including bees.
- Costs to limit the massive and inappropriate use of pesticides.
- Investments to spread the culture based on chemical agriculture.

The estimates are given as an example and certainly do not provide the right dimension of the problem: it is very difficult to estimate the cost generated by the extinction of some species from more or less vast territories, or from the entire Planet. It is an anthropocentric vision to give an economic value to everything, including human health. The simplification is based on a principle widespread in capitalism, that of being able to put a price on anything and, consequently, to be able to buy everything. In this way, human health and environmental protection automatically become a commodity and, at the same time, a luxury.

INTRODUCTION TO PESTICIDE DAMAGE

The exposure to pesticides may occur through ingestion (e.g. fruit), contact and inhalation (there are also other indirect exposure routes such as foetal exposure). Some alterations generated by pesticides in living beings are as follows (often they are side effects as they are different from the primary action, i.e. the one that justifies agricultural use): [724]

- Genetic modifications. Blood cancers such as leukaemias (e.g. from the exposure to organochlorine agents such as aldrin, chlordane, DDT, heptachlor, lindane and from the exposure to mancozeb and toxaphene); non-Hodgkin's lymphomas [from the exposure to lindane, cynazine, 2,4-D (2,4-dichlorophenoxyacetic acid)]; multiple myeloma from the exposure to various molecules such as glyphosate. Melanoma is not only related to the exposure to sunlight, but also to the exposure to pesticides, in particular carbamates

and toxaphene. Prostate cancer has been associated with the exposure to certain pesticides such as methylbromide and organochlorines.
- Mitochondrial and endoplasmic reticulum dysfunction.
- Alterations in ion channels that generate disturbances in nerve conduction.
- Neurological and cognitive impairments such as attention deficit hyperactivity disorder, or reduced intelligence in children (e.g. due to organophosphate insecticides).
- Altered activity of enzymes (e.g. acetylcholinesterase, cytochrome P450).
- Oxidative stress.
- Modification of the absorption capacity of nutrients (e.g. through the modification of cell permeability).
- Damage to the immune system.
- Reproductive damage, such as reduced male fertility.
- Endocrine system damage (such as thyroid damage).
- Alterations in infant health due to in utero exposure (otitis, asthma, respiratory distress, decreased fetal growth, reduced duration of gestation, certain types of malformations). For example, organophosphate compounds have been associated with reduced head circumference at birth (an indirect measure of possible delay in brain development), growth retardation, impaired cognitive abilities and autism. [1271]

These are just some of the alterations produced by pesticides, but they are sufficient to highlight the multiplicity of pathologies they may generate.

It is now very clear that pesticides alter the balance in organisms such as human beings, even at very low doses, measurable in millionths of a gram: malfunctions of the endocrine system, the nervous system, the reproductive system, the immune system, and also renal, respiratory and cardiovascular dysfunctions. Consequently, there is a correlation between exposure to this category of molecules and many diseases such as cancer, Parkinson's, Alzheimer's, autism, attention deficit hyperactivity disorder, diabetes, amyotrophic lateral sclerosis (ALS), reproductive problems (e.g. foetal malformations, infertility) and thyroid dysfunction. [491, 500] If the exposure occurs early in life, the risks are even more significant, such as reduced IQ, behavioural, sensory, and motor dysfunction. [516]

DETERMINATION OF THE ACCEPTABLE LEVEL OF RISK

One of the crucial points in a risk assessment is the definition of the acceptable risk threshold. The estimation has to consider different toxicological, epidemiological, but also political, economic, and social evaluations. In some contexts, such as carcinology, a risk may be considered acceptable, depending on the case, if the expected increase in mortality for the general population is between one case in ten thousand and one case in a million per year. For workers this level of risk is, in some cases, increased by 10 to 100 times.

Exposure routes may be:

- Inhalation and contact with airborne material (this exposure mainly affects workers).
- Direct ingestion (through water and food).
- Indirect ingestion through the food chain (bioaccumulation, e.g. in meat and meat products).

For the purpose of determining the acceptable level of a risk, it may be useful to establish a tolerable pollution or contamination value. A threshold reference value is the highest concentration at which no adverse effects are measured (NOAEL, no-observable adverse effect level). This dose is derived from experimental models. Some NOAELs calculated for exposure by ingestion in the rat, in parts per million, are: mercury 15 ppm; cadmium 25 ppm; lead 300 ppm; nickel 500 ppm; chromium 3,000 ppm. In rare cases the NOAEL may be derived directly

in humans, for example, as a result of occupational exposures. The concentration may refer to the exposure by ingestion, inhalation or contact.
A possible interpretation, in the absence of other specific information, could be the following: material containing pollutant concentrations below the NOAEL presumably may be used without restrictions. This conclusion has the limitations of an approach that does not consider all possible assessments and interactions. It does not consider special risk situations, such as that for workers or vulnerable people. Summative effects or interactions between different exposures are not assessed, and it is difficult to predict what happens in the long term.
The NOAEL, in most cases, has the limitation of being estimated in animals, for short times, for a single route of exposure and does not consider the simultaneous presence of other factors. For these reasons too, conclusions drawn from animal models in the laboratory are transferred to humans by applying safety factors (100-1,000); for example, to calculate concentrations that can be taken over a lifetime without measuring adverse effects on human health.
It should be borne in mind that any risk assessment will always be characterised by different levels of uncertainty, especially when predicting effects at low doses (e.g. carcinogenic, endocrine, reproductive or nervous system effects), from multiple substances (e.g. plant protection products) and over a lifetime.

Another parameter used to identify safe concentrations is the TLV (Threshold Limit Value). This acronym indicates the limit value set (for example by the ACGIH, *American Conference of Governmental Industrial Hygienists*) for exposure to airborne substances or radiation, and provides an estimate of the concentration to which workers may be exposed on a daily basis without causing negative health effects. In particular it defines:

- TLV-TWA (Time Weighted Average): time-weighted average concentration, over a conventional 8-hour working day and 40-hour working week, to which most workers may be repeatedly exposed, day after day, without adverse health effects.
- TLV-STEL (Short Term Exposure Limit): a 15-minute weighted average exposure that should never be exceeded during the workday, even if the 8-hour weighted average is below the TLV-TWA. Exposures at the TLV-STEL must not occur more than 4 times per day, with at least 60 minutes between successive exposures. This is considered a concentration to which workers may be exposed continuously, for short periods of time, without experiencing irritating, chronic or irreversible effects. This exposure value (concentration) is usually higher than the TLV-TWA.
- TLV-ceiling (TLV-C, ceiling limit): is the concentration that must not be exceeded during work activity, even for a very short period of time.

Some individuals may be more sensitive to exposure to certain substances and, therefore, may not be sufficiently protected when concentrations are below TLVs, for several reasons: genetic predisposition, age, previous exposure, intake of medicines, smoking, alcohol, drugs and underestimation of risks (insufficient knowledge).

Another acronym used in toxicology is TEQ or toxicity equivalent. This is a toxicological quantity that expresses the concentration of a harmful substance in terms of the amount equivalent to a compound with which it can be compared. The TEQ expresses the amount of a contaminant as the concentration of the reference substance, which is capable of generating the same toxic effects. The TEQ is related to the actual concentration of a given substance through the toxic equivalency factor (TEF), which when multiplied by the actual concentration gives the TEQ. Simply put, one gram of substance A, which is twice as toxic as substance B, has the same toxic equivalent as two grams of substance B. The toxic equivalent is commonly used to quantify dioxins and related or assimilated compounds, such as furans. Legal limits for the emissions of these substances also use the unit of measurement commonly expressed in ng/Nm^3 (nanograms, i.e. billionths of a gram, per cubic metre under normal conditions, i.e. fixed temperature and pressure) of tetrachloro-dibenzo-p-dioxin equivalent toxicity. The World Health Organisation has identified at least 17 harmful polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans and 12 polychlorinated biphenyls, assigning them a toxic equivalency factor relative to tetrachloro-dibenzo-p-dioxin (TCDD), the most dangerous of the dioxins (at least 210 dioxins have been identified).

Particular attention must be paid to professional users of pesticides and to workers in production factories. In Lombardy, some herbicides are used in rice and maize cultivation (molinate, propanil and terbuthylazine), which occupy hundreds of thousands of hectares. Farmers are exposed to pesticides during different phases such as the preparation of the mixture in the distribution machine (e.g. the sprayer), the distribution, the activities carried out in the areas where the substances have been distributed and the cleaning of the equipment and devices used. Operators may absorb molecules contained in pesticides through skin and respiration, which may be found in urine (e.g. propanil and its derivative, 3,4-dichloroaniline or 3,4-DCA; terbuthylazine and its derivative, des-ethyl-terbuthylazine or DET). Dangerous molecules may enter the body through the skin (e.g. hands) and as a demonstration of the danger of this route of exposure, some pesticides are found in the urine of agricultural workers who, in Lombardy, have distributed them on rice or corn crops. [1253] Even 3 mg of active ingredient per each kilogram used may be deposited on the body of workers (the body and clothing could be contaminated by a few tens of millilitres of solution containing the mixture). The occupational contamination of farmers may exceed the level of exposure considered tolerable (Acceptable Operator Exposure Level or AOEL). These molecules may also be found in the urine of the general population (3,4-dichloroaniline which is a metabolite of propanil up to a concentration of 6.2 µg/L).

Imidacloprid and a metabolite of imidacloprid (6-chloronicotinic acid) may be found in all the urine samples of the agricultural operators examined (46 Chinese farmers). [1257] It is estimated that the daily dose absorbed by imidacloprid users ranges from 0.52 to 248.05 µg/kg, while the dose that guidelines suggest not to exceed in an adult is 60 µg/kg per day: it follows that users of this molecule in agriculture may reach levels of contamination certainly dangerous for health. [1257] This neonicotinoid insecticide is present in urine even before the application in the fields, but after this operation the concentration in urine increases 3-4 fold. In China neonicotinoids are molecules recorded with a high frequency in fruit and vegetables so also the dietary exposure for the general population is significant.

PESTICIDES ARE TOXIC SUBSTANCES

Pesticides are toxic, persistent, mobile, bioaccumulative substances and have a negative impact on soil properties and on the entire ecosystem. In Italy an average between 5 kg and 6 kg of pesticides are distributed per hectare each year. As a result, pesticide residues are found in at least half of the fruit and vegetables that reach our plates and drinking water every day. At least 131 active molecules and their metabolites have been identified in drinking water in Italy, and 36.6% of samples have been contaminated above the maximum safe levels that have been established. [724]

Unfortunately, it is very difficult to prove a cause-and-effect relationship between disease and occupation, between lifestyle and disease, or between environmental contamination and health problems. In some cases, effects have been reported even at infinitesimal doses: for atrazine, effects have been described at doses 30,000 times lower than legal limits. [724]

A limitation of a risk analysis is that effects are assessed for each individual active substance, even though in reality we are exposed to mixtures of molecules. In some cases, the effects become apparent after many years or even in the next generation, while toxicological studies are carried out for short periods and at doses usually higher than those to which we are exposed.

For example, the organochlorinated insecticide DDT, which has been banned for years, is still present in environmental matrices and is correlated with an increased risk of breast cancer if exposure occurs in the pre-pubescent age.
Particularly serious is the increased risk of adult neurodegenerative diseases, such as Parkinson's, following the consumption of water contaminated by pesticides such as chlorpyrifos and damage to the thyroid gland due to exposure to mancozeb. In Italy, lymphomas in the age group between 0 and 14 years show an annual increase higher than the average annual increase in Europe. The sad record that Italy holds for the incidence of cancer in childhood, especially for lymphomas, should make us reflect with great attention. Especially in crucial phases of development such as those of intrauterine life or childhood, some substances act at infinitesimal doses. Unfortunately, pesticides are now permanently present in our environment, particularly in water and in our bodies: in breast milk, urine, the umbilical cord and blood. So we are exposed all along our lives and even before we are born. It is useful to remember (ISPRA reports on water) that the more than 150,000 tons of pesticides sprayed every year in Italy have contaminated not only surface water, but also groundwater and drinking water. Exposure to pesticides promotes chronic diseases and various types of problems such as lower birth weight of newborns, the presence of birth defects and a reduced sperm count.
Organophosphates may act as endocrine disruptors, organochlorines have been linked to cancer, neurological damage and endocrine problems. Birth defects, learning disabilities and autism are also affected by harmful chemical mixtures that we are exposed to even before we are born.
Determining the association between single-molecule contamination and disease is also difficult because of the concomitant exposure to other categories of substances that may have different effects, such as neurotoxicity from polychlorinated biphenyls in children or from manganese in adults (that may generate parkinsonism). [327]
There are numerous studies showing an association between chemical agriculture, pesticides, disease, and disasters to the biosphere. For example, pyrethroids can be detoxified by enzymatic systems which, if inhibited, increase toxicity. [193] Pyrethroids (such as permethrin) generate several adverse effects in mammals: [527]

- neurotoxic,
- genotoxic and cytotoxic,
- immunotoxic,
- reproductive,
- hepatic,
- digestive,
- cardiac.

Pyrethroids are found in wild mammals such as dolphins (in liver, placenta, and milk) confirming that they are mobile, persistent and bioaccumulable.

Some effects of pesticides recorded in the scientific literature are summarized below: [160, 261, 280, 282,283, 286, 287, 288, 289, 290, 293, 297, 298, 299, 300, 301, 302, 321, 336, 477, 491, 495, 502, 505, 509, 515, 516, 528, 724, 725, 1254, 1256]

- Exposure to pesticides such as some herbicides, fungicides, and insecticides generates DNA damage, and dangerous interactions between these substances and at least 300 different genes have been identified (some of these substances are carcinogenic). For some genes the alteration generated by the presence of certain pesticides has been demonstrated, confirming a mutagenic action that constitutes one of the steps for the tumoral transformation of cells. Children, due to their different constitution, have a risk of registering tumours even 10 times higher than adults.

- Glyphosate (an herbicide) according to the World Health Organization is a probable human carcinogen.
- Propoxur and permethrin generate chromosome translocation, which involves moving pieces of DNA containing up to hundreds of genes to other chromosomes, causing serious and often fatal alterations.
- The use of pesticides in the home immediately before conception, during pregnancy, and immediately after increases the risk of registering leukaemia in the children (e.g. those used in gardens or insecticides for flies, mosquitoes, cockroaches or ants in the home; pesticides used on pets with soaps).
- Women who work with pesticides are more likely to experience ovarian cancer and skin cancer.
- The occupational use of pesticides promotes certain diseases such as non-Hodgkin's lymphomas, multiple myelomas, breast cancer, ovarian cancer, eczema, and neurological diseases.
- Some pesticides (phenoxy herbicides, carbamate insecticides, organophosphorus insecticides, and organochlorinated insecticides such as lindane) have been associated with non-Hodgkin's type lymphoma, while herbicides (glyphosate and phenoxy herbicides) have been related to B-cell lymphomas.
- Studies in the USA and Spain have determined the association between intensive agriculture and childhood cancers.
- Exposure of parents, especially mothers during pregnancy, increases the likelihood of recording brain tumours in their children. The occupational exposure of fathers also increases the risk in their children, and this increase is important since very often occupational exposure is more frequent in men.
- Parents' exposure to pesticides before conception has negative consequences for the next generation and sometimes for grandchildren, such as reproductive and germ cell changes, brain tumours and other cancers. Occupational exposure of mothers to certain pesticides during pregnancy or of fathers before conception increases the risk of registering leukaemias in their children (cancers that can be registered before the age of five).
- Exposure of mothers to some pesticides has been associated with birth defects (carbaryl, chlorpyrifos, diazinon, atrazine, methyl bromide, DDT, HCH, DDE, aldicarb, dimethoate, and phorate). The organophosphate insecticide diazinon generates neurotoxicity, oxidative stress, cardiotoxicity, and vascular toxicity. Metabolites of diazinon can be found in the urine of American children. The toxic effects of diazinon have the characteristic of occurring selectively, more easily and with greater intensity in male mice than in females. In this case the adverse health effect is sex-related.
- The presence of the herbicide atrazine in drinking water (in the USA it is found in 94% of samples) has been associated with birth defects, decreased fertility, and cancer.
- Women exposed during pregnancy to endosulfan are more likely to have autistic children.
- Exposure to certain pesticides such as organochlorine insecticides has been associated with earlier puberty in females (e.g. breast appearance) and in males with the failure of one or both testes to descend into the scrotal sac (this is termed cryptorchidism).
- Girls exposed to DDT before puberty are five times more likely to develop breast cancer.

- Children exposed during pregnancy to chlorpyrifos experience changes in brain architecture.
- Cognitive and neurodevelopmental damage has also been documented: a study conducted in California, in the period 1998-2010 (on 2,961 cases of autism and 29,610 healthy individuals), associated the exposure of mothers, determined by living within 2 km from agricultural areas, with an increased risk ranging from +10% to +16% for glyphosate, chlorpyrifos, diazon, malathion, avermectin, and permethrin; in the 445 cases of autism associated with intellectual disability the risks were significantly higher (+33% glyphosate, +27% chlorpyrifos, +41% diazon, +46% permethrin).
- A California study of 238 mother-child pairs associated the use of organophosphate insecticides within 1 km of residence during pregnancy with a reduction in children's IQ (intellectual quotient).
- Exposure during pregnancy to organophosphate insecticides increases the likelihood of recording fetal death and exposure to herbicides (chlorophenoxy) increases cases of miscarriage. Maternal exposure to pesticides is associated with reduced birth weight and earlier delivery, as is the case for organophosphate insecticides (chlorpyrifos and diazinon). Some birth defects have been associated with the maternal exposure to fungicides and herbicides.
- Farmers who use pesticides are more likely to have prostate cancer, brain cancer, and more frequently have Parkinson's disease than the general population: dieldrin, diquat, parquat, rotenone, maneb, ethylene bis-dithiocarbamates, organophosphates, and organochlorinated pesticides had a strong association with Parkinson's disease.
- Organochlorine insecticides, such as DDT and lindane, increase the likelihood of Parkinson's disease by 2.4 times. Living within 500 m from fields treated with the fungicide maneb and/or the herbicide paraquat (association evidenced in California) increases the risk of Parkinson's disease. The risk increases with a simultaneous exposure to both molecules.
- In the USA, an increased likelihood of Parkinson's disease has been demonstrated following the exposure to glyphosate, used within a one kilometre radius from the home; an increase in cognitive impairment has also been recorded in relation to a previous exposure to organophosphate insecticides. In the Netherlands, living within 100 metres from fields means an increase in Parkinson's disease due to the exposure to 21 substances, including paraquat. Paraquat is a non-selective herbicide with a desiccant action, used in the preparation of seedbeds or transplantation, within the inter-row of tree crops, in embankments, drains, and roadsides. Paraquat poisoning may result in acute respiratory failure. Some studies have confirmed that this substance may lead to an increased risk of Parkinson's disease in people living in areas adjacent to the treated land. Given the proven danger, the European Union has banned the marketing and use of this pesticide in the Community territory. The product is still produced in Europe for export to markets where there is no ban on its use.
- Pesticides also alter the immune system and disrupt its responses. Molecules that are capable of damaging the immune system include organochlorines such as DDT, lindane, endosulfan and dieldrin.
- Pesticides favour allergies and autoimmune reactions; in the latter case the immune system loses the ability to distinguish foreign cells from those of its own organism and kills the latter. Among the molecules for which some studies have underlined, since the 90s, a causality between exposure and effects (e.g. occupational skin

manifestations) are atrazine, parathion, maneb, and dichlorvos. In industrialised countries the predisposition to develop an allergy affects between 20% and 30% of the population.

- In the USA it has been found that living within 500 metres of places where 2,4-D, paraquat, and pendimethalin are used results in an increase in urogenital malformations. Exposure to glyphosate, cyhalothrin, S-metolachlor, mepiquat, and pendimethalin resulted in an increase in heart malformations.
- In Andalusia, an increase in spontaneous abortions, a reduction in birth weight, and male urogenital malformations have been observed as a result of the use of endocrine disrupting pesticides.
- Organochlorine insecticides have been associated with an increased likelihood of recording obesity.
- The herbicide atrazine alters the action of lymphocytes in rats and alters the ability to resist an infection in salmons.
- In cetaceans and seals some immune system problems have been associated with the presence of persistent pesticides. This alarm has been raised since the 1990s.
- Males of some frogs exposed to pesticides become females.
- Pesticides are also the cause of at least 0.5% of diseases recorded in animals by veterinarians and 0.04% of all reported deaths: these figures underestimate the problem as, for example, they only concern cases of poisoned animals recorded by veterinary services.

The list could go on and on, but in addition to the effects on health, the enormous impact that the current agricultural model of industrialized countries has on the entire Planet should not be overlooked. The food production system represents a global risk, by threatening ecosystems and the stability of the Earth's system.

With a reasonable certainty it is possible to state that the relationship between pesticides and human health has been extensively investigated and that, especially with regard to neuropsychological damage in children and cancer risks (in particular haematological cancers), a causal link is difficult to question. [724]

In many cases the lack of certainty about causal relationships removes responsibility, i.e. there is no assumption of liability on the part of the incriminated companies. This means that in the event of an occupational disease being registered, it will not be possible to receive compensation for the damage suffered. This situation in fact ensures impunity for the producers of poisons and encourages the underestimation of environmental diseases. It should be noted that in many cases the composition of all the molecules that make up the formulation of a pesticide is not known, as this information may be protected by industrial secrecy and other rules that protect the economic interests of very strong individuals. The principle of law according to which there is a presumption of innocence, until guilt is proven, is applied. Currently all ecosystems are contaminated by pesticides and, consequently, also our tissues, so it becomes difficult to identify individual responsibilities. In Italy the consumption of pesticides per hectare is among the highest in Europe: about 33% of all insecticides used in Europe. According to an authoritative institution (Endocrine Society) we are exposed daily to more than 85,000 substances artificially created by man and for only about 1% of these molecules we have some information about their safety. The massive, widespread, and constant contamination constitutes a major obstacle to the identification of responsibility and generates an effective but at the same time lethal protective shield.

The solution exists and it is agro-ecology, that is to say, a model that must be able to meet environmental, economic, and social sustainability through the least possible use of external resources: pesticides, fertilizers, fossil fuels, and irrigation. We need to encourage agricultural systems based on biodiversity, family farming, short supply chains, and local resources, and to encourage the exchange of knowledge between operators, citizens, and scientists. At stake are not only fundamental values such as the protection of health, biodiversity, water resources, soil fertility, and climate, but also participation, democracy, and social peace.

The European law has protected great private interests in a dangerous way. Companies have been allowed not to disclose the place of production of food, not to indicate the ingredients, not to provide information of ethical, environmental, and worker protection relevance. [845] As a result, ecologically sensitive consumers have been deprived of the opportunity to recognise local products and to select the most attentive and forward-looking entrepreneurs. The food safety obligation has therefore been one big media scam that has led to the opposite direction: the absence of information for consumers and freedom of action for unscrupulous entrepreneurs. [752]

PESTICIDES INTERFERE WITH THE HORMONAL SYSTEM

It is both interesting and worrying to take a closer look at some of the effects of pesticides and other substances that act as endocrine disruptors. [211] Endocrine disruptors are man-made substances that alter important physiological functions of animals and plants, at very low concentrations, by mimicking natural molecules such as hormones. Hormones are substances that act by activating or deactivating fundamental mechanisms and perform these functions at very small doses. The timing of exposure is crucial in determining effects that may manifest themselves many years later and even in the next generation. During growth there are very sensitive windows of vulnerability.

Endocrine disruptors interfere with the mechanisms of action of the more than 50 hormones produced by the glands in our bodies: they can inhibit hormones or they can trigger mechanisms at the wrong time and with a different intensity. In practice, false messages are produced that may interfere with the main functions such as brain or reproductive functions (e.g. the quantity of spermatozoa per millilitre of semen decreases): from the 1940s to the 1990s, some research revealed that the number of spermatozoa was halved. [280] The organochlorine insecticides (aldrin, DDT, HCH, heptachlor, chlordane, endosulfan) have a structure related to steroid hormones and act on the same receptors. [443]

Like natural hormones, endocrine disruptors are characterised by the fact that they do not have activation thresholds and may also cause adverse effects in offspring. They are a heterogeneous group of man-made substances that can interfere through various mechanisms (receptor-mediated, metabolic, etc.) with the functioning of the endocrine system, especially with the homeostasis of sex steroids and the thyroid. [724] Thousand of different molecules are involved, including pesticides, flame retardants, polychlorinated biphenyls (PCBs), phthalates or bisphenol A, which is used in the plastics industry. [468] Bisphenol A is active at minute concentrations as an endocrine disruptor: it affects fertility, favours obesity, increases the risk of certain cancers such as prostate and breast cancer, and promotes nervous diseases. [982] Bisphenol A is added to plastics used for beverages or to wrap cans internally (e.g. tomato cans) and, consequently, it may also be found in 93% of urine samples (of 2,517 Americans). [837]

In summary some of the major harms to human health, concerning endocrine disruptors, are: [464, 501, 502]

- damage to the immune system such as autoimmune disorders;
- some kinds of obesity;
- heart and lung diseases;
- nervous dysfunction, behavioural disorders and cognitive deficits;

- neurodegenerative diseases;
- increased risk of cryptorchidism[I] and hypospadias [II];
- reproductive dysfunction such as decreased male fertility;
- birth defects;
- miscarriages, endometriosis[III], extrauterine pregnancy, pre-term delivery;
- increased risk of cancers such as prostate, lung, and breast cancer (the risk of breast cancer increases at least 4-fold in women who have DDT metabolites in their blood; the risk of prostate cancer increases in agricultural workers exposed to organochlorines);
- hormonal dysfunction (especially thyroid) and early pubertal development;
- diabetes.

An association between the use of certain pesticides (aldrin, chlordane, heptachlor), for at least 100 days in a lifetime, and increased likelihood of having diabetes has been recorded. [500] Other molecules have also been linked to diabetes (organophosphates and organochlorines including: DDE, DDT, HCB, oxychlordane, heptachlor, β-HCH, mirex, aldrin, dieldrin, chlordane, alachlor, pentachlorophenol, parathion, phorate, phonophos, trichlorfon, cyanazine; and herbicides such as 2,4,5-T/2,4,5-TP). [491, 511, 1159]

These are some of the problems that may be generated by hundreds of molecules produced by the chemical industry in large quantities. It is known that many of these agents also have a mutagenic and carcinogenic action. Some monitoring showed a significant increase in breast cancers since the 1990s: from one case per 20 women in 1960 to one case per 8 women in 1995. [502]

Some of the most important features of the mechanisms of action of endocrine disruptors are as follows:

- They work in very low doses.
- They act very precisely and during specific time windows.
- Children and early stages of reproduction may be much more vulnerable.
- The effects can be measured in generations following the one that was exposed.
- The doses assessed as tolerable by the regulations (e.g. maximum permitted concentrations of pesticide active molecules in food) have been determined without weighing up the endocrinological risk and are therefore unable to protect our health from these dangers. The concentrations at which endocrine disruptors generate significant biological effects may be much lower than those established by evaluating, for example, the dose that generates the death of 50% of animals (in a few hours). [668] The concentrations capable of generating endocrine effects are more than a thousand times lower than those capable of producing acute toxic effects. In addition, epidemiological studies on endocrine disruptors are difficult to implement, since for some categories (e.g. molecules that interfere with estrogen) it has become impossible to

[I] Cryptorchidism is the most frequent anomaly of the male urogenital system in childhood and may be associated with other abnormalities of the genitourinary tract. During fetal life the testicle descends from the abdominal cavity to the scrotum, through the inguinal canal, but may stop at any point on the way down. This is accompanied by alterations in the structure of the testis, reduced sperm production and hormone production in adulthood, causing damage to fertility and general health. Risk factors are prematurity and low birth weight, as well as diabetes during pregnancy and tobacco smoking. The determinants of cryptorchidism are hormonal, genetic, and environmental. [726]

[II] Hypospadias is a congenital anomaly of the penis due to an insufficient development of the urethra, whose outlet (meatus) is not located at the apex of the glans, but is located on the ventral face of the penis or, in severe cases, in the scrotum or perineum. [727]

[III] Endometriosis is the presence of endometrium, a mucous membrane that normally covers only the uterine cavity, outside the uterus and may it affect women as early as their first menstruation (menarche) and accompany them until menopause. In Italy, 10-15% of women in their reproductive age are affected by endometriosis; the disease affects about 30-50% of infertile women or those who have difficulty in conceiving. At least 3 million women are diagnosed with endometriosis. [728]

find samples of non-exposed people, because they are ubiquitous pollutants. Current experimental models applied by companies before pesticides are marketed are not able to assess endocrine effects.

- Endocrine effects also occur in animals. For example, male frogs exposed to 25 parts per billion of atrazine (herbicide) show a 10-fold reduction in testosterone concentration. [516] This reduction is probably due to the activation of a protein (the aromatase enzyme) that converts testosterone into estrogen.

Many of the effects recorded in humans are similar to those determined in bees and described in the preceding pages: these are sub-lethal effects, i.e. from a very low dose, and lifetime exposures. Risk assessment for both bees and humans suffers from the same limitations as it is difficult to predict long-term consequences on the whole community in the laboratory. Moreover, additive and synergistic effects cannot be determined in advance in the laboratory and it is impossible to think of having information in advance on the damage generated in hundreds of thousands of wild species. Therefore, as in the case of insects, we are acting as guinea pigs because we do not have sufficient knowledge for most of the pollutants we come into contact with on a daily basis. On the contrary, many alarm bells have been ringing for a long time, both with regard to the effects on pollinators and on humans and the entire biosphere.

More than 1.000 molecules are known to be potential endocrine disruptors due to their chemical characteristics such as diethylstilbestrol, polychlorinated biphenyls (PCBs), dioxins, perfluoroalkyl compounds, solvents, phthalates, polybrominated diphenyl ethers, and some pesticides including lindane, dihedrin, DDT, DDE (organochlorine insecticides), fungicides (vinclozolin, prochloraz and linorun), triazoles (cyproconazole), imidazoles (imazalil), herbicides (simazine and atrazine), organophosphates (chlorpyrifos), dithiocarbamates (mancozeb), pesticide co-formulants such as alkyl phenols. [468, 500, 501, 516, 598] The following pesticides are also definitely endocrine disruptors: alachlore, aldicarb, benomyl, chlordecone, chlorpyrifos-methyl, dicofol, endosulfan, mancozeb, methoxyclore, nitrphene, procymidone, toxaphene, vinclozolin, 2,4-D. [260, 309] At least 105 pesticides have been recognized as endocrine disruptors: 46% are insecticides, 31% fungicides, and 21% herbicides. [464] To get a better representation of this severity, it is useful to point out that for example, in Denmark, it is estimated that at least 50% of the fungicides used are endocrine disruptors (e.g. they inhibit ergosterol biosynthesis). [598]

In some cases, the metabolites are more dangerous than the starting molecules. For example, vinclozolin in soil, plants and animals forms degradation products that have an anti-androgenic activity. [464]

Traces of endocrine disruptors, such as molecules contained in plastics or pesticides, may be found in most of our bodies. Being exposed to additional doses of hormones from food or contaminated water alters delicate balances. The effect of the exposure to a complex mixture over a lifetime should not be underestimated. Synergistic and additive effects are largely unknown. These substances can act on pregnant women, the foetus, and the cells involved in the reproduction of the foetus, and can thus manifest their effects on grandchildren, i.e. for three generations.

Exposure to pesticides such as organochlorine pesticides (DDT) contributes to an increased risk of heart disease, according to the Endocrine Society. [467] The association between exposure to organochlorine pesticides, birth defects, and increased risk of type 2 diabetes has been shown. The presence of estrogenic endocrine disruptors in the bloodstream increases complications generated by obesity such as heart disease and inflammatory processes. [467] Even the most widely used herbicide in the World, glyphosate, has been found to be an endocrine disruptor for humans (it alters aromatase activity) and not only. [552]

Endocrine disruptors can alter fragile balances by promoting obesity and behavioural disorders. For example, in children, autism and related disorders have seen a 50-fold increase in frequency in less than 40 years. [463]
Endocrine disruptors have been linked to decreased IQ (intellectual quotient) which may be associated with disability, autism, attention deficits, and hyperactivity. Other diseases for which an association with endocrine disruptors has been reported are obesity, diabetes, and male infertility. [501] In Europe, it is estimated that the economic damage caused by endocrine disruptors amounts to hundreds of billions of euros per year (at least 1.2% of the GDP or Gross Domestic Product).

PESTICIDES AND THYROID

Occupational exposure (e.g. farmers distributing them) to organochlorine insecticides (aldrin, chlordane, DDT, heptachlor, lindane), herbicides (2,4-D, 2,4,5-TP, alachlor, dicamba, paraquat), organophosphate insecticides (diazinon and malathion), carbamate insecticides (carbofuran) or fungicides (chlorothalonil, maneb/mancozeb and benomyl) increases the risk of hypothyroid disease. [498, 506] Fungicides (maneb/mancozeb) have also been associated with hyperthyroidism. Women who had married a farmer, who used pesticides professionally, were more likely to register hypothyroidism-related diseases than the general population. Exposure to some pesticides such as organochlorine insecticides also damages the thyroid gland of other animals.
Many other categories of molecules can damage the thyroid, such as halogenated compounds including polychlorinated biphenyls, dioxins, and furans. [506] In the USA, thyroid diseases are recorded in 7.5% of the population (they are more common among women) and this is probably an underestimate, as less severe cases are not recorded; in Brazil, thyroid diseases are recorded in 5.4% of the population. [498] According to some estimates, 5-9% of the population registers subclinical, i.e. not very evident thyroid problems, while between 0.8% and 7.5% registers evident problems and therefore requires treatment. [506]

ALTERATIONS IN MALE FERTILITY

Endocrine disruptors are related to the increased frequency of emerging diseases such as those of the male reproductive system. [459, 460] The quality of semen may be altered by pesticides (e.g. organophosphates) in several ways: reduction in density, reduction in the number of spermatozoa, inhibition of spermatogenesis, increase in DNA abnormalities, alteration of testosterone and pituitary hormone levels. Some undesirable effects are an increase in miscarriages, altered male to female ratio, and demasculinization (aldrin, atrazine, chlordane, DDT, dieldrin, endosulfan, and vinclozolin). [500] It has been known for over 70 years that DDT, when administered to roosters, generates testicular atrophy and feminization. [308]
In males living in the USA and Europe, the number of spermatozoa decreased by 50% between 1938 and 1990. [502] In France, between 1989 and 2005, the concentration of spermatozoa in 35-year-old men decreased by 32%. [461] Other studies also show a reduction in the quality of spermatozoa, which may suggest an increase in the demand for assisted reproduction. [462] In general, in Europe and the USA, a reduction in the number of spermatozoa is estimated at up to 2% per year. [502]

Dibromochloropropane (1,2-Dibromo-3-Chloropropane or DBCP) is a pesticide used against worms (nematodes), which are pests in tropical crops such as pineapple, sugarcane and bananas (it has been used in the soil as a fumigant in quantities of 10-120 kg/ha or in irrigation water). [554] It must be remembered that nematodes in the soil are very important and contribute to nitrogen mineralization, i.e. they help plants to feed themselves. Dibromochloropropane was authorized as a fumigant in the USA in 1964, although the negative effects on rodent testicles tested in laboratories were already known in 1961 (e.g. reduction in sperm count). [554] In 1977 the same effects were recorded among the workers of the factory producing this nematicide: they could not have children (because of oligospermia or azoospermia). In 1990 tens of thousands of agricultural workers reported damage to their reproductive system and recorded a ruduction of the ability to procreate. In the USA, the use of dibromochloropropane was stopped (in 1979) but not the production. Therefore, the industries continued to sell the nematicide in the tropical countries where it was most used. It is also known (since 1975) that it is carcinogenic and persistent in soil for at least 2 years. [555, 556] In North America, 20 years after the prohibition of the use of this substance, it is still possible to find it in drinking water (in 38 cities in California). [554] This story, like many others concerning the use of dangerous chemicals, should help us to understand the importance of applying the precautionary principle, at least following the first scientific or medical results.

In conclusion, there are many signs that fertility is declining in Europe and in the USA. For example, in the USA, between 1976 and 1998, the number of childless women (aged 35-39) doubled (from 10.5% to 19.8%), the number of medical consultations for male infertility increased and pregnancies in young women decreased. Pesticide exposure can adversely affect male semen quality. The presence of herbicides (alachlor, atrazine, metolachlor) and insecticides (diazinon) in urine has been associated with recording a decrease in sperm quality. [1251] They are probably one of the factors responsible for the reduction in male fertility. Also female fertility and embryonic development may be negatively affected by the exposure to some hazardous substances (e.g. diethylstilbestrol, phtalates). [1251] Phthalates may be found in children at high concentrations: higher than those measured in the urine of their mothers. [1255]
Environmental exposure to man-made molecules may promote a reduced fertility. Knowledge is incomplete, but suspicions should be sufficient, since it is a question of safeguarding mechanisms that are fundamental for our survival.
If diffuse endocrine disruptor pollution continues, at the current rate of fertility reduction, the threshold of infertility will be reached for part of the population within a few decades. This assumption is quite reasonable.

UNDERESTIMATED KNOWLEDGE

The problems generated by endocrine disruptors, such as some active molecules of pesticides and adjuvants that are part of the commercial formulation, have been known for a long time: some molecules since the 1990s. Unfortunately, this knowledge, which in some cases has been confirmed by adverse events (diseases among farmers or pesticide producers) and epidemiological studies, has not led to the application of appropriate preventive measures. The scientific literature on the delicate hormonal balances is substantial, and it is enough to alarm us to the point of pushing us to adopt different strategies from those applied until today. The knowledge and evidence provided by disease registration is only the tip of the iceberg. We are exposed, even before we are born and throughout our lives, to variable mixtures made up of thousands of artificial chemical compounds, at doses that are wrongly considered safe from political and economic criteria, rather than health and environmental ones. The effects, such as additive, synergistic and long-term effects, of an endless number of possible combinations are still largely unknown. We are therefore subjecting ourselves and the biosphere to a worrying experiment. Some of the results reported in the scientific literature are summarised below.

- A survey conducted in France and published in 2006 examined the presence of pesticides in the urine of 546 pregnant women. Fifty-two pesticide molecules were searched for (including 32 organophosphates, 12 triazines, 6 amides, and 2 carbamates). Only 1.6% of the samples had no traces of these 52 molecules, while 54% of the samples contained at least 8 molecules, 10% of the samples contained at least 13 pesticides, and one urine sample was recorded with the simultaneous presence of 28 pesticides. [319]

- In the United States, the urine of children and their parents may contain atrazine, chlorpyrifos, metolachlor, and glyphosate. [320]

- In California, 35% of pregnant women living in rural areas report dangerous concentrations of organophosphate metabolites in their urine. [1254]

- A research conducted in the USA shows the existence of a correlation between the professional use of pesticides and the increase of children with birth defects (e.g. abnormal number of fingers). Among the incriminated molecules, some herbicides (chlorophenoxy such as 2,4-D or MCPA) and fungicides were highlighted. [329] Some herbicides are probably teratogenic (e.g. the herbicide 2,4-D). Teratogenesis (from the Greek "*generation of monsters*") means the abnormal development during pregnancy of certain organs in the fetus, resulting in the birth of a child with birth defects. [330] In this research, the herbicide trifluralin and the fungicides mancozeb, maneb, and tributyltin are found to be endocrine disruptors. [329] Several scientific works, since the '90s had reported an association between pesticide exposure and an increase in the number of children born with defects. [331, 332]

- A study published in 2004 confirms that the exposure to aldrin and lindane predicts an increased likelihood of having breast cancer. [325]

- Neonicotinoid insecticides (acetamiprid, clothianidin, thiacloprid, imidacloprid, thiamethoxam, and dinotefuran) are among the most widely used pesticides in the World: at least 24% of the global market in 2014. Studies have shown that neonicotinoids reduce the population of insects and insectivorous birds, but they can also be toxic to mammals with effects on reproduction, the liver, the nervous system, and they are genotoxic. [433, 1249] They can damage neurons, the brain and associated functions such as memory and learning. [1250] Laboratory (in vitro) studies have shown adverse effects on mammalian sperm and alterations in embryonic development, so they could also adversely affect reproduction in the human species. Some studies therefore raise the suspicion that these molecules are very dangerous for mammals like us. Unfortunately, neonicotinoids enter our bodies and may be measured in urine. In the Japanese population, from 1991 to 2011, the concentration of neonicotinoids in urine increased: more than 65% of samples were positive for imidacloprid. [1249] Neonicotinoids are also present in the urine of the Sri Lankan population (at concentrations between 0.086 to 2.6 ng/mL), in the urine of Spanish volunteers and in 100% of the Chinese population living in rural areas, and 95% of the Chinese population living in urban areas (a total of 295 urine samples were analysed). [1249] China is both a producer and user country of neonicotinoids. The detection of 6 neonicotinoids (acetamiprid, clothianidin, thiacloprid, imidacloprid, thiamethoxam, and dinotefuran) in 324 urine samples from the general population residing in 13 different Chinese cities reported a lot of alarming results: [1249]

- All urine samples contained neonicotinoids: clothianidin (99%), thiamethoxam (98%), imidacloprid (97%), dinotefuran (96%), acetamiprid (96%), thiacloprid (92%).
- Clothianidin was found at the highest (median) concentration: 0.24 ng/mL (and at the highest concentration of 17 ng/mL).
- Dinotefuran is the active ingredient measured with the highest concentration: 18 ng/mL.
- Males record higher total concentrations.

These results demonstrate a high level of exposure of the Chinese urban population to dangerous molecules that unfortunately are also found in the urine of children. In urine also

organophosphates may be measured, at concentrations higher than those of neonicotinoids, as well as metabolites of pyrethroids.
One aspect that should be of great concern is that there is not much information about the transformations that neonicotinoids undergo in our bodies. The metabolites could be much more toxic than the starting substances (thiamethoxam is known to be transformed into clothianidin by insects such as *Spodoptera frugiperda* larvae, mice, and plants such as rice and cotton). The enzyme complex Cytochrome P450 (also present in humans) can transform thiamethoxam into clothianidin. Unfortunately, there is insufficient knowledge to consider these levels of exposure, which occur early in development, as non-hazardous. Synergistic, additive and sub-lethal effects are difficult to detect with the available pre-market information but could hold many bad surprises. It should be noted again that there is a possibility of a positive correlation between the detection of pesticides in urine and certain reproductive problems (e.g. reduced sperm count). [1251]

- Imidacloprid was authorized for use in Japan in 1992 and in the USA in 1994. Since then, neonicotinoids have been authorized in more than 120 countries. In Japan the use of neonicotinoids has also been encouraged by the emergence of insects resistant to organophosphate insecticides and pyrethroids. Due to widespread use, pesticides enter our tables through food and water. Unfortunately, insecticides or their metabolites (neonicotinoids, organophosphates, and pyrethroids) are harmful to our health. Exposure to organophosphates promotes neurotoxic effects (mental retardation, attention deficit, and reduced intellectual quotient), and the exposure to pyrethroids is associated with tumours (paediatric leukaemia). [1252] Unfortunately, these insecticides were found in the urine of 703 Japanese children of three years of age: 58% recorded neonicotinoids, 90% organophosphates, and 92% pyrethroids. [1252] The highest concentrations of organophosphates were associated with fruit consumption and professional pesticides used by family members. Most of the Japanese children examined had traces of the hazardous substances sought in their urine, confirming the dangerous exposure.

- Dangerous concentrations of several pesticides may be found in the Italian population not professionally exposed. The organophosphate insecticides may be transformed into several metabolites that are found in urine [dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP), methamidophos (METH which is also derived from acephate), 3,5,6-Trichloro-2-pyridinol (TCP, derived from chlorpyrifos and chlorpyrifos-methyl)]. The half-life in our bodies following dermal exposure and ingestion may be quite long: 27 days for TCA derived from chlorpyrifos and chlorpyrifos-methyl. [1256] A metabolite of fungicides (ethylenthiourea derived from ethylene-bisdithiocarbamate), that may be measured in urine, alters the thyroid function. Some metabolites of pyrethroid insecticides (permethrin, cypermethrin, deltamethrin, and fenvalerate) may also be measured in urine. Thus, both carbamates, organophosphates, and pyrethroids may be determined, via metabolites, in urine. A study conducted in Italy looked for these molecules in the urine of non-occupationally exposed people living in Florence or Ragusa. Of 69 urine samples, only one was found to be devoid of the 10 metabolites sought, 50% of the subjects recorded at least 6 different molecules and one sample contained 9 substances. [1256] These results confirm that through food one may be exposed to dangerous substances that are transformed and subsequently eliminated with urine.

- In Italy, in pregnant women living in Rome, glyphosate was found in urine at concentrations between 0.43 ng/mL and 3.48 ng/mL. [500] This herbicide also acts as an endocrine disruptor (anti-estrogenic profile) and has negative effects on reproduction (e.g. on sperm production and pregnancy). [492] Endocrine effects are recorded at concentrations 800 times lower than those permitted in some foods or feeds (e.g. those derived from plants genetically modified for resistance to the herbicide where it will therefore remain at high concentrations), and at concentrations at least 10 times lower than those causing the first evident acute toxic effects. [492]

Toxicity studies conducted in human liver cell lines report synergistic effects generated by the other substances present in the commercial glyphosate product. Adjuvants present in the commercial herbicide formulation may increase its toxicity and may be more dangerous for some effects than the active ingredient itself. These results highlight how important it is to test the entire commercial product as adjuvant molecules may be as or more hazardous and may enhance the adverse effects of the active ingredient.

We are exposed to tens of thousands of different chemical molecules and, assuming that only a few hundred have endocrine effects, the additive or multiplicative effects of millions of possible combinations are unpredictable. Some of the adverse effects such as those on reproduction (e.g. birth defects) and infertility, in both women and men, have been associated with the exposure to certain pesticides belonging to all of the widely used categories: organochlorines, organophosphates, and pyrethroids. [491] The adverse reproductive and developmental effects of pesticides (and others) are particularly risky as they occur in subsequent generations. This is all the more reason to act: ethical and moral principles should spur us on. [571]
The following is a non-exhaustive list of some pesticides for which endocrine disrupting effects have been documented. [464]

Interferents of androgen activity	**Interferents of estrogen activity**	**Alteration of aromatase activity**	**Thyroid interferents**
2,4-D (E), Aldrin (I), Atrazine (E), Bitertanol (F), Chlorothalonil (F), Chlordane (I), Chlorpyrifos methyl (I), Cyproconazole (F), DDT and metabolites (I), Dichlorvos (I), Dicofol (I), Dieldrin (I), Endosulfan (I), Fenarimol (F), Fenitrothion (I), Lindane (I), Heptachlor (I), Hexaconazole (F), Methiocarb (E), Myclobutanil (F), Penconazole (F), Procymidone (F), Propiconazole (F), Tebuconazole (F), Vinclozolin (F)	Acetochlor (E), Alachlor (E), Aldicarb (I), Bendiocarb (I), Benomyl (F), Bitertanol (F), Captan (F), Carbaryl (I), Carbendazim (F), Chlordane (I), Chlorfenviphos (I), Cypermethrin (I), Cyproconazole (F), DDT and its metabolites (I), Deltamethrin (I), Diazinon (I), Dicofol (I), Dieldrin (I), Endosulfan (I), Fenitrothion (I), Glyphosphate (E), Heptachlor (I), Hexaconazole (F), Methiocarb (E), Methomyl (I), Myclobutanil (F), Penconazole (F), Permethrin (I), Propiconazole (F), Tebuconazole (F).	Atrazine (E), Benomyl (F), Bitertanol (F), Carbendazim (F), Cyproconazole (F), Endosulfan (I), Fenarimol (F), Glyphosphate (E), Hexaconazole (F), Methomyl (I), Myclobutanil (F), Penconazole (F), Propiconazole (F), Tebuconazole (F).	Acetochlor (E), Dimethoate (I), Fenbuconazole (F), Lindane (I), Malathion (I), Trichlorfon (I)
Alteration of melatonin production	**Altered gonadotropic hormone production**	**Interferents of catecholamine activity**	**Interferents of insulin activity**
Parathion (I)	Parathion (I)	Malathion (I), Parathion (I)	Dimethoate (I), Lindane (I)
Hypothalamic interferents	**Interferents of progesterone activity**		
Acephate (I), Atrazine (E)	Alachlor (E), Aldicarb (I)		

Note: E = herbicide; F = fungicide; I = insecticide.

HONEY AND PESTICIDES

At least 1,100 pesticides (insecticides, acaricides, rodenticides, fungicides, and herbicides) are marketed in Europe. These substances are mainly used for food production and contaminate the environment. Contaminated foods include honey, which may contain several molecules at the same time. The potential daily intake of honey (0.8 g per kilogram of body weight per day: for a 60 kg adult, this means consuming 48 g of honey per day) implies health risks. A bibliographic review of pesticides found in 2,620 honey samples from all over the World reports that 52 different insecticides may be recorded, of which 19 belong to the organophosphorus category (e.g. coumaphos, parathion, parathion-ethyl, phorate, chlorfenvinphos, chlorpyrifos, dimethoate, dichlorvos, malathion), 13 belong to the organochlorinated category (e.g. p,p′ DDT, 4,4-DDE, p,p′ DDE, p,p′ DDD, heptachlor, dieldrin, methoxychlor, lindane, dieldrin), 7 to neonicotinoids (e.g. acetamiprid, clothianidin, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam), 6 to pyrethroids (e.g. cypermethrin and permethrin), 5 to carbamates (e.g. aldicarb, carbofuran, carbaryl, and pirimicarb). [1220] Therefore, through the consumption of honey we may consume substances classified as hazardous to our health: aldicarb, coumaphos, parathion, parathion-ethyl, carbofuran, azinphosmethyl, and dichlorvos. In honey, some of these molecules may be found in dangerous concentrations such as parathion (detected for example in French, Italian and Portuguese honeys) and carbofuran (found in dangerous concentrations in France and Portugal). [1220] Residues of acaricides used by beekeepers may also be found in honey. In some cases, they are found in high concentrations, such as amitraz, phosmet, and monocrotophos. Residues of 19 fungicides and 12 herbicides may also be found in honey.

It is known that pesticides can impair the reproductive capacity in humans by negatively affecting sperm quality. For example, organophosphate insecticides have been associated with reduced motility, decreased sperm volume, and sperm count (in occupationally exposed workers in pesticide factories). [1220] Occupational exposure (farmers) to glyphosate has also been reported to decrease semen quality. Other molecules suspected to decrease semen quality are some organochlorines and pyrethroids. Organophosphates and carbamates can alter testosterone production. In animal studies, some organochlorines can inhibit the biosynthesis of testosterone. In women too, the occupational exposure to pesticides increases the likelihood of infertility problems (e.g. exposure to 2,4-dichlorophenoxy acetic acid in women in Canada). Organochlorine insecticides are suspected of increasing the production of certain hormones (such as the follicle stimulating hormone and the leutinising hormone).

Some of these effects are very evident in animal models such as mice. For example, pesticides that in the laboratory interfere with testosterone biosynthesis (in mice and rats) are: acetamiprid, chlorpyrifos, cypermethrin, diazinon, permethrin, dimethoate, endosulfan, esfenvalerate, and glyphosate. [1220] In the experimental rat model, sperm count and sperm motility are reduced by: cypermethrin, amitraz, carbaryl, carbendazim, carbofuran, chlorpyrifos, diazinon, dimethoate, endosulfan, fenvalerate, and glyphosate. Studies in rats show that pesticide residues can induce testicular damage by altering mitochondria (acetamiprid, clothianidin, cypermethrin, carbendazim, and glyphosate).

Unfortunately, these molecules are contained in honey, plant foods, and in some cases contaminate water and also other foods (e.g. bioaccumulated organochlorines in animal by-products). Thus, the consumption of contaminated honey may also contribute to reproductive problems in animals, including humans.

NEUROTOXIC EFFECTS OF PESTICIDES

Many of the active molecules used to control animals such as insects act on fundamental and ubiquitous mechanisms of the nervous system. Therefore, it is not surprising that these molecules are capable of exerting adverse effects that may occur in all animals with alterations in nervous system-dependent functions. Neurotoxic effects may be very severe, especially following foetal exposure (e.g. to insecticides).

Adverse effects on the nervous system may be very diverse and depend on multiple factors such as doses, duration of exposure, and mode of contamination. Some symptoms of intermediate severity associated with the exposure to organophosphates are headache, nausea, vomiting, and contraction of the pupils. In severe intoxication, bronchospasm, changes in heart rate, muscle aches, cramps, leg muscle paralysis, altered sensory abilities, and convulsions may be reported. [1254] Some symptoms may also occur several years after the exposure. Possible long-term effects include depression, Parkinson's disease (organophosphates, glyphosate, paraquat, dieldrin, diquat, rotenone, maneb, and ethylene bis-dithiocarbamates), and deficits in the cognitive function. [1254]

Chemicals such as pesticides are not tested for neurotoxic effects during development before they are marketed because they are considered safe unless proven otherwise. It is very difficult to investigate the neurotoxic effects of molecules by evaluating them individually, especially if their effects are weak (e.g. reduction of intellectual quotient by a few points and, therefore, decrease in intelligence). It is even more difficult to study the synergistic and additive effects of dozens of molecules such as neurotoxic pesticides to which we are exposed daily.

Organochlorine insecticides are persistent and bioaccumulative molecules in the food chain. Due to the widespread use of these molecules, their neurotoxic effects may occur in the early stages of development. In Japan, 21 molecules of organochlorine compounds have been found in the serum of pregnant women (some are also found in the adipose tissue and the umbilical cord). The exposure of mothers (not occupationally exposed) to organochlorines (e.g. cis-heptachlor epoxide or p,p'-DDE) has been shown to cause measurable neurotoxic effects in their children as early as 18 months of age (reduced mental and psychomotor development). [1158]

At least 200 molecules (environmental pollutants) have been identified as generating neurotoxic effects, including many pesticides. The presence of organochlorine pesticides in breast milk (e.g. DDT and derivatives) is probably associated with neurodevelopmental problems in infants exposed during lactation (an association has been found between the exposure to organochlorine insecticides through breast milk and the concentration of thyroid and growth hormones in infants). [1163] The exposure of infants may be higher than that of their mothers due to bioconcentration in breast milk. Polychlorinated biphenyls accumulating in adipose tissue through breast milk may result in 100 times higher exposure of infants (calculated on the basis of body weight). [327]

Mothers who reside near agricultural fields where pesticides are used increase the likelihood of recording neurodevelopmental problems in their children. [1162] In 7-year-old children, the incidence of cognitive problems (e.g. reduced intellectual quotient, verbal comprehension, memory capacity) increases if the mothers were exposed (not professionally but as residents within 1 km of treated fields during pregnancy) to neurotoxic pesticides (e.g. organophosphates, pyrethroids, neonicotinoids, and manganese-based fungicides). [1162] The exposure of mothers to some pesticides (e.g. pyrethroids and organophosphates) has also been linked to an increased likelihood of recording birth defects in infants. [1162]

Dozens of molecules classified as organophosphates are registered for use in agriculture (about 40 in the USA) and are among the most widely sold pesticides in the World. In California, it has been found that 40% of children working in agriculture show signs of

organophosphate and carbamate poisoning (low levels of cholinesterase in the blood). [502] The exposure during pregnancy to certain pesticides, such as organophosphate insecticides (measured in the urine of women), can reduce the intelligence of children as young as 7 years old. [513] In the urine of children, aged 8 to 15 years, the detection of organophosphate insecticides (and their metabolites) has been associated with an increased likelihood of recording attention deficits and hyperactivity disorder. [514] In the USA, 94% of children's urine (1,139 subjects) records the presence of at least one organophosphate or its metabolite. [514] Therefore, the exposure is systematic and begins in the early stages of development. The frequency with which attention disorders with hyperactivity were recorded was proportional to the concentrations of insecticides found in the urine.
In the elderly population of Mexican Americans living in California, the possible association between exposure to organophosphates insecticides - because they live near treated fields - and cognitive decline and mortality has been highlighted. [1159] A prolonged exposure to low concentrations of organophosphate insecticides generates sub-lethal effects, measurable in the elderly with a reduction in the cognitive ability. Neurotoxic effects have also been demonstrated following acute intoxication, i.e. at higher doses. [1159] These effects are measured with higher frequencies in people exposed professionally or because they live near the treated fields (less than 500 m) as they record high concentrations of these molecules in urine. The organophosphate insecticides (they inhibit the acetylcholinesterase enzyme) are used in great quantities (at the time of publication of this study they constituted 35% of all insecticides distributed in the USA) and have recorded, in addition to problems with the central nervous system, other negative effects such as those on the mitochondria and neuro-inflammatory ones (they generate a reduction in the concentration of the hormone adiponectin which is related to type 2 diabetes and other health trouble). [1159] The exposure to higher doses of organophosphates, for example among people who use these molecules for professional reasons, has been reported to cause memory problems, reduced attention span, mood depression, and cognitive deficits.

At least 90 pesticides are toxic to the brain, and in children they are likely to be related to increased attention disorders and hyperactivity (e.g. organophosphates, such as chlorpyrifos and pyrethroids). [326, 327, 491, 513]
The process of formation and development of the nervous system is very vulnerable to chemicals. Studies show that concentrations of glyphosate, similar to those tolerated in drinking water, can alter the development of the nervous system (0.1 µg/L in Europe and 700 µg/L in the USA). [1274] Glyphosate is able to cross the blood-brain barrier and has been associated with Parkinson's disease, behavioural disorders, autism, and anxiety. In the laboratory, it has been reported to affect gene expression in nerve cells (it inhibits the production of certain tubulins and cytochrome proteins that are important in detoxification processes). [1274] Many studies confirm that the safety thresholds established by pre-marketing risk assessments are not able to ensure the protection of our health.

Chronic pesticide exposure has been associated with neurobehavioural abnormalities such as anxiety, depression, deficits in short-term memory, learning, and attention. [500, 516, 522] These alterations may be caused by imbalances in ion channels important for neurotransmission (caused by aldrin, chlordane, hexachlorobenzene, DDT, DDE, pyrethroids), by damage to mitochondrial functions or by the inhibition of enzymes (e.g. acetylcholinesterase). [500] Therefore, the neurotoxic effects are the consequence of the primary mechanisms of action of the active molecules, which are known and are the desired ones as they are necessary to induce the death of the insects. Again we find analogies with the problems recorded in the insect from pesticides. Sub-lethal effects may occur due to the alterations induced in the nervous system and generate devastating consequences in the long time, in humans as well as in bees. The basic mechanisms of cells are identical, the neurotransmitters, their receptors, enzymes, the functioning mechanisms of neurons, ion channels are very similar and ubiquitous in the animal kingdom. Therefore, it is not surprising that some of the symptoms generated by sub-lethal doses in insects are very similar to those recorded in humans.

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized by difficulty in maintaining attention, excessive activity and/or difficulty in controlling one's own behaviour (impulsivity) that does not appear appropriate for the person's age: about 4% of the school-age population is affected by ADHD and it is more frequent in males. In Italy at least 1% of children between 6 and 18 years of age have these disorders. [512, 539, 540] People with Attention Deficit Hyperactivity Disorder may also have other health problems such as sleep disorders and anxiety. [540]

The exposure of mothers, during the second or third trimester of pregnancy, to certain pesticides (organophosphates such as chlorpyrifos, neonicotinoids such as imidacloprid, and pyrethroids) has been associated with an increased likelihood of registering autism. [491]

Several studies reveal that harmful substances can promote autism, hyperactivity, dyslexia, and learning problems in children; probably up to one child out of six is affected by these disorders and at least 3% of sufferers are due to exposure to pollutants. [326, 327] Some pesticides (chlorpyrifos, DDT/DDE) and others (lead, methyl-mercury, polychlorinated biphenyls, arsenic, toluene, polybrominated diphenyl ethers, phthalates, and bisphenol A) have been identified as incriminating substances. A study conducted in California has recorded the association between the manifestation of autism and prenatal exposure to the following molecules (mothers living within 2 km from treated fields): chlorpyrifos, diazinon, glyphosate, malathion, methyl bromide, myclobutanil, permethrin. [1161] Exposure in the first year of life makes things worse by promoting intellectual disabilities. Therefore, in order to hope to decrease the incidence of these developmental disorders of the central nervous system, it would be necessary to reduce the environmental exposure of mothers and of children in the first year of life. The molecules used in the fields are found in the dust inside the house, in the bodies of mothers and in the air of residents within 1,250 m from the treated fields (in California). [1161]

Some neurodegenerative diseases such as Parkinson's disease, Alzheimer's, and Amyotrophic Lateral Sclerosis have been associated with the chronic exposure to certain substances such as paraquat, maneb, diedrin, organophosphates, and pyrethroids. [518, 519] Workers exposed to organophosphates and organochlorinated insecticides, or fumigants and defoliants have been found to be more likely to register Alzheimer's. [491]

Parkinson's disease is a slow-onset but progressive neurodegenerative disorder that primarily affects certain functions such as movement control and balance. The disease is part of a group of pathologies called "*Movement Disorders*" and among them Parkinson's disease is the most frequent. Before the age of 20 it is extremely rare. Above the age of 60 years it affects 1% or 2% of the population, while the percentage rises to 3-5% when the age exceeds 85 years. [544] The prevalence[I] of the disease in industrialized countries is about 0.3%. [546] The structures involved in Parkinson's disease are located in deep areas of the brain. The disease occurs when the

[I] Prevalence is the ratio of the number of health events detected in a population at a defined time (or over a short period of time) to the number of individuals in the population observed over the same period. There is a distinction between point prevalence and period prevalence i.e., for example, for a 12-month period; in the former the observation of the number of individuals who are ill and may develop the disease refers to a defined time (e.g., December 31 of a year). In the second it refers to a short time frame that can be even a whole life (lifetima prevalence). [1296]

Prevalence does not allow the probability of falling ill to be estimated. The concept of estimating the probability of getting sick is related to the incidence of the disease. In fact, it is necessary to define the new cases of the disease in a certain time interval. Incidence represents the change in one quantity (the new sick) with respect to the change in another quantity (time); therefore, it is a dynamic measure and is a true rate because at least two surveys are needed. Incidence can be seen as a way to measure the rate of transition from the healthy state (absence of disease) to the diseased state in a population.

production of dopamine in the brain drops consistently. The risk of the disease increases with exposure to substances such as certain pesticides (e.g. paraquat), hydrocarbon-solvents (e.g. trichloroethylene) and in certain occupations (such as welding) that expose workers to metals (iron, zinc, copper). [544] In France, in 2013, Parkinson's disease was recognized as an occupational disease, i.e. resulting from exposure to neurotoxic molecules (many German farmers also had Parkinson's recognized as an occupational disease). [500, 1295]

The main motor symptoms of Parkinson's disease are tremor at rest, rigidity, bradykinesia (slowness of automatic movements), and, at a later stage, postural instability (loss of balance); these symptoms occur asymmetrically, with one side of the body being more affected than the other. The most frequently observed non-motor symptoms are: vegetative disorders (alteration of viscera functions), disorders of smell, sleep and mood. Other symptoms are fatigue, pain, anxiety, depression and apathy. With an appropriate treatment, life expectancy is considered to be similar to, or only slightly reduced from that of the general population. There is no known cure for Parkinson's disease, however there are several treatments that can control its symptoms. [545] Parkinson's has been associated with the exposure to insecticides (e.g. organochlorines such as DDT, dieldrin, heptachlor) and herbicides. [519] Some of the pesticides, which at least in one epidemiological study are suspected to contribute to an increased likelihood of recording Parkinson's disease (e.g. due to long-term residence within 1,000 m or 500 m of treated fields) are: alachlor, atrazine, chlorpyrifos, diazinon, glyphosate, maneb, methomyl, metolachlor, paraquat, propargite, rotenone, simazine, ziram, 2,4-D, β-HCH. [500, 1160]

Amyotrophic lateral sclerosis (ALS) is a very serious disease that leads to the paralysis of voluntary muscles and even respiratory muscles. [543] Motor neurons that connect the brain to the spinal cord and motor neurons that connect the upper motor neurons from the spinal cord to all muscles in the body are involved in the disease. These neurons communicate by sending electrical messages from one neuron to another until they reach the desired target, which is the muscles. In amyotrophic lateral sclerosis, this line of neuronal communication collapses; motor neurons are unable to carry electrical information from the brain and spinal cord to the muscle, which becomes inactive (paralyzed). If a muscle is inactive for a long period its mass begins to decrease, i.e. it atrophies. This is why muscle deterioration is a common symptom of amyotrophic lateral sclerosis. About half of the people living with this disease may experience difficulties in learning, speech, and concentration. [543] In Italy about 6,000 patients are registered and the incidence of this disease is between 1.5 and 2.4 cases per 100,000 inhabitants; in Europe the prevalence is 4-8 cases per 100,000 inhabitants. Life expectancy after diagnosis is on average 3-5 years. The onset of this disease has also been associated with the use of pesticides such as organochlorine insecticides (e.g. aldrin, dieldrin, DDT, toxaphene) and pyrethroids. [518]

There are at least 200 substances for which neurotoxic effects are well documented, but probably those for which there is a reasonable suspicion are more than 1,000 (thanks to experimental studies in the laboratory). [326, 327] The lack of definitive confirmations creates an unbridgeable gap, especially if we rely on the mechanism according to which a substance (e.g. an insecticide) cannot be considered dangerous until proven otherwise. Usually this evidence comes from deaths and illnesses recorded during accidents or occupational exposures: when it is already too late.

Below is a list of substances for which some scientific studies have documented neurotoxic effects. [326, 327]

NEUROTOXIC SUBSTANCES		
Pesticides	**Metals and inorganic compounds**	**Organic substances other than pesticides**
Acetamiprid - Aldicarb - Aldrin - Amitraz - Avermectin - Bensulide - Bromophos - Carbaryl - Carbofuran - Carbophenothion - α-Chloralose - Chlordane - Chlordecone - Chlorfenvinphos - Chlormephos - Chlorpyrifos - Chlorthion - Coumaphos - Cyhalothrin - Cyolane - Cypermethrin - Deltamethrin - Demeton - Dialifor - Diazinon - Dichlofenthion – 1,2-Dibromo-3-chloropropane (DBCP) - Dichlorodiphenyltrichloroethane (DDT) – 2,4-Dichlorophenoxyacetic acid (2,4-D) – 1,3-Dichloropropene - Dichlorvos - Dieldrin - Dimefox - Dimethoate - Dinitrocresol - Dinoseb - Dioxathion - Disulphoton - Edifenphos - Emamectin - Endosulphan - Endothion - Endrin - EPN - Ethiofencarb - Ethion - Ethoprop - Fenitrothion - Fensulphothion - Fenthion - Fenvalerate - Fipronil Fonofos - Formothion - Glyphosate - Heptachlor - Heptenophos - Hexachlorobenzene - Hexaconazole - Imidacloprid - Isobenzan - Isolan - Isoxathion - Leptophos - Lindane - Merphos - Metaldehyde - Methamidophos - Methidathion - Methomyl - Methyl bromide - Methyl demeton - Methyl parathion - Mevinphos - Mexacarbate - Mipafox - Mirex - Monocrotophos - Naled - Nicotine - Oxydemeton-methyl - Parathion - Pentachlorophenol - Permethrine - Phorate - Phosphamidon - Phospholan - Propaphos - Propoxur - Pyriminil - Sarin - Schradan - Soman - Sulprophos – 2,4,5-T - Tebupirimfos - Tefluthrin - Terbufos - Thiram - Toxaphene - Trichlorfon - Trichloronat - Trichloronate	Aluminum compounds - Arsenic and arsenic compounds - Azide compounds - Barium compounds - Bismuth compounds - Carbon monoxide - Cyanide compounds - Decaborane - Diborane - Ethylmercury - Fluoride compounds - Hydrogen sulphide - Lead and lead compounds - Lithium compounds - Manganese and manganese compounds - Mercury and mercuric compounds - Methylmercury - Nickel carbonyl - Pentaborane - Phosphine - Phosphorus - Selenium compounds - Tellurium compounds - Thallium compounds	**SOLVENTS**: Acetone - Benzene - Benzyl alcohol - Carbon disulphide - Chloroform - Chloroprene - Cumene - Cyclohexane - Cyclohexanol - Cyclohexanone - Dibromochloropropane - Dichloroacetic acid – 1,3-Dichloropropene - Diethylene glycol - N,N, N-Dimethylformamide - 2-Ethoxyethyl acetate - Ethyl acetate - Ethylene dibromide - Ethylene glycol - n-Hexane - Isobutyronitrile - Isophorone - Isopropyl alcohol - Methyl butyl ketone - Methyl cellosolve - Methyl ethyl ketone - Methylcyclopentane - Methylene chloride - Nitrobenzene - 2-Pentropane - 1-Pentropane - 1-Pentropane - 1-Pentrol ketone - 2-Pentropane - 1-Pentropane - 1-Pentropane - 1-Pentrol ketoneNitropropane - 1-Pentanol - Propyl bromide - Pyridine - Styrene - Tetrachloroethane - Tetrachloroethylene - Toluene – 1,1,1-Trichloroethane - Trichloroethylene - Vinyl chloride - Xylene. **OTHER ORGANIC SUBSTANCES**: Acetone cyanohydrin - Acrylamide - Acrylonitrile - Allyl chloride - Aniline - 1,2-Benzenedicarbonitrile - Benzonitrile - Butylated triphenyl phosphate - Caprolactam - Cyclonite - Dibutyl phthalate - 3-(Dimethylamino)-propanenitrile - Diethylene glycol diacrylate - Dimethyl sulphate - Dimethylhydrazine - Dinitrobenzene - Dinitrotoluene - Ethylbis(2-chloroethyl)amine - Ethylene - Ethylene oxide - Fluoroacetamide - Fluoroacetic acid - Hexachlorophene - Hydrazine - Hydroquinone - Methyl chloride - Methyl formate - Methyl iodide - Methyl methacrylate - p-Nitroaniline - Phenol

RESPIRATORY PATHOLOGIES

Occupational exposure to pesticides increases the likelihood of experiencing respiratory problems such as sore throat, cough, asthma, and chronic bronchitis. [517] These are diseases recorded more frequently in those who manufacture, transport, prepare, and apply pesticides, such as users of: alachlor, aldicarb, benomyil, chlorpyrifos, carbaryl, carbofuran chlorothalonil, coumaphos, cypermethrin, dichlorvos, DDT, dimethoate, malathion, mancozeb, methomyil, parathion, permethrin, propineb, S-ethyl-dipropylthiocarbamate, tetramethrin, trifluralin. The occupational use of pesticides is also associated with a decreased functional capacity of the lungs (e.g. organophosphate and carbamate insecticides). Lung tumours have also been associated with the use of certain pesticides and it must be remembered that carcinogenic substances other than the active molecules may be present in commercial formulations (e.g. dioxins in mixtures based on phenoxy herbicides such as: 2,4-D, MCPA and 2,4,5-T). [517]

Although some pesticides have been banned for decades, they are still found and cause damage to our health. In Canada, the presence of DDT (found in 10% of the subjects) and its metabolite DDE (in more than 99% of the subjects) has been correlated with lung dysfunction (e.g. reduced respiratory capacity), bronchitis and asthma. [541] Organophosphates and carbamates were associated with chronic bronchitis. [500]

In the general population the existence of a correlation between the exposure to some insecticides, such as organophosphates, pyrethroids, and organochlorines (e.g. DDT), and respiratory system dysfunctions has been detected. [542] Therefore, a very worrying signal has been evident for some time, since usually this type of problem (asthma and bronchitis associated with exposure to pesticides) was recorded in occupationally exposed subjects and not in the general population. [581]

The parental use of herbicides or insecticides increases the likelihood of diagnosing asthma in children, before the age of five. [516] In the USA at least 15% of farmers who distribute pesticides suffer from asthma, chronic sinusitis, and/or chronic bronchitis. [502] Among American male farmers asthma has been recognized as one of the most frequent occupational diseases, and has been associated with the use of coumaphos, heptachlor, parathion, and dibromoethylene. In the case of women, asthma has been associated with the use of carbaryl, coumaphos, DDT, malthion, parathion, permethrin, 2,4-D, glyphosate, and metalaxyl. [500]

THE ANTIBIOTIC ACTION OF ORGANOPHOSPHATE INSECTICIDES

Pesticides that harm bacteria also have negative effects on our health. Alterations in the composition of the community of human intestinal microorganisms have been associated with some pathologies such as obesity, diabetes, cardiovascular diseases, colon cancer, and liver cirrhosis. [495] The impairment of the intestinal microbiome reduces the ability to detoxify some substances, the ability to digest others (e.g. some carbohydrates) and reduces the availability of useful substances produced by symbiotic microorganisms (e.g. some vitamins).

In this regard, it is useful to report the change in the composition of the bacterial community in human and rat intestines generated by chlorpyrifos or diazinon which are organophosphate insecticides. [493, 495, 524] In rats, prenatal exposure to chlorpyrifos (during gestation) generates several alterations such as reduced size at birth, changes in the intestinal epithelium (it will be thinner) and in the composition of the intestinal bacterial flora; its distribution is also altered as the bacteria will move more easily to other parts of the body by crossing the epithelia. [524] It should be remembered that at least 10^{14} bacteria (the number one followed by 14 zeros), belonging to at least 400 different species, reside in the human intestine. The exposure to pesticides in the uterus, e.g. to organophosphate insecticides, may damage the intestine's ability to absorb. Disruption of the microbial balance in the gut predisposes to various diseases. Unfortunately, these insecticides are widely used and consequently are easily found in food. The average exposure by ingestion of the population in the USA is considered to be between 8 x 10^{-6} and 34 x 10^{-6} mg per kilogram of body weight per day, while in France in adults it is estimated to be between 0.01 and 0.14 mg per kilogram of body weight per day. In children, in France, the exposure was estimated to be up to 0.15 mg per kilogram of body weight per day (in 2011); it should be noted that children are more sensitive than adults and that these insecticides cross the barrier formed by the placenta. [524]

Diazinon, another organophosphate insecticide, causes alterations in the composition of mouse gut microorganisms differently in the sexes, with males registering the main adverse effects. [495] This information suggests that experimental models for preliminary toxicity testing should consider sex and age.

In rare cases, microorganisms can use pesticides as nutrients and, therefore, will be favoured. For example, staphylococci in the soil can degrade chlorpyrifos. [524] In some cases, microorganisms transform pesticides into derivative molecules that may be equally or more toxic (derivatives may act negatively with different mechanisms of action than the starting molecule).

GLYPHOSATE AND INTESTINAL MICROORGANISMS

It may be useful to remember that some pesticides are also registered as antibiotics, as is the case of glyphosate. Other molecules used for bactericidal action are triazine-S-triones and agents that release chlorine. [517] Substances that alter the composition of the microbiome produce different effects. Potentially dangerous intestinal bacteria (e.g. *Salmonella* spp and *Clostridium* spp) can be very resistant to the antibiotic action of glyphosate while others, potentially very useful (e.g. *Bifidobacterium* spp and *Lactobacillus* spp), can be susceptible. [524] This effect has been observed in the intestinal flora of chickens, where pathogenic bacteria may be resistant to glyphosate and, therefore, be favoured when it is present in feed and water (*Salmonella gallinarum, Salmonella enteritidis, Salmonella typhimurium, Clostridium perfringens*, and *Clostridium botulinum*). [552]

In cattle, the increase in disorders caused by the presence of a bacterium, the agent of botulism (*Clostridium botulinum*), is favoured by the ingestion of molecules with an antibiotic action such as glyphosate. This herbicide inhibits the growth of intestinal bacteria such as lacto-bacilli and enterococci that are normally present in the intestine and are able to counteract the development of pathogens such as *Clostridium botulinum*. In cattle, the ingestion of glyphosate damages the microorganisms that constitute the natural defence against the attack of pathogenic bacteria. [534] Many bacteria in the intestines of cattle are capable of producing bacteriocins (natural antibacterial agents) that are active against *Clostridium botulinum*. Ingestion of the herbicide promotes bacterial diseases by microorganisms resistant to the biocidal action of this pesticide, such as the botulinum agent, and reduces the defences by natural antagonists.

Concentrations of the herbicide glyphosate considered safe in drinking water have recorded adverse effects in laboratory animals. The administration of glyphosate at the concentration considered safe for drinking water in the USA (1.75 mg/kg bw/day) recorded significant changes in the composition of the gut flora measured in rat faeces. Small concentrations may adversely affect the composition of the gut microbiome, favouring some microbes and decreasing the concentration of others (*Lactobacillus*). [1174] These negative effects have also been recorded in early developmental stages. The microbiome is fundamental for

maintaining health as it performs important functions: these effects may be expected at very low concentrations that are considered safe.
Glyphosate is also found at worrying concentrations in breast milk (up to 166 mg/L). [1174] As a result, newborns may be exposed during breastfeeding, negatively affecting the intestinal microbial ecosystem (and others) early in life.

The antibacterial action generated by some substances affects symbioses that have evolved over millions of years between microorganisms, and between microorganisms and multicellular organisms such as insects and mammals. The microorganisms that perform useful functions, such as those in the intestine, are similar or the same in the animal kingdom. So the antibiotic action generates devastating effects in apparently very different organisms like humans, cows, chickens, and bees. Again we find similarities with the sub-lethal effects generated by some pesticides to bees. Unfortunately, the antibiotic action of pesticides also damages the soil and the plant kingdom. The symbioses between microorganisms and plants are important and necessary, so the anti-bacterial action widens the lethal range of pesticides to a much greater part of the biosphere, sparing no one: plants, animals, fungi, and all terrestrial, aquatic, and marine ecosystems. Negative changes are dangerously amplified by this kind of chemical arsenal: we do not need more information to predict disastrous effects.

MICROORGANISMS IN THE SOIL

Plants interact with thousands of microorganisms that live in the rhizosphere, the area of the soil surrounding the roots, or on the leaves. The numbers in the plant microbiome are very high: up to 10^{11} microbial cells per gram of root. [579]
The name mycorrhizal fungi identify a remarkably heterogeneous group of mycetes that establish associations, called mycorrhizae, with the roots of almost all plants found on Earth. The function of mycorrhizal symbioses is an exchange: the plant improves its mineral nutrition, recording a positive effect on growth and, in return, gives sugars to the fungus. Thanks to mycorrhizal symbioses, the plant becomes more resistant to biotic or abiotic stresses, increases tolerance to lack of water or presence of pollutants, and reduces its susceptibility to the most common pathogens. A type of mycorrhizal symbiosis is the so-called arbuscular symbiosis, which is the most ancient one (structures similar to arbuscular mycorrhizae are documented by fossils dating back to the Devonian, that is 400 million years ago); arbuscular mycorrhizae involve most of the cultivated herbaceous plants such as rice, maize, and tomato. [579] The development of the symbiosis may be divided into three main stages: a first stage in which the two organisms, which have not yet established a direct contact, exchange chemical messages in a real molecular dialogue; a second stage in which the contact takes place and, finally, the colonization event itself, with the formation of the intra-root arbuscules and the acquisition of the full functionality of the symbiosis. Some known examples of mycorrhizal symbioses are those between truffles and oaks, or between boletus mushrooms and chestnut trees. The host plant reacts to the fungal signals, to the contact, to the colonization, and then to the formation of the arbuscules with extraordinary changes, both in the cellular organization and in the gene expression. The mycorrhizal symbiosis generates an increased resistance to pathogenic bacteria and improves the quality and quantity of the fruits (the gene expression will be modified). A significant increase in antioxidant compounds has been recorded in tomatoes derived from mycorrhized plants. [579]
The impact of symbiotic fungi on the physiology of the root and of the plant as a whole is extraordinary: many functions are activated, such as those related to the uptake and transfer of nutrients from the soil to the plants, defense processes, morphogenetic processes (such as those related to the development of the root system), and surprisingly, events controlling plant

fertility are also favoured. Soil microorganisms such as mycorrhizal fungi are certainly important components of plant health and must be safeguarded.
The fertility of the soil depends on the state of health of the organisms that reside in a few centimetres of depth and we can say that our survival depends on this thin ecosystem that we are degrading very quickly.

GLYPHOSATE AND THE RHIZOSPHERE

Plants have developed different types of symbiosis with microoganisms and, like us, give rise to billions of bacteria: in the leaves or in the barks (phyllosphere), in the spaces between cells (endophytes) but the largest number is located around the roots. [986] Some plants also dedicate 40% of the energy obtained through photosynthesis to support symbioses such as the root symbiosis: the rhizobiome. Through the donation of sugars and other substances, plants favour certain micro-organisms useful for their own survival. So plants have the ability to select the most useful microorganisms around their roots: i.e. the rhizosphere. We could say above all that some plants breed the most useful soil micro-organisms to satisfy their demands.

Pesticides may alter the microbial composition in the soil. In the USA, soybean plants genetically modified for resistance to the herbicide glyphosate were marketed in 1996. In 2008, other plants resistant to the herbicide were marketed through different genetic modifications. [535] Glyphosate damages the micro-organisms that live in symbiosis with the roots. [552] The use of the herbicide glyphosate in soybean plants (*Glycine max*) which have been genetically modified to be resistant to its phytotoxicity are adversely affected by its effects on soil microorganisms. Glyphosate damages the complex interactions between soil micro-organisms and the roots of the soybean plant, leading to a decrease in root growth. Other effects recorded by glyphosate in soybean crops resistant to this herbicide were: [535]

- Reduction in chlorophyll photosynthesis leading to a decrease in lignin content.
- An increase in the presence of phytopathogenic fungi in the roots such as *Fusarium* (it produces rots). This effect in genetically modified soybean plants was observed even when using the lowest recommended doses of glyphosate (720 g/ha, as stated on the commercial product label). *Fusarium* fungi are also of health concern as they can produce mycotoxins harmful to humans and animals. Soybean plants that are not resistant to the herbicide have a minor colonization by these phytopathogenic fungi. This effect, i.e. increased colonization of the roots by the *Fusarium* fungus following glyphosate treatments, has also been observed in herbicide-resistant cotton plants (these are genetically modified plants).
- Some soil microorganisms (e.g. *Pseudomonas*) produce chemicals that regulate plant growth (phytohormones such as auxins). The presence of glyphosate reduces the production activity of these hormones useful for the growth of plants such as soybeans and decreases the presence of some bacteria (such as *Pseudomonas*).
- The reduction in the weight of the shoots and roots of the soybean plant: especially the biomass of the roots is reduced considerably.

Traditional agriculture makes an extensive use of herbicides because mechanical weeding is not possible (crops in full vegetation) or is more expensive. With the spread of agricultural practices involving minimal tillage in some crops, the use of herbicides has increased. In crops such as cereals this implies an increased use of herbicides such as glyphosate. [536] In plants, and in some fungi and bacteria, this herbicide inhibits the production of certain aromatic amino acids; in plants these amino acids may be used to produce phytohormones or defense system molecules.

Some bacteria present in the soil and rhizosphere are resistant to the biocidal action of glyphosate and, therefore, will be favoured by the use of this herbicide having an antibiotic activity. Thus, the use of glyphosate modifies the soil ecosystem by favouring microorganisms such as bacteria and fungi that are resistant to its action. In Canada, in wheat and pea crops, the influence of glyphosate on mycorrhizae has been measured. The use of glyphosate generates alterations through two mechanisms: it favours microorganisms resistant to the active ingredient and modifies the physiology of symbioses. Glyphosate favours the development of plant pathogenic fungi and also damages the symbioses between nitrogen-fixing microorganisms and leguminous plants. [536]
Tolerance to glyphosate is more widespread among bacteria than among fungi in the rhizosphere, consequently some bacteria will be favoured (e.g. glyphosate-resistant Gram-negatives such as *Pseudomonas*) and the composition of the soil microorganism community will change. The soil ecosystem is altered due to the monocultures perpetuated in large areas and the systematic use of herbicides. It should be noted that about 72,000 species of fungi have been classified and that they are probably less than 5% of the species estimated to exist. Therefore, it is reasonably foreseeable that this category of living beings, like many soil microorganisms, will be damaged and destroyed by chemical agriculture before they are even identified. In conclusion, the application of the herbicide glyphosate alters the equilibrium of the rhizosphere by encouraging the development of pathogens and reducing the growth of the plants it is supposed to protect.

CANCER

The word cancer has been attributed to Hippocrates (460-370 b.C.) who observed its ramifications and associated them to the shape of the crab (from which the term in Greek originates) and, according to some research carried out since the 1920s, cancer was a disease almost unknown to primitive peoples. [280] The incidence of cancer has been measured since 1880. Although past data are not as comprehensive as more recent data, it still indicates a continuous increase (since the incidence of cancer was first recorded). More than 200 tumour pathologies have been classified. They may occur in any part of the body and are characterized by an abnormal cell growth, unrelated to the normal control mechanisms of the organism. [572]
The process of transformation of a normal cell into a neoplastic one occurs through various stages with the accumulation of genetic, functional, and morphological abnormalities. Proliferation (cell division) is a fundamental physiological process: normally there is a balance between proliferation and programmed cell death (apoptosis). Mutations in the DNA leading to cancer lead to the destruction of these ordered processes, favouring uncontrolled cell division and tumour formation. The transformation process may require more than one mutation in different classes of genes: those that control cell division, cell death and DNA repair processes. The human body is able, through repair processes and the activation of the immune system, to counteract the transformation processes but when this ability fails the cell is transformed through various stages into a tumour cell. Therefore, the activation of genes that promote growth (oncogenes) and the inactivation of genes that inhibit growth (oncosuppressors) are necessary. Carcinogenesis is a long and complex process: rarely a single genetic alteration is sufficient for the development of cancer. Usually a carcinogen acts on the cellular DNA and causes a process of initiation (rapid and irreversible), followed by a phase of promotion of neoplastic growth (slow and irreversible). Other factors must intervene to promote the progression of the disease, which in most cases takes several years.
In Italy, data from the National Institute of Statistics (ISTAT) indicate, for 2016 (the latest data available at the time of writing this paragraph), that there are just over 179,000 deaths

attributable to cancer, among the approximately 600,000 deaths that occurred that year. Cancers are the second leading cause of death (29% of all deaths), after cardiovascular disease (37%). In males, cancers and cardio-circulatory diseases cause approximately the same number of deaths (34%), while in females the weight of cardio-circulatory diseases is more relevant than that of cancers (40% against 25%). In the Italian areas where Cancer Registries are active, the frequency of deaths caused by cancer is on average, every year, about 3.5 deaths per 1,000 men and about 2.5 deaths per 1,000 women (overall about 3 deaths per 1,000 people). In Italy at least 485 people die of cancer every day. [572, 573] data concerning the areas covered by the Tumour Registries indicate that the first cause of cancer death in the population is lung cancer (12%): the first cause of cancer death among men is lung cancer (27%), while among women it is breast cancer (17%).

THE SPREAD OF CANCER IN EUROPE AND ITALY

To understand the seriousness of the phenomenon, it is useful to have some information on the frequency of registration of different types of tumours. It should be emphasized that it is the order of magnitude that is the information to be grasped and not the exact number.

In 2014, in Europe (EU-28), 1.3 million people died from cancer, more than a quarter (26.4%) of the total number of all deaths. Cancer had a slightly higher share (29.7%) of deaths among men than among women (23.2%). [305] The share of cancer deaths, in the total number of deaths, exceeded 30% in Denmark, Ireland, the Netherlands, and Slovenia (in Slovenia, among men, this share reached 36.1%).

In Italy, the number of deaths due to cancer out of the total number of deaths is 28.3%, with a prevalence of men: 32.9% against 24% in women.

In Europe (EU-28) the standardized mortality rate for cancer was 261.5 deaths per 100,000 inhabitants, i.e. lower than the rate for circulatory diseases, but higher than the rate for most other causes of death. Industrialized countries may record more than 300 deaths from cancer per 100,000 inhabitants (e.g. France, Australia, Canada): in Italy 246.6. [280, 500] According to the information collected by the National Cancer Registry, in Italy, the probability of being diagnosed with cancer during one's lifetime (0-84 years) is one in two men and one in three women (currently the life expectancy at birth is 84.6 years for girls and 80.1 for boys). [572] For Italians the probability of dying of cancer during their lifetime is 1 in 3 for men and 1 in 6 for women.

In 2019, in Italy, 371,000 new cases of cancer were diagnosed and, in 2016, 179,000 people died of cancer. [572] In detail, considering the entire population and excluding skin carcinomas, the most frequently registered cancer sites were: breast (14%), colorectal (13%), lung (11%), prostate (10%), and bladder (8%). Excluding skin cancer, the five most frequently diagnosed cancers among men were prostate (19%), lung (15%), colorectum (14%), bladder (12%), and stomach (4%); among women: breast (30%), colorectum (12%), lung (12%), thyroid (5%), and uterus (5%). [572]

In Italy, lung cancer is the most frequent cause of death from neoplasia, followed by colorectal, and breast cancer. In Italy, over a lifetime (0-84 years), one in eleven males is likely to have lung cancer, one in nine prostate cancer and one in nine women breast cancer.

Prevalence is the ratio between the number of health events detected in a population at a defined point in time (or over a short period of time) and the number of individuals in the population observed over the same period (https://en.wikipedia.org/wiki/Prevalence; 03/09/2022). There is a distinction between point prevalence and period prevalence, i.e. over a period of 12 months; in the former, the observation (of the number of individuals who are ill

and may develop the disease) refers to a defined point in time (e.g. 31st December of a year). In the latter, it refers to a short period of time, which can also be a lifetime prevalence.
Prevalence does not make it possible to assess the probability of falling ill. The concept of estimating the probability of falling ill is linked to the incidence of the disease. It is necessary to define the new cases of the disease in a certain time interval. In epidemiology, incidence represents the proportion of individuals who are affected by the disease in a given time period. Incidence can be seen as a way of measuring the speed of transition from the state of health (absence of disease) to the state of disease in a population. Incidence represents the variation of one quantity (new illnesses) with respect to the variation of another quantity (time); it is therefore a dynamic measure and is a true rate because at least two surveys are needed.
In Italy, in 2019, regarding prevalence there are about 3,460,000 people alive with a previous diagnosis of cancer: 30% of prevalent men had a previous diagnosis of prostate cancer and 44% of prevalent women had a previous diagnosis of breast cancer. In 2019, in Italy, there were 60,391,000 residents of which 55,175,000 were Italian citizens: about 6% were diagnosed with cancer. [573]
In Italy, the 5-year survival from cancer diagnosis has increased, compared with that of cases diagnosed in previous five-year periods, for both men (54% in 2005-2009, compared to 51% in 2000-2004, 46% in 1995-1999, and 39% in 1990-1994) and women (63% survive compared to 60%, 58%, and 55% for the same years). [572] In Italy, the 5-year survival after cancer diagnosis is lower than in the United States and Australia. [572]
In Italy, according to an official report, updated in 2014 and published in 2019, the incidence of cancer is decreasing in both men and women. In particular among men, the incidence of all cancers has shown a significant decrease (-0.9% per year) in the period 2003-2014. [572] Some cancers, however, are increasing, such as breast and lung cancer in women, testicular cancer, brain cancer, lymphomas, and tumours in children and adolescents. [500, 572]
In Europe (EU-28), in 2015, about 7.3 million patients admitted to hospitals were treated for neoplasms (2014 data for Belgium, no data for Greece or the Netherlands). [305] The highest discharge rate recorded for all tumours was in Austria: after diagnosis or treatment of malignancies, 2,900 patients per 100,000 inhabitants were discharged on an inpatient basis. In Germany, Hungary, Bulgaria, and Estonia (2014 data), this rate exceeded 2,000 per 100,000 inhabitants. Italy had a rate of about 1,500 patients per 100,000 inhabitants. Thus, each year, between 1.7% (in Italy) and 3% (in Austria) of the population registers a cancer. In Italy about 16% of new cancer patients die (246/1,500). Unfortunately, some types of cancer increase with frequencies much higher than 2-3%, that is the average value reported. For example, in France the incidence of prostate cancer increased by 6.3% from 1980 to 2005, and that of thyroid cancer by 6%. [280]
Worldwide, the incidence (standardized rate) of cancer in children (0-14 years) has increased from 124 cases per million children in 1980, to over 140 in 2010. [500] Cancer is the leading cause of death in children worldwide. [521] The most frequent cancers in children (between 0 and 14 years) are leukemia, brain tumours, and lymphomas, those in boys, between 15 and 19 years, are lymphomas, epithelial tumours, and melanomas. [521]
In the USA, cancer (leukaemia and brain tumours) is the second leading cause of death in children between the ages of one and fourteen (the first being accidental death). [280] Studies show that the exposure of mothers to certain substances increases the likelihood of cancer in their children. Mothers' occupational exposure to insecticides and herbicides increases the likelihood of their children developing leukaemia. [328]
In Italy, in the young, the most frequent tumour is represented by testicular cancer, while in the 50 - 69 year old class and in the over-70s the most frequent tumour is that of the prostate. [572] In the first decades of life, the frequency of tumours is in fact very low, equal to a few tens of cases per 100,000 children per year; after 35 years of age there are more than a hundred cases,

while after 60 years of age there is a sharp increase in incidence, reaching over a thousand cases per 100,000 people/year. [572]
Some areas of Italy (the registers of Umbria, Romagna, Modena, and Parma) report an incidence of cancer in children (0-14 years) of 200 cases per million children each year, compared to a national average of 140 in 2010. [531] The survival for paediatric tumours has increased a lot in the last decades, but it is also true that in the course of life survivors have complications in two thirds of cases (serious in about 25% of them; the incidence of complications increases with age), and hospitalization rates, calculated up to 30 years after diagnosis, higher than the general population.
Progress has had the side-effect of increasing the frequency of some diseases, although in some cases this increase is related to factors such as increased life expectancy, improved diagnostics or genetic predisposition. Regarding the genetic origin, a study, which examined 44,788 pairs of twins in Northern Europe to assess the risks for 28 types of cancer, concludes that environmental causes are more important than genetic ones. [306] Compelling evidence of anthropogenic causes also includes the increase in recorded diseases among workers since the beginning of the industrial revolution era. One recalls scrotal cancer recorded in the late 1700s among chimney sweeps because they were in contact with soot. In 1830 a terrible disease was recorded among workers in match factories (phosphorus industry). [280] A study of more than 200,000 twins, included in a Scandinavian registry, estimated that the hereditary component of cancer is 33%, with enormous variability between different types of cancer. [572] Parental exposure, occupational or domestic, increases the likelihood of registering cancers (e.g. leukaemia) in the offspring. [509, 515]
The resources put into combating this health crisis are based on investment in diagnosis and treatment (pharmaceutical, surgical and radiotherapy) rather than on reducing causes such as pollution and lifestyles. In order to achieve strong reductions in the registration of these diseases, it is necessary to act on prevention. However, investment in diagnosis and treatment, for some cancers, has increased the chance of survival.

TOBACCO SMOKING AND LIFESTYLES

In 2017, worldwide, tobacco consumption was probably responsible for 14.5% of total recorded deaths (1.2 milion deaths attributable to passive smoke out of 8.1 million deaths) and thirty-eight million years lived with a disability. [574] It is estimated that there are about 1.3 billion smokers in the World and, in Italy, probably one person out of four smokes: the average daily consumption in Italy is about 11 cigarettes, but almost 2 smokers out of 10 consume more than a packet a day. [572, 575, 576, 985]
In 2015, in Italy, at least 16% of cigarettes were traded illegally, while in Europe this trade accounted for approximately 10% of total cigarette consumption (smuggling and counterfeiting; Italy is the leading European tobacco producer). [987]
According to the World Health Organization, tobacco is capable of killing up to half of its users: at least eight million people a year, which is more than three times the number of Covid-19 victims registered in 2020. Smoking is the leading cause of easily avoidable death across the Planet. A heavy smoker, on average, has a life expectancy 10 years less than if he or she did not smoke.
At least 7,000 different chemicals have been identified in cigarette smoke (arsenic, cadmium, lead, pyridine, hydrogen cyanide, nicotine, ammonia, etc.), hundreds of which are very dangerous and at least 70 of which are carcinogenic (e.g. vinyl chloride, formaldehyde, polonium-210, nitrosamines, polycyclic aromatic hydrocarbons such as benzo-a-pyrene). The

exposure to cigarette smoke can be measured by determining the concentrations of toxic substances in urine.
There are several hundred additives intentionally added during the manufacturing of cigarettes with the intention of promoting consumption and addiction (sugars, flavorings such as licorice, ammonia, bronchodilators, analgesics, substances that control the speed of combustion, etc.). Quitting smoking is very difficult: 70% of smokers say they want to stop, 40% try to stop at least once a year, 80% of those who have tried to stop smoking start again within 30 days of their last cigarette; in conclusion, less than 3% manage to reach the goal of stop smoking. [1287]
Two-thirds of all current smokers started smoking at the age of 20, and 89% of new smokers develop an addiction before the age of 25. This is a problem because the success of the fight against smoking depends largely on keeping young people away from traditional cigarettes and all alternative products. It is very likely that people who do not smoke before the age of 25 will remain non-smokers throughout their lives. [1288] Trying to give up smoking in centres that provide medical and psychological support and anti-smoking therapies can triple the probability of success.

Growing, producing and marketing tobacco is a huge waste of resources. Cultivation uses pesticides, fertilizers, and contributes to the reduction of biodiversity: cultivation occupies at least 4 million hectares in 124 countries. The greatest tobacco growers are, in descending order: China, India, Brazil, and United States; Italy is the first tobacco grower in Europe (at least 17,000 hectares in 2019, mainly in Campania, Veneto, Umbria, and Tuscany). According to the United Nations, the smoking industry employs at least 100 million workers, a large proportion of whom cultivate tobacco in countries where malnutrition and hunger are a serious problem. Another negative aspect is child labour, which is widespread in tobacco cultivation and is also denounced in Italy together with the exploitation of migrants. [1287]

About 3.7 litres of water are used to produce one cigarette and to compensate for the emissions produced by one smoker in one year, it is necessary to plant more than 130 trees and let them grow for at least 10 years. [1287] Up to a third of the waste on beaches consists of cigarette butts, which are not biodegradable.

In the USA, tobacco smoking is responsible for 33% of neoplasms; another 33% is linked to the so-called lifestyles: diet, overweight, alcohol abuse, and physical inactivity. [572] In the USA, occupational factors are responsible for 5% of tumours and infections for 8% (*Papilloma virus*, Epstein-Barr virus, hepatitis B and C viruses, etc.). In the UK, tobacco is responsible for 19% of cancers and diet for another 19%. [572]
In Italy, it is estimated that in 2017, 14.5% of all deaths were attributable to tobacco use (amounting to 90,000 deaths, of which 7,000 were attributable to passive smoking) and were responsible for 552,000 years lived with disability. [574]

In the USA, it is estimated that, in 2014, 42% of all cancers reported in adults over 30 of age were attributable to seven risk factors:

- active and passive cigarette smoke,
- excess body weight,
- alcohol consumption,
- diet,
- ultraviolet radiation,
- viral infections.

It should be emphasized that, according to official statistics, between one third and 50% of tumours in Italy could be prevented by acting on lifestyles: smoking, sedentary lifestyle, exposure to ultraviolet rays (including tanning lamps that promote melanomas), alcohol, and diet. By acting on culture, knowledge, and consequently preventing and modifying bad habits, we could hope to reduce up to 50% of all cancers recorded. The failure in this challenge confirms the inability to defend the society from itself and in particular from behaviours promoted by a few big companies such as those of tobacco, pesticides, and some food industries.

In our daily experience we can easily perceive paradoxical social behaviours, i.e. devoid of logic, as they cause damage to our health, to the environment, and to future generations. Actions of this kind are easily detected by anyone, both in the experience of daily life and in collective actions. Starting from afar with this discourse, it is possible to note that some of the major sources of health expenditure, in Italy and in Europe, are the result of individual actions. These actions are favoured by an ailing system, which could be easily cured, generating an economic gain (and not only) for the whole society. It is very evident, on many issues, that society and individuals, know the problem, perceive it and are able to identify it effectively, but the actions generated to contain it are completely insignificant and contradictory. There is a sort of divergence between the ability of science and technology to provide information, knowledge and innovation, and the ability of society to use this wealth for the best applications. Artificial rules, such as economic and financial ones, are privileged over those that nature teaches us or science suggests. For example, data from the World Health Organization (WHO) report that 86% of deaths and 75% of health spending in Europe and Italy are caused by chronic diseases favoured and aggravated by four main risk factors: [888]

- smoke,
- alcohol abuse,
- improper nutrition,
- physical inactivity.

In the World, cardiovascular diseases, tumours, respiratory diseases and diabetes, according to WHO data, are responsible for most deaths, suffering and health costs. These are chronic diseases that have in common some risk factors (as just written: smoking, alcohol abuse, improper diet, sedentariness) linked, for the most part, to unhealthy behaviours.

In the World today, more people die from overweight and obesity than from malnutrition. Obesity is favoured by bad eating habits and an unhealthy lifestyle. Nutrition and lifestyle education, already among young people, is one of the best preventive measures. Health education is the most cost-effective strategy to implement in order to counter the supply of models that encourage obesity (foods with high calorie content and low nutritional value, sometimes dangerous due to the chemical technology used: flavourings, colourings, preservatives, and thousands of other possible authorized substances). The lack of regulation and information encourages the abuse of poor quality foods and indirectly incentivises health care costs such as pharmaceuticals and medical treatment.

It would be appropriate to promote food education campaigns in schools with the aim of:

- reducing consumption of carbonated, sugary, and nervine drinks;
- reducing consumption of fried and high-calorie foods (pre-packaged confectionery products);
- encouraging the consumption of fruit and low-processed foods;
- reducing meat consumption (e.g. zero consumption of animal by-products one day a week);
- promoting local food products;
- reducing the use of packaging;
- encouraging a less sedentary, more outdoor life;
- improving the ability to understand the messages written on food labels;
- ensuring a greater knowledge of the negative impacts generated by food production (e.g. virtual water, use of pesticides);

- improving knowledge about the methods used to produce and process food (everyone should have visited intensive livestock farms and slaughterhouses at least once in their lives: this first-hand knowledge would probably encourage a decrease in the consumption of food of animal origin).

Cultivating knowledge about nutrition, food production and, in general, health education may significantly reduce environmental impacts but also health problems (e.g. obesity and overweight). With an eco-information we make visible what surrounds us but it remains invisible as long as we continue to ignore it.

Most of the behaviours that favour avoidable diseases are easily modifiable in a positive sense, but also in a negative sense. Often the economic, social and environmental context in which people live and work provides incentives for negative behaviour, as those that encourage the development of harmful habits. Some sectors of our society benefit from the existence of these problems, and a small part of the community gains richly from them. The majority are harmed, but the collective actions of self-defence that should result are totally inadequate. The inconsistency and inadequacy of the strategies that could be planned and implemented to reduce the problems generated by just four factors are very evident.

Combating smoking or overweight in young people certainly produces more economic benefits than inaction. Yet we do not act as we should and could. This is one of the paradoxes of our time. The private interests of a few override the principle of safeguarding the community. The economic rules impose to privatize the advantages and to distribute on the whole community the problems and the resulting costs, in a blind, unsustainable and ethically incorrect way.

PESTICIDES AND CANCER

Several epidemiological studies that have attempted to demonstrate the association of 43 diseases, which can be grouped into six major groups, with pesticide exposure have recorded various correlations. In particular, associations between pesticide exposure and diseases have been recorded for: carcinogenicity, neurotoxicity, reproductive problems, metabolic problems, lung toxicity, and developmental problems. [491] Cancers and neurotoxic effects are among the most studied diseases associated with pesticide exposure. The cancers for which the evidence is most significant are those of the brain (chlorpyrifos, bufenacarb, paraquat, coumaphos, metribuzin), prostate, breast, intestine (colorectal), pancreas, and lung. Insecticides are the class of pesticides most frequently associated with cancer.

Carcinogenic effects have been reported for several molecules in laboratory animal tests, such as some organochlorinated insecticides (aldrin, chlordane, DDT, dieldrin) and some solvents used for the preparation of formulations. Molecules belonging to different groups [phenoxyacetic acid (herbicides), organochlorines, organophosphates] have been associated with non-Hodgkin's lymphomas. [1254] Some leukaemias and other cancers are more frequently reported among pesticide users. [1254]

Studies, already in the 1990s, showed that at least 18% of insecticides and most fungicides used in the USA are potentially carcinogenic. [502] Pesticides can promote cancer in several ways: they can damage DNA (e.g. fragmentation, chromosomal alterations, mutations) or they can reduce the ability to repair DNA damage, they can generate mitochondrial dysfunction, they can alter gene expression or phenotype without changing the DNA sequence (epigenetic alterations). [487] Small doses of pesticides in the long term promote lesions in the genetic code (DNA) contributing to the onset of cancer (lung, prostate, and hematopoietic system) or diseases of the nervous system. [487] The exposure to pesticides may significantly increase the incidence of the following cancers: adult and child brain tumours, oesophageal, stomach, liver, bone, prostate, breast, ovarian, lung, thyroid, and cervical cancers. [491, 500, 528]

Some active substances probably implicated in the increase of cancer incidence are: aldrin, chlordane, heptachlor, lindane, cyanazine (banned or not approved by the European Union), mancozeb, glyphosate, pyrethroids, chlorpyrifos. [500, 507] For some molecules such as glyphosate (herbicide) and chlorpyrifos (an organophosphate insecticide) there is a well-founded suspicion that they may promote cancer by generating dysfunctions in the mitochondria. [477] Oxidative stress is among the problems resulting from mitochondria malfunction.

In agricultural production, operators who distribute pesticides may be exposed for more than 10 years but also for 30 years to high doses and are therefore sentinels of possible risks. The failure to record diseases in this population group constitutes a loss of information that is essential to improve risk management and prevention.

The occupational exposure of applicators or workers in the manufacturing industries of some pesticides (aldrin, azinphos, carbaryl, chlordecone, coumaphos, DDT, diazinon, dichlone, phonophos, lindane, malathion, maneb, methyl bromide, simazine, terbufos, triazine herbicides, ziram) has been associated with an increased likelihood of registering prostate cancer. [491] Malathion and diazinon are classified by IARC as probable carcinogens (category 2A like glyphosate). [507] Female farmers who have used organophosphates are more likely to develop breast cancer. The concentration of organochlorinated insecticides (e.g. DDT and its metabolites such as DDE) in blood and adipose tissue has also been positively correlated with a significant increase in breast cancer, which is the most important malignant tumour in women. [491]

The occupational use of some pesticides (acetochlor, terbufos, dicamba, metolachlor, diazinon, chlorpyrifos, pendimethalin) has been related to lung cancer. The occupational use of alachlor, malathion, and atrazine has been associated with thyroid cancer. The use of acetochlor, maneb, parathion, and carbaryl has been correlated with skin cancer. [491]

The exposure to pesticides by farmers has been associated with an increased probability of registering some cancers as in the case of the following molecules: alachlor, aldicarb, carbaryl, chlorpyrifos, diazinon, dicamba, S-ethyl-N,N-dipropylthiocarbamate, imazethapyr, metolachlor, pendimethalin, permethrin, trifluralin. [1156] In some cases the epidemiological evidence is not conclusive but toxicological studies in animals confirm probable risks of increased tumour incidence for the molecules: alachlor, carbaryl, metolachlor, pendimethalin, permethrin, and trifluralin. Some cancers are suspected to be associated with the exposure to particular molecules: [1156]

- lung cancer for chlorpyrifos, diazinon, dicamba, dieldrin, metolachlor, and pendimethalin;
- colon cancer for aldicarb, dicamba, and trifluralin;
- rectal cancer for chlorpyrifos, chlordane, pendimethalin, and toxaphene;
- leukaemias for chlorpyrifos, diazinon, and phonophos;
- melanoma for carbaryl and toxaphene;
- brain tumour for chlorpyrifos;
- prostate cancer for phonofos and methylbromide.

Of the 32 pesticides analyzed in 28 epidemiological studies, 12 were associated with an increased risk for at least one type of cancer.

The term follicular lymphoma includes a group of tumour forms (with non-Hodgkin's characteristics) of mature B lymphocytes. [549] These tumours result from genetic mutations or a translocation between chromosomes 14 and 18. The occupational exposure to pesticides increases the probability of registering these tumours. The translocation between chromosome 14 and chromosome 18 that is related to follicular lymphomas has been recorded in French farmers. [520] Despite the problems involved, translocations, like all mutations, play an important role in evolution. There are two main types of translocations: intra-chromosomal, that is, within the same chromosome (we have 23 pairs, half from the father and half from the mother), or inter-chromosomal, that is, between two different chromosomes (one person in five hundred may be a carrier of a translocation). [547] Chromosomes seem to break and reunite quite often during sperm and egg formation, and in sometimes some problems ensue. These mutations occur in most cases without being able to prevent them. [548] A known translocation is the one that affects chromosome 21 and generates a high probability of registering the Down syndrome. Other disorders associated with translocations are cancer and both male and female infertility. [547]

Living near cultivated fields (less than 1 km away) where pesticides are used increases the probability of registering tumours in children. This is what has been found in Spain, where childhood cancers are the leading cause of death from disease in children (between 1 and 14 years). [1157] The most important cancers in children associated with living near treated fields are leukaemia, followed by central nervous system tumours and lymphomas.

Several data confirm that living near agricultural areas where pesticides are used increases the probability of registering certain types of cancer in children under 14 years of age. [1164] In this study, differences in the frequencies of some cancers were measured that were found to be influenced by living near soybean rather than oat fields or combinations of different crops.

Studies show that the fathers' pre-conception exposure to pesticides increases the likelihood of recording brain tumours in their children. [491, 500] Mothers' exposure to pesticides increases the likelihood of their children developing leukemia. [491] The occupational exposure to some pesticides (dichlorvos, famphur, methoxychlor, diazinon, and pyrethroids) has also been associated with increased leukemia in adults.

The exposure of women during pregnancy to the organochlorine insecticide DDT exposes the fetus to this carcinogenic molecule. The in utero exposure to DDT increases the likelihood of breast cancer for daughters after 50 years. [410] This study is the result of 53 years of work involving 9,300 daughters of mothers exposed during the 1960s (and 354 unexposed daughters used as controls). DDT is an endocrine disruptor, and women's exposure to it during fetal life predicts a four-fold increase in breast cancer risk. There is a window of time during fetal life when the female body is sensitive to exposure to endocrine disruptors (DDT alters the estrogen function) and the consequences are measured decades later (breast cancer). Prenatal exposure to medium or high levels of DDT has also been associated with an increase in the onset of hypertension in women after the age of 50. [508] In this regard, it may be recalled that in the USA and South Africa, more than 20% of the population suffers from hypertension, while in Germany and Spain more than 50%. [508]

Glyphosate has been shown to be genotoxic to human and other mammalian cells. [492] Another herbicide, atrazine, induces breast and prostate cancer in animals (in the laboratory). [309] The herbicide glyphosate induces apoptosis (programmed cell death) and necrosis, and is a likely promoter of non-Hodgkin's lymphomas. [500, 523, 526] Glyphosate inhibits the proliferation and differentiation of human cells (e.g. into adipocytes). [526] Human cells exposed to the glyphosate-based formulation (all components of the mixture), at doses lower than those allowed as residue in food and feed, are killed in 24 hours: the mixture of the formulation stimulates apoptosis of the treated cells. [523] The interesting thing demonstrated by this work is that cellular damage is not proportional to the concentration of glyphosate but depends on the adjuvants. That is, cellular toxicity is generated to a greater extent by the other components of the mixture, other than the active ingredient. [523] Severe cellular damage could be generated in the presence of glyphosate residues considered acceptable in food and could be favoured by molecules considered inert or harmless.

Apoptosis is programmed cell death: the acaricide amitraz, which is also used by beekeepers, can induce cell apoptosis. [482, 550] In contrast to necrosis, which is a form of cell death resulting from acute stress or cell trauma, apoptosis is carried out in an orderly manner and regulated by genes, it requires energy consumption and during the life cycle of the organism it leads to an advantage; for this reason it is sometimes referred to as altruistic death. To give an example, during its development, the human embryo presents the growth of "palmate" hands and feet: for the fingers to differentiate it is necessary that the cells that constitute the interdigital membranes die. [550]

Apoptosis may also occur when a cell is damaged beyond its ability to repair itself or because it is infected by a virus. Defective processes of apoptosis affect many diseases. An excessive programmed death activity may cause cell loss disorders (e.g. in some neurodegenerative diseases), while deficient apoptosis may involve uncontrolled cell growth (mechanisms underlying neoplasms). Understanding the process by which a cell kills itself could make it possible to prolong its life or hasten its death, a coveted goal for the treatment of several human diseases.

The information available to us, although partial and incomplete, is sufficient to sound the alarm. For this reason, it is necessary to limit the use of molecules for which the harmful action, such as carcinogenicity in laboratory animals, has been ascertained, even when there is no confirmed evidence in human beings (e.g. evident carcinogenicity in animals for parathion and malathion). [507] The effects of occupational exposures are also an important indicator that should lead to the application of the precautionary principle. In conclusion, many studies show a correlation between the exposure and an increase in the probability of registering certain types of cancer. The application of the precautionary principle could avoid unsustainable costs and health problems.

THE CANCER CENSUS

The various systems for monitoring the causes of cancer suffer from the inability to know in advance the triggers to which one was exposed years earlier. It is therefore very difficult to identify the chemicals responsible. This is also why official reports attribute a very small fraction of all recorded cancers to environmental pollution: 2% in the USA in 2012. [572]

Another indication of insufficient information is the official classification of carcinogenic substances by international organisations. Of the tens of thousands of potentially carcinogenic molecules to which we are exposed throughout our lives, only 120 have been identified by the International Agency on Cancer as definitely carcinogenic to humans (although for thousands of substances there is at least a suspicion validated by scientific research and/or epidemiological information). This Agency was founded in 1965 so it has been able to identify 120 carcinogens in over 50 years, just over two substances per year.

The incompleteness of the information at our disposal is also highlighted by the fact that only in some geographical areas the registration of cancer occurs with a certain efficiency. For example, the network of Italian Cancer Registries (AIRTUM or Italian Association of Cancer Registries) covers about 70% of the Italian population, therefore almost one third is excluded. [577]

Preventive activities, in the different geographical areas, are carried out with different intensity and energy: in Italy there is a gap between the North and the South, since in the North systematic diagnoses are more active. [572] To give another example on the incompleteness of the information available to us, we report the case of mesothelioma resulting from the exposure to asbestos: there are no ISTAT data available on deaths from malignant mesothelioma in Italy (2019), although it is believed to be responsible for 4% of oncological deaths (in Italy there are about 5,615 people with a previous diagnosis of mesothelioma). [572] In Italy, 6,000 asbestos-related deaths were recorded in 2017, but due to long latency times, deaths are likely to peak around 2030. [582] While in America, according to the Association of Diseases Caused by

Asbestos, thousands people die each year from diseases attributable to this mineral. History does not teach and unfortunately repeats itself: many countries still use asbestos.
Epidemiological information on the risks arising from the occupational exposure to particular substances (e.g. farmers or beekeepers) and on the possible association between cause and effect is often insufficient and fragmentary. The cocktail effect unfortunately also exists for carcinogenic molecules, and additive and synergistic effects are difficult to investigate.
In the analysis of cancer incidence, the estimation of less serious cases is often absent and they escape the filing and cataloguing system. For some important cancers (prostate, kidney, thyroid, melanoma, lymphomas) few risk factors are identified. Other weaknesses are the differences in the ability to diagnose and register a tumour in different territories, which translate into the inability to determine the causes of death for many cases. The trend in general mortality, even in cases where the causes are not known, should however be a wake-up call.
In the latest available report (at the time of writing this paragraph) on cancer incidence in Italy (2019), the words pesticides, insecticides, fungicides or neonicotinoids do not exist among the risk factors for cancer. [572] Herbicides are mentioned among the risk factors for soft tissue sarcomas, which are rare cancers as they register an incidence of 5 cases per 100,000 inhabitants per year and do not represent more than 1% of cancers (they are herbicides with a phenoxy-acetic or chloro-phenol structure together with their contaminants such as dioxins). Being a farmer and handling chemicals is mentioned as an occupational risk for one type of melanoma: this is not sufficient.
Among the risk factors, even air pollution, which is recognized to have carcinogenic effects, is mentioned only for lung cancer. Yet while in industrialized countries there are about 3,000 cancer deaths per year per million inhabitants due to air pollution, in Italy deaths due to air pollution are estimated at about 1,500 per million inhabitants: a half. [196, 572] A systematic study conducted in 188 countries, covering the period 1990-2013, reports that air pollution generates a number of deaths equivalent to those generated by cigarette smoking, as well as an equivalent number of years lived with disabilities (it is estimated that there are over one billion smokers in the World and, in Italy, probably one person in four smokes). [572, 575, 576]
The concept of environmental pollution as a cause of cancer diseases is mentioned in the paragraph dedicated to the *environmental causes of cancers*: less than 4 of the total 388 pages are superficially dedicated to it (AIRTum Data Bank, 2019). [572] Yet in the document, the increased risk of lung cancer occurrence is recognized for increases in the atmospheric concentration of particulate matter (by 22% for every 10 $\mu g/m^3$ of PM_{10}, and 18% for increases of every 10 $\mu g/m^3$ of $PM_{2.5}$, which are the finest dusts: 2.5 thousandths of a millimeter in diameter). Among the risk factors for some cancers (e.g. breast, uterus, and prostate) a role of hormones (estrogens and androgens) is mentioned and endocrine-derived cancers are discussed (testicle, ovary, breast, thyroid, prostate), but no reference is made to endocrine disruptors (pesticides, bisphenol and hundreds of other substances); endocrine disruptors are mentioned as a possible cause of the increase in cancers in the population living near contaminated sites to be remediated. Much more attention should be paid to health problems caused by pollution such as chemical pollution (of water and food) or atmospheric pollution, and to occupational hazards.

MULTIPLE CONTAMINATIONS IN EARLY STAGES OF LIFE

Pesticides kill and sicken humans in synergy with hundreds of other xenobiotics: hundreds of toxic substances produced by human activities may be found in our bodies, for example in blood and urine, including pesticides. [311] A study in Europe looked for 101 dangerous chemicals in urine and blood and found 76 of them, with an average of 37 different products per sample. [310] These substances can also be found in umbilical cords, indicating an

exposure early in life and therefore much more dangerous. [312] Metabolites of very hazardous pesticides, such as diazinon and chlorpyrifos, may be found in 95% and 100%, respectively, of samples of first faeces produced by infants (meconium): these are neurotoxic substances. [313]
Up to 10 different pesticides may be found in the umbilical cord at the same time as the 20 molecules sought (in Chinese infants from rural areas). The presence of the active molecules in the umbilical cord has been associated with a reduced birth weight (vinclozolin, a fungicide, and acetochlor, an herbicide). [1256] Exposure to organophosphates also showed the same effect in newborns. These studies demonstrate the existence of hazardous fetal exposure.
The search for toxic molecules in the umbilical cords of 230 newborns and in the blood of their mothers living in New York produced the following results: 22 of the 29 pesticides searched for were found including the organophosphate insecticides chlorpyrifos and diazinon (in the USA, the EPA estimated that 75% of diazinon and 50% of chlorpyrifos were used in urban settings), the carbamates bendiocarb and 2-isopropoxyphenol (a metabolite of propoxur), the fungicides dichloran, phthalimide (a metabolite of folpet and captan) and tetrahydrophthalimide (a metabolite of captan and captafol). Neonicotinoid insecticides (acetamiprid, clothianidin, dinotefuran, flonicamid, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam) are found in blood and urine.
The exposure to multiple toxic and persistent substances undoubtedly generates synergistic and additive effects, and dangerous interactions, especially if the exposure occurs early in life as indicated by the discovery in the umbilical cord of traces of over 200 dangerous substances including pesticides. [835]

SYNERGISTIC EFFECTS

We are potentially exposed to more than 129 million organic and inorganic substances, to which at least another 4,000 new ones are added every year. These molecules include at least 6,400 pesticides (comprising at least 700 active molecules authorized in Europe in 2017 and hundreds of additives added to improve the action of the active molecules). [443, 446] The number of chemicals derived from human activities is increasing exponentially: 15% per year in the last 10 years. [443] At least 150 million tons of chemicals are produced in Europe per year: many of these molecules alter functions important for living beings at doses in the order of thousandths of a gram or smaller. For the vast majority of these molecules we do not have sufficient information on their safety.
Exposure to mixtures of harmful compounds such as pesticides and tens of thousands of other molecules undoubtedly produces adverse effects, some of which are amplified by the concurrent lifetime exposure. Several studies confirm this hypothesis: two herbicides (procymidone and vinclozolin) and a medicine prescribed to treat prostate cancer (flutamide), which individually have no effects as endocrine disruptors, together enter into synergy and, by interfering with the function of the male hormone testosterone, they generate alarming effects in laboratory animals. [323] That is, very small doses, at which individually there are no effects, generate dangerous changes when combined. This research demonstrates the existence of additive and/or multiplicative effects. Synergistic effects (additive and/or multiplicative) in the immune system, nervous system, and endocrine system are recorded in laboratory animals ingesting water containing aldicarb, atrazine, and nitrates at concentrations permitted by the legislation. [324] The simultaneous ingestion of these molecules generates effects that would not be recorded otherwise.

In the USA, the Centers for Disease Control and Prevention (CDC), in a 2004 report summarized very alarming results. [321] This document presents the results of the search for toxic substances in the bodies of 9,282 people. A total of 116 substances were found in the blood and urine, including 34 pesticides. A metabolite of the insecticide chlorpyrifos was found in 93% of the tested samples and the metabolite of DDT in 99% of the people [high levels of the DDT metabolite (p,p-DDE) in mothers have been associated with lower birth weight babies and lactation problems]. 18 pesticides were found in at least 50% of samples (including 2,4-D) and 100% of samples (blood and/or urine) contained at least three pesticides. Children between 6 and 11 years of age were exposed to doses higher than those considered acceptable for chlorpyrifos and methyl-parathion. [321] In 2004, when these results were published, there were 1,200 active molecules registered in the USA, marketed in more than 16,000 commercial products and formulations.

Sirens have been sounding for over 20 years: data published in 2001 confirmed the presence of pesticides in the urine of 102 children, aged between 3 and 13 years. [318] Carbamates (and related compounds), atrazine, malathion, chlorpyrifos (and related compounds) were present in 93% of the urine samples.

A study that tested 214 substances found 102 of them in blood and urine, including polychlorinated biphenyls, dioxins, pesticides, mercury, and arsenic compounds. [874]

Research carried out in France and published in 2010 revealed a very worrying fact: children ingest at least 81 harmful molecules through food in a single day, including 36 pesticides (of which 18 are classified as possible carcinogens and 14 as endocrine disruptors). [322] 47 of the harmful substances ingested daily are suspected of being carcinogenic and 37 are suspected of being endocrine disruptors (in detail 28 substances are classified as probable carcinogens). It is not surprising that one in two men and one in three women in France will develop cancer in their lifetime (as in Italy).

As already mentioned, interactions between different pesticides and other molecules in formulations can be additive or multiplicative. [497] Studying and estimating these effects is complicated and, above all, very expensive. In some cases, fortunately, useful information is available. For example, the exposure to organophosphate insecticides may enhance the simultaneous exposure to pyrethroid insecticides and herbicides (triazines). [497] Synergism between pyrethroid insecticides and carbamates is also known. The oestrogenic effects generated by the simultaneous exposure to organophosphorus and organochlorine insecticides are cumulative. In addition, interactions between pesticides and other xenobiotic substances must be considered. We are certainly exposed to dangerous mixtures as the monitoring conducted in Italy recorded pesticides in 75% of 629 samples of fruit and 47% of 467 samples of vegetables. Among the pesticides found most frequently (among those searched) in vegetables, in decreasing order, we highlight: boscalid, propamocarb, chlorantraniliprole, difenoconazole, azoxystrobin, cyhalothrin, lambda, fluopyram, thiamethoxam, cyprodinil, dimethomorph, chlorpropham, imidacloprid, pyraclostrobin, and acetamiprid. [496] In fruit, the following pesticides were found most frequently and in a decreasing order: boscalid, etofenprox, captan, chlorantraniliprole, tebuconazole, acetamiprid, fluopyram, pyraclostrobin, fludioxonil, indoxacarb, imazalil, thiacloprid, spinosyn a, chlorpyrifos, triflumuron. 24% of vegetable samples and 58% of fruit samples from traditional agriculture recorded several residues at the same time (even more than 5). [496] The fruit and vegetable types in which more than 50% of the samples recorded residues were apples, pears, oranges, strawberries, grapes, peaches, carrots, potatoes, and spinach. Pesticide residues were also found in 5% of 40 samples of organic vegetables and 2% of 48 samples of organic fruit. [496] Despite the fact that it is impossible to search for all molecules that are used in agriculture (and their derivatives) and those that could contaminate food due to other pollutions, these studies highlight the presence of dangerous exposures. Food monitoring data cannot provide a measure of the severity of the problem. In the USA, to take another example, food monitoring typically looked for fewer than 40 active molecules among the more than 600 approved. [502] Laboratory analytical research is expensive, it is impossible to search for all possible pollutants and their derivatives, analytical methods are not always able to measure very small concentrations but are still capable of triggering adverse effects, many hazardous substances are unknown or very difficult to find. Therefore, it would be necessary to encourage the collection of upstream information such as the quantities produced, marketed, used, and distributed in the environment in each territory.

ACCIDENTS

There are at least 26 million cases of pesticide poisoning per year, including at least 300,000 in the USA (this is an optimistic estimate). [1243] The World Health Organization records at least 280,000 deaths per year from pesticide poisoning such as organophosphates and organochlorinated pesticides, which are the most frequently implicated pesticides among the causes of poisoning. [280, 500] Reducing the use of pesticides may only have beneficial effects.

Among the side effects generated by the production of pesticides, there are also accidents. One may recall the accident at the insecticide factory located in Bhopal, India (in 1984), which in the days immediately following the disaster caused 3,000 victims. In the following years there were between 20,000 and 30,000 deaths, in addition to at least 250,000 injured and intoxicated people; at least 10,000 people were permanently disabled (figures are uncertain). [175, 280, 500]

Worldwide, pesticides are the cause of at least 3 million hospitalized intoxicated people, 750,000 chronically ill people and at least 25 million agricultural workers who experience unintentional poisoning every year (over 250 million people in 10 years). [443, 502] In addition, there are between one million and two million cases of unintentional poisoning each year due to pest control accidents.

Worldwide, in 2004, it is estimated that at least 346,000 individuals died as a result of ingestion, inhalation or contamination through unintentional contact with chemicals, 19% of whom were children (30,000 children died as a result of poisoning that occurred in the workplace); 71% of these deaths from unintentional poisoning were classified as easily preventable. [528] Asbestos was among the incriminating substances, and in 2004 it generated at least 107,000 deaths from various types of cancer such as mesothelioma, whereas lead accounted for 143,000 deaths and caused mental retardation and cardiovascular disease. [528] Overall, in 2004, at least 526,000 people died in the World due to intentional or unintentional poisoning by chemicals. In the same year, 2004, this research estimates the death of at least 1,152,000 people due to air pollution in urban environments and at least 872,000 people due to indoor pollution generated by the domestic use of fire to generate heat and cooking (it must be remembered that at least half of the World's population uses solid fuels, such as wood, indoors). [528] On the whole, it is estimated that about 4.9 million people died in the World in 2004 due to chemical contamination (8.3% of all deaths recorded in that year). These are certainly underestimates, but they give a dimension to some important problems such as those generated by pesticides, asbestos, and lead in children. Many adverse health effects are underestimated or undetected such as those requiring costly surveillance and diagnostic systems (e.g. measurement of behavioural changes, cognitive defects and generally sub-lethal effects such as those on reproduction).

Another dramatic fact is that pesticides are used to commit suicide: at least 186,000 cases were recorded in the World in 2002. [528]

In the 2000s, in the USA, it is estimated that there were at least 300,000 cases of poisoning per year. [502] In the USA, being employed in agriculture is 34 times more likely to record pesticide illnesses (18.2 illnesses per 100,000 agricultural workers) than being employed in another job (0.53 pesticide illnesses per 100,000 workers). [517, 581] In the USA, pesticides are the ninth most common category of substances involved in the registration of health problems by poison control centres and 45% of all pesticide poisonings involve children. [500, 516]

Fortunately, the activities of some organizations signal the existence of a new awareness about the effects of pesticides on human health. Of particular note is the French site *victimes des pesticides* which reports the testimony of hundreds of farmers and citizens who have experienced health problems caused by pesticides. [272] In France, the victims of pesticides have organized themselves in an association. [273] Campaigns are promoted on websites in different territories to collect the signatures needed to abolish the use of pesticides. These are popular movements to oppose and limit the use of pesticides. Testimonies are collected by organizations in other countries as well. [274] Some very sad testimonies about the stories of pesticide victims are collected in documentaries and publications. [280] The spread of greater attention to the negative effects of pesticides is likely to influence policy makers to accelerate processes that should lead to a less and more careful use of thousands of substances toxic to humans and the environment.

SOME LIMITATIONS OF PREVENTIVE TOXICOLOGICAL EVALUATION

Some of the limitations of toxicological risk assessment for human health are as follows:

- Information about the testing carried out by the companies marketing the pesticides is secret, as it is protected by trade rules. [1295]
- It is almost impossible to test before marketing the synergistic and additive effects of the different substances to which we are exposed at the same time and throughout our lives.
- Extrapolating conclusions about health risks from the results of animal experiments is not easy and there is a risk of underestimating the problems.
- Pesticide toxicity is influenced by several factors, including individual genetic characteristics. Some genes (PON1 and Cytochrome P450) express proteins that may increase or decrease certain risks. [1254]
- The toxicological evaluation is carried out on single doses of a single active substance and is related to a 70 kg adult. Children cannot be considered as lighter adults and there is a risk of underestimating effects that are much more pronounced in women, the elderly or the sick. Many pollutants may be found in children (e.g. in urine) at higher concentrations than those measured in mothers. [1255]
- Some effects such as those on behaviour, cognitive deficits, endocrine dysfunction, reproduction and on the immune system are difficult to assess.
- The other components of the commercial pesticide mixture are not considered and metabolites are not evaluated, partly because they may not be known, especially in the preliminary stages. The same applies to degradation products. For example, the adjuvants present in the preparation containing the herbicide glyphosate have been found to be more toxic than the herbicide itself in cell studies. [523, 551] A comparison of the toxicity of 9 active molecules and their commercial formulations (i.e. mixtures of the active molecules with other substances) in three human cell lines (human placenta, liver, and embryo cells) showed increased toxicity of the mixtures. [525] Three herbicides were tested: glyphosate, isoproturon, fluroxypyr; three insecticides: pirimicarb, acetamipirid, and imidacloprid; three fungicides: prochloraz, tebuconazole, and epoxiconazole. Fungicides were the most toxic to cells and showed adverse effects at concentrations 300-600 times lower than those recommended for agricultural use. In terms of toxicity to cells, fungicides were followed by herbicides and then insecticides. Of the herbicides and insecticides tested, the active ingredient glyphosate was found to be the most toxic. Eight of the nine commercial formulations tested were more toxic than the active molecules: that of glyphosate, called *Roundup*, was found to be 125 times more toxic; that of

tebuconazole 1,056 times more toxic. [525] In the case of the commercial formulation of the glyphosate-based herbicide (*Roundup*), an ethoxylated adjuvant was much more toxic than glyphosate. It must be remembered that the introduction in agriculture of genetically modified plants resistant to glyphosate has made it one of the most sold pesticides in the World and, therefore, also one of those that most easily contaminate soil, water and food. [552] In this study, the only formulation that was not found to be more toxic than the active ingredient was the one with isoproturon. [525]

In conclusion, the toxicological assessment of individual active substances does not reflect reality. Adjuvants and other substances in marketed mixtures can significantly increase the toxicity of active substances. [523, 551] Adjuvants are often wrongly assumed to be harmless and their toxicity is usually not measured using the methods used for active substances. In addition, they are not declared as ingredients on the label. Thus, the estimation of the acceptable dose, for example in food, does not assess the risks from adjuvants, which may be more toxic than the active substance and present in higher amounts. It should also be assessed whether metabolites and degradation products are more poisonous than the starting products. Degradation products may be generated in the environment due to hydrolysis, which may occur in the soil, or may be generated from photo-degradation by light. [677] Metabolites are those produced within target organisms or in soil microorganisms through demethylation, hydroxylation, and conjugation reactions with amino acids. Some examples of metabolites more toxic than the starting products are those of the fungicide prothioconazol (its most toxic metabolite is called prothioconazole-desthio), the metabolite of fipronil (generated by photo-degradation), omethoate which is derived from dimethoate and the derivative (sulfoxide) from N-methylcarbamates. [677] It should be remembered that theoretically, substances such as metabolites or degradation products of pesticides, which may be found in food at concentrations above 0.01 mg/kg, require a toxicological evaluation. A safety assessment is important but often difficult if not impossible. In many cases, metabolites and degradation products are not known at the time of marketing new molecules. Sometimes the toxicity of molecules (such as genotoxicity, the effects of which do not have a safe threshold) may be predicted from the chemical structure as it may be compared with that of known compounds.

The toxicological assessment almost automatically considers the active molecules as the most dangerous part of the commercial product when in many cases it is not so. It is necessary to point out that adjuvants can also prolong the resistance of the active substance in the environment, delaying its degradation and facilitating its absorption into living beings. Adjuvants declared on the label include naphtha, xylene, 1-methyl-2-pyrrolidinone, N,N-dimethyldecanamide: all have recorded dangerous effects. [525]

Due to the difficulties inherent in the toxicological assessment, which are aggravated by bureaucratic mechanisms and influenced by great economic interests, it happens that the Agencies delegated to the protection of the collective interest provide discordant assessments. We report the case of the herbicide glyphosate that was classified as a *probable carcinogen* (category 2A) by the International Agency for Research on Cancer (IARC, 2015) while, after a few months, the European Agency for Food Safety (EFSA, 2015) concluded that "*it is unlikely that glyphosate constitutes a danger of carcinogenicity to humans*". The different conclusions are also a consequence of the fact that EFSA and IARC used different publications and scientific results as reference. Probably EFSA has focused on toxicological work carried out by agro-chemical companies that, among other things, are not public and therefore not available for consultation. [500] It should be remembered that glyphosate causes DNA and chromosome damage in mammals, and its metabolites may be found in the blood and urine of people exposed to it. The USA Environmental Agency (EPA) also concluded in 2016 that glyphosate *is not a probable carcinogen to humans* based on non-occupational exposures. [712] These important differences in evaluations and conclusions highlight critical issues and weaknesses in

both the scientific World and the Health Authorities across the Planet. However, there is evidence that glyphosate may promote cancer, that it is bioaccumulative and that it has several adverse effects in the biosphere. [712]
Unfortunately, the great economic interests and the lack of transparency in the decision-making processes (e.g. on the conflict of interest between the evaluators and on the scientific literature reviewed) constitute a huge obstacle to democracy and the safeguarding of the interest of the community. In general, the scientific community and decision-makers have underestimated the health problems generated by pesticides and, even more, the damage generated to the biosphere. The current system of rules and practices tested for at least 50 years exclude the possibility for a territory to defend itself from the hegemony of these large chemical companies. The inhabitants of a territory, for example of a Province, of a Region but also of a State, have to face bureaucratic, administrative and legal difficulties considered insurmountable in order to limit the use of chemical arsenals and strategies that undermine sovereignty and food security. Private interests have been adept at building insurmountable barriers for who would like to ensure greater protection of health and environment. The right to defend oneself, by unjust and unsustainable laws and economic rules, has long been undermined.

One aspect that could be explored is the health status of beekeepers using pesticides (e.g. acaricides and fungicides). One type of epidemiological study that could be implemented is the prospective study: starting from a given time, a population exposed to a given factor (e.g. beekeepers exposed to pesticides they use on their bees), is followed even for decades, in order to record life habits and health problems. The results should be compared with control groups, i.e. subjects not exposed to the same factors but in similar health, living and environmental conditions. Epidemiological studies are a very demanding and expensive tool, but they are often the best available system for measuring trends and providing evidence.
The current methodology for analysing risks to our health has many limitations as it is based almost exclusively on short-term studies that do not consider low concentrations of exposure of very different mixtures over a lifetime. Unfortunately, the information generated by scientific studies on the toxicity of individual substances is not sufficient to provide guarantees.

THE PRECAUTIONARY PRINCIPLE

The knowledge available to us confirms that exposure to pesticides worsens our health. We have no doubt. We can argue about the intensity of the problems but not about the general effects and the overall direction. Many studies confirm the danger to animals but the projection of these results to humans may lead to underestimating or, worse, ignoring consequences that may be very serious.
Progress has produced an epidemic of avoidable chronic diseases such as cancer: prudence is the more courageous strategy that can be implemented. A paradigm shift in public health management is urgently needed. The health crisis is part of the wider global environmental crisis. There is an urgent need for action as we do not need new warning signs, the ones we refuse to accept are already very worrying. In 2018, for the first time in decades and for the third consecutive year, the average life expectancy at birth, in the US, declined. [333] If this figure will be validated it will confirm the serious environmental and health crisis. This is one of many obvious signs that economic and financial rules cannot neglect. Political and economic practices neglect human and environmental health in order to prioritise short-term profits. Businesses ignore, underestimate or conceal the social and environmental costs of their activities, externalising them to the community as a whole and, in particular, to future generations. The costs avoided by the non-occurrence of a disease, of environmental disasters or of damage to the food production system are not given the necessary weight. The application

of the precautionary principle would save gigantic economic resources and the lives of many of us. The precautionary principle does not apply to hazards that have already been identified, but to potential hazards, of which we do not yet have certain knowledge. Where there is a risk of serious or irreversible damage, the absence of full scientific certainty must not be a reason for postponing appropriate measures to prevent environmental degradation and health impairment. [335] Invoking the precautionary principle is a decision that should be exercised in conditions where scientific information is insufficient, inconclusive or uncertain and, at the same time, there are indications that the possible effects on the environment and human, animal and plant health may be potentially dangerous and incompatible with the chosen level of protection. The logic of the precautionary principle is opposed by business interests, as it would reduce freedom of action. Preventive and corrective measures are often not taken even when there is no longer any doubt. In order to change the paradigm, it is necessary to spread information, because knowing can help the necessary revolution.

INEFFECTIVE SOLUTIONS

In order also to try to meet the demands of citizens, in some cases, ineffective and/or impossible solutions are proposed. For example, it is proposed as an objective to be achieved by 2020 in Europe (the Seventh Framework Programme) that only pesticides that do not generate adverse effects on humans and do not have unacceptable effects on the environment should be used. [276] In addition, it is stated as a good intention to use them in a sustainable way. These objectives are unattainable because the purpose of using, for example, insecticides is to exterminate insects and the mechanism of action by which they act is the same even in non-target organisms. Effects linked to the main mechanism of action or other secondary actions, which may be even more harmful, are also regularly recorded in non-target living beings, including humans. Achieving the goal of exterminating insects without generating side effects is therefore a utopia.

The agro-industry and the politicians underestimate the fact that sooner or later the risks associated with chemical agriculture affect those who cause them. Other ineffective solutions include countering the effects of toxic insecticides, such as neonicotinoids distributed in seeds, by proposing only to modify the sowing machines. [678] Among other things, imidacloprid manufacturers claim that there is no risk to insects during sowing, as environmental contamination is negligible; someone else claims that risks only exist if the sowing machine is faulty. [700, 869] Unfortunately, several aspects are underestimated: these molecules are persistent and can move in the biosphere; superficial attempts are made to play down and divert our attention by focusing on exceptional accidents.

THE STOCKHOLM CONVENTION

At an international level, many actions have been proposed to reduce health risks, the most important of which are those provided for by the Stockholm Convention on Persistent Organic Pollutants, established during a conference held in Stockholm in 2001. [390, 404] This international agreement aims to eliminate and reduce the use of certain substances that are harmful to human health and the environment, known as *Persistent Organic Pollutants* (POPs). These are chemical compounds with toxic properties which, due to their poor degradability, remain in the environment for a long time. In the Stockholm Convention, restrictive measures were suggested for at least 15 pesticides, dioxins and polychlorinated biphenyls. The Convention was adopted in 2001, entered into force in 2004 and covered the following substances:

- Pesticides such as aldrin (an organochlorinated insecticide), chlordane (a chlorine-based insecticide), chlordecone (an insecticide), dichloro-diphenyl-trichloroethane (DDT), dieldrin, endrin, heptachlor (an organochlorinated insecticide), mirex (an organochlorinated insecticide), toxaphene (a reaction mixture of about 200 organic compounds, obtained by chlorination of camphene to a chlorine content of 67-69% by weight), hexachlorophene (a disinfecting and bactericidal substance, also used in formulations for external use such as soaps and for disinfection of the oral mucosa such as toothpastes) and hexachlorocyclohexane (lindane).
- Flame retardants: pentabromodiphenol and hexabromodiphenyl. [391, 393]
- Polychlorinated biphenyls (PCBs) used as thermal and electrical insulators or as lubricants. [402]
- Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) or more commonly known as dioxins are formed during combustion and are generated by certain thermal and industrial processes as unwanted and unavoidable by-products [e.g. copper, iron, steel, aluminium, zinc production, the production of plastics (PVC), and chemical compounds such as certain herbicides based on chlorine derivatives]. [403, 405] Sludge from water purification (they may be used as a fertilizer) and compost (that contains unwanted but unavoidable waste and leaves that have bioaccumulated dioxins from the atmosphere) may contain dioxins. The physico-chemical characteristics of dioxins are at the origin of their behaviour in environmental and biological matrices, and their ability to transfer and accumulate in living organisms. They are extremely stable compounds, resistant to heat, chemical and biological degradation, and are highly liposoluble. They are readily bioaccumulated in the food chain, increasing in concentration at each step. The primary mechanism of entry of dioxins into the terrestrial food chain is atmospheric deposition on plants, and on the surface of soils, resulting in ingestion by animals of contaminated fodder and soil (from grazing herbivores). Animals accumulate these substances in the adipose tissue of the body, making them available for human consumption, through products such as milk and meat. They are characterised by having a long persistence in the human organism: 4-16 years. The persistence of some dioxins in soil may be high. For example, the dioxin known as TCDD (or 2,3,7,8-tetrachlorodibenzo-p-dioxin), may halve its concentration in the surface soil layers in 9-15 years, while the estimated half-life for the deeper layers is 25-100 years.

Subsequently, it was proposed that other molecules should also have a restriction in their manufacture, such as the insecticide endosulfan, the insecticide methoxychlor, the flame retardant dechlorane plus, plasticisers, metalworking additives, and flame retardants such as chlorinated paraffins, perfluorooctane sulphonic acid and its salts, and perfluorooctane sulphonyl fluoride (used as insecticides, in hydraulic fluids for aviation, photography, and imaging, clothing, etc.). [405, 406, 407]

This Convention aims to curb some environmental and health damages, and has received the adhesion of 181 countries including the member states of the European Union. Italy, which signed it in 2001, by the 9th of August 2019 has neither ratified it nor put it into practice: it is the only European signatory state to have disregarded the Convention, which has also not been ratified by Malaysia, Israel, India, and the USA, while China and the Russian Confederation accept limitations only for some of the molecules regulated in the Convention. [403, 404, 409]

Even if this kind of agreement tries to limit or ban the production of very few substances, among the hundreds for which there is sufficient information on the hazard to justify bans, it fails to generate widespread application. In fact, molecules such as DDT continue to be manufactured or imported, or are used to produce chemically similar molecules such as dicofol, which is produced from DDT and has practically the same chemical structure: it is imported and marketed in Italy (2004). [408] In Italy DDT is still found in drinking water and surface water, generating the suspicion of illegal use. DDT is certainly used in great quantities in Asia and Africa. [410]

THE CHALLENGE OF HEALTHCARE COSTS: INTELLECTUAL DISABILITIES

Estimating the costs of health damage caused by pesticide use is a difficult task and may lead to highly questionable underestimates. A study published in 2005 reported that in the USA pesticides generated costs for environmental damage and health problems of at least 12 billion dollars. In those years, approximately 600 different pesticides were used in the USA at a cost of about 10 billion dollars. [502, 537] The biggest costs generated by the massive use of pesticides, according to this estimate, are those generated by several problems (in descending order): [502, 537]

- The damage to human health.
- The emergence of pesticide-resistant organisms.
- Crop reduction. For example, from 1945 to 2000, the use of insecticides (organochlorines, organophosphates, and carbamates) increased at least 10-fold but insect-generated damage increased from 7% to 13%.
- Increased mortality in birds. This has been known for some time; for example, in the 1990s it was estimated that diazinon caused the death of between one and two million birds every year. Some estimates calculate that pesticides in the US kill between 0.25 and 9 birds per hectare per year. To this problem one must add the reduction of food sources (e.g. insects), the effects on reproduction (e.g. organochlorinated insecticides at sub-lethal doses).
- Groundwater contamination.

In 24 European countries, 13 active molecules used in viticulture, fruit-growing and vegetable cultivation generate a loss of about 2,000 years of life per year, which could correspond to 78 million euros per year. [494] One year of life lost, in this estimate, has been valued at 39,000 euros; this simplistic hypothesis of monetizing life would open a long reflection on how much our health can be worth, in monetary terms. This approach is based on the assumption that life and death also have a monetary value and, therefore, are a possible bargaining chip.

In order to be able to estimate the effects generated by the use of pesticides, it would be very useful to know the substances and products sold and used in the territories, with details for every single crop and for each hectare or fraction thereof. Unfortunately, this information is insufficient or absent.

Several methods have been designed to try to establish the benefits obtained from different prevention strategies. For example, prevention of the presence of pollutants such as lead in children's blood generates different benefits depending on the threshold concentrations that are established (in terms of economic estimates). Costs generated by lead pollution include reduced IQ (intelligence quotient), and health costs for testing and treatment (for at least 10% of French children, such as those with blood concentrations exceeding 100 µg/L). Subjects with reduced IQ may need special training and generate increased educational costs, and will be less productive at work. Exposure to lead has also been associated with an increased likelihood of recording criminal acts during one's lifetime. A study in France estimates that if the maximum blood lead concentrations of children aged zero to six years remain below 15 µg/L the economic and social benefits are at least 22 billion euros (4.7 million children were registered in France in 2008). [503] In France, 50% of children have blood concentrations of lead above 15 µg/L. The direct health costs, resulting from the presence of lead in children's blood, are

estimated to be about 300 million euros. According to this estimate, the health costs are slightly higher than the costs that could be incurred to reduce lead exposure in the home. Overall, in France, it is estimated that the IQ reduction generated by lead produces costs of more than 20 billion euros per year (this is an underestimate as it considers only neurotoxic effects and does not assess environmental damage). [326] The Authors of this study consider that in children blood concentrations of lead between 24 µg/L and 100 µg/L cause a reduction in IQ of 3.9 points for every millionth of a gram per liter (µg/L) increase, while it decreases by one point between 15 and 24 µg/L (always per millionth of a gram per litre increases). [503]
In the USA, the damage generated by the neurotoxic effects of the exposure to atmospheric lead from oil combustion is well documented for the generation born between 1960 and 1980. Harmful lead exposure has affected millions of children, resulting in a significant reduction in IQ and costs estimated between $110 and $319 billion per year. [327] It is estimated that the introduction of unleaded gasoline in the 1980s generated an economic benefit of over $200 billion per year. [326] Probably for every dollar invested in reducing lead exposure, benefits between $17 and $220 are produced (this reduction in health care costs may be comparable to or greater than the benefits produced by some vaccines). [326]
These results confirm the often underestimated notion that when considering health and social costs, both direct and indirect, prevention and depollution to reduce the exposure to pollutants also pays off economically: removing lead from paints, replacing drinking water pipes, and reducing emissions from metal-processing industries. Environmental costs such as the effects of lead on flora and fauna are often not properly considered. It should be remembered that the effects of lead poisoning were already known to the Romans and 100 years ago an epidemic affecting children in Australia was well described. [327]

For increases in the concentration of mercury compounds (methyl-mercury) of 6 µg/g in hair, a reduction in IQ (by about 3 points) has been estimated. [327] In Europe, methyl-mercury generates a loss of at least 600,000 IQ points; this reduction translates into costs for treatment, education, and reduced work productivity estimated at least 10 billion euros each year. [326] For every IQ point lost due to prenatal exposure to mercury, there is a loss of economic value estimated at 17,000 euros (assuming it is possible to quantify so precisely the value of health lost). [500] Other molecules that have been reported to cause cognitive impairment in children (e.g. reduced IQ) are polychlorinated biphenyls (taken with breast milk), organophosphate insecticides and fluoride. [327]

In Europe, prenatal exposure to organophosphate pesticides generates an annual loss of 13 million IQ points and 59,300 cases of intellectual disability. [501] Overall, endocrine disruptors could generate costs equal to one-sixth of all those attributed to brain disorders in Europe. Nervous disabilities resulting from IQ impairment caused by endocrine disruptors are attributed an economic value of about 146 billion euros. In this case too, a value for intelligence is estimated that could be the source of numerous criticisms and interesting reflections (2,400 euros for each new disability: 146 billion/59,300 new cases per year). These considerations are an interesting starting point for reflecting on the possible evaluations carried out at a political level, in the decision-making moments; one can perceive how superficial and fragile the evaluation based on the criterion of monetizing health or intelligence can be. The same study estimates that, in Europe, endocrine disruptors generate between 126 and 631 new cases of autism per year and between 19,400 and 31,200 new cases of attention deficit hyperactivity disorder. [501]
In Europe, the exposure to phthalates (present in plastics such as those used to make drinking water bottles) promotes male infertility, leading to over 600,000 new requests for assisted reproduction each year and causing over 53,000 new cases of obesity. [501] These are estimates (the Authors calculate a probability range between 40% and 69%), so they could turn out to be wrong. Systematic disclosure could probably have the positive effect of encouraging a

reduction in the use of these substances. To have faith in arid and simplistic economic rules is a dangerous simplification: we risk to commodify health and environment by accepting buying and selling as a politically correct practice.

According to another criterion for monetizing health, in the US, for every dollar spent on pesticide purchases, at least two dollars are spent on externalized costs such as public health. [500] In Brazil, it is estimated that the costs generated by the damage to the health of farmers who grow beans and corn correspond to 25% of the value of the revenue. [501]

These attempts at economic evaluation are undoubtedly underestimates and are not sufficient to give an idea of the seriousness of the situation, but they are, however, a useful starting point for reflection on the simplistic and reductive criteria used to systematically arrive at the same conclusions, namely: *there are insufficient elements to justify a change of course*. Economic and financial rules also dominate over health. The situation is even more complex and underestimated because of its importance when it comes to assessing environmental damage.

PESTICIDE SALES DATA IN ITALY AND WATER MONITORING

Some information on pesticides is summarized below highlighting that sales data is current as of 2012, while water analysis was published in 2016. [500, 504, 523, 533, 534, 535, 551, 697]

Pesticides	**Tons sold in Italy in 2012 and percentage on total sales**	**Percentage of findings in surface waters in Italy**	**Percentage of findings in groundwater in Italy**	**Possible carcinogen or mutagen**	**Suspected endocrine disruptor**	**More information**
2,4-D or 2,4-Dichlorophenoxyacetone (E)	55.7 (0,4%)	**9.9**	**2.5**	*X*	*X*	*Respiratory tract irritation, allergenic, impaired reproduction. Suspected correlation with Parkinson's disease.*
Acetamiprid (neonicotinoid, I)	2.32 (0.02%)	**11.2**	**3.2**	-	-	*Neurotoxic.*
Atrazine (E), **banned since 1992**	-	**4.1**	**5.6**	-	*X*	*Persistent in the environment and bioaccumulative. The presence of the herbicide atrazine in drinking water (in the USA it is found in 94% of samples) has been associated with birth defects, decreased fertility and cancer.*
Atrazine-2-hydroxy (atrazine metabolite)	-	**<u>66.7</u>**	**<u>33.8</u>**	-	-	*Persistent in soil.*
Atrazine desethyl (atrazine metabolite)	-	**5.1**	**9.9**	-	*X*	-
Atrazine desisopropyl (atrazine metabolite)	-	**-**	**4**	-	-	-
Bentazone (E), benzothiadiazine	48.8 (0.37%)	**<u>18.7</u>**	**-**	-	-	*Damage to reproduction.*
Boscalid (F), carboxyanilid	37.65 (0.1%)	**<u>29.4</u>**	**<u>5.7</u>**	*X*	*X*	*Persistent in soil and water.*
Cadusafos (I, N, organophosphate), **withdrawn in the**	-	**<u>40.7</u>**	**<u>12.1</u>**	-	-	*It affects neurodevelopment. It is suspected to be*

EU since 2008						*related to Attention Deficit Hyperactivity Disorder.*
Carbaryl (I, carbamate) **withdrawn since 2009, no use authorized in the EU**	0.31 (0.002%)	**<u>11</u>**	**<u>2.2</u>**	*X*	*X*	*Parental exposure has been correlated with birth defects. Lethal to earthworms at doses 10 times lower than recommended for agricultural use. Its metabolites are more toxic than the starting molecule (formed in humans and soil).*
Carbendazim (benomyl, F), **no use allowed since 2003**, it is a metabolite of the fungicide thiophanate-methyl that is suspected of being carcinogenic	-	**<u>45.3</u>**	**<u>12.3</u>**	*X*	*X*	*Persistent in water. In 2016, ARPA Lazio found it in apricots, pears, apples, grapes, kiwis, and peaches.*
Chlorpyrifos (I, organophosphate)	464 (**3.45%**)	**4.3**	**1.5**	*X*	*X*	*It affects neurodevelopment. It is suspected to be related to Attention Deficit Hyperactivity Disorder, and autism. Its metabolites can be 3,000 times more toxic to the nervous system than the starting molecule (e.g. chlorpyrifos-oxon).*
Chlorpyrifos-methyl (I, organophosphate)	7.9 (0.59%)	**1**	**0.3**	*X*	*X*	*Toxic to earthworms and birds.*
Cyproconazole (F, triazole)	7.74 (0.06%)	**11.9**	**12.3**		*X*	*Toxic to aquatic organisms.*
Dicamba (E, benzoic acid derivative)	43.4 (0.33%)	**11.9**	**2.7**	*X*		*Stable in water.*
Dichlorvos (I, organophosphate), **withdrawn since 2008**	0.44 (0.003%)	**<u>2.1</u>**	**<u>0.8</u>**	*X*	*X*	*It is suspected to be related to Attention Deficit Hyperactivity Disorder.*
Diflubenzuron (I, growth regulator, azotorganic)	2.85 (0.02%)	**40.9**	**8.3**	-	*X*	*It is very toxic to aquatic organisms.*
Dimethoate (omethoate, I, organophosphate)	100 (0.75%)	**11**	**0.3**	-	*X*	*It affects neurodevelopment. Suspected to be related to Attention Deficit Hyperactivity Disorder.*
Dimethomorph (F, morpholine)	11.6 (0.38%)	**36.6**	**5.7**	-	*X*	*Impairs the activity of soil bacteria (e.g. nitrifying bacteria). Toxic to aquatic organisms.*
Dithianon (F, fumigant, thiocyanoquinone)	249 (1.85%)	**50**	**7.4**	*X*	-	*Toxic to fish.*
Diuron (E, phenylurea), **withdrawn since 2008**	0.01 (<0.001%)	**<u>18</u>**	**<u>2.6</u>**	*X*	*X*	*In soil it is converted to the more toxic and persistent 3,4-dichloroaniline. It is*

						very toxic to aquatic organisms.
Esaflumuron (I), **not approved in the European Union and withdrawn in Italy in 2004**	-	**-**	**<u>20.4</u>**	-	-	*Persistent in soil and water.*
Fenazaquin (F, quinazoline)	0.0014 (<0.001%)	**18.2**	**17.8**	*X*	-	*It is very toxic to aquatic organisms.*
Fluazifop (E, aryloxyphenoxy propionate)	-	**66.7**	**22.4**	-	*X*	--
Fluazifop-butyl (E, aryloxyphenoxy-propionate)	32.74 (0.25%)	**14.3**	**-**	-	-	*Harmful to reproduction. Very toxic to aquatic organisms.*
Glyphosate (E, organophosphate), **banned in 2016**	**1,795** (13.4%)	**<u>70.9</u>**	**<u>5</u>**	*X*	-	*It has been associated with an increased risk of miscarriages, reproductive disorders and malformations. It alters the intestinal flora of bees, cattle and pigs (it was registered as an antibiotic). In the soil it damages nitrogen-fixers.*
AMPA (glyphosate metabolite, E)	-	**<u>70.9</u>**	**<u>4</u>**	-	-	*Reproductive disorders in earthworms.*
Imidacloprid (I, neonicotinoid)	30.5 (0.1%)	**53.5**	**10.1**	-	-	*It has been associated with autism. It is neurotoxic to birds.*
Ioxinil (E, dicarboximide), **not approved since 2016**	7 (0.05%)	**<u>68.8</u>**	**<u>21.7</u>**	*X*	*X*	-
Metalaxyl (F, acylalanine)	16.28 (0.05%)	**27.8**	**5.9**	*X*	-	*Toxic to reproduction. Dangerous for aquatic organisms.*
Metalaxyl M (F, acylalanine)	20.9 (0.16%)	**36.5**	**-**	-	-	*Toxic to reproduction.*
Methomyl (I, carbamate)	2.54 (0.0%2)	**29**	**10.3**	*X*	*X*	*It has been associated with Parkinson's disease. It is very toxic to aquatic organisms. It is a metabolite of the herbicide nicosulfuron.*
Metolachlor (E, chloroacetamide), **withdrawn since 2003**	0.88 (0.003%)	**<u>38.3</u>**	**<u>4.3</u>**	*X*	*X*	*It turns into equally dangerous metabolites.*
Metolachlor-s (E, chloroacetamides)	402 (2.99%)	**38.3**	**10.3**	*X*	-	*Very toxic to algae. Damages reproduction. Turns into equally dangerous metabolites.*
Metolachlor esa (E, chloroacetamide), a metabolite of metolachlor and	40 (1.33%)	**55.6**	**30.3**	-	-	*Persistent in soil and toxic to aquatic organisms.*

alachlor. The latter is carcinogenic and **withdrawn since 2006**						
Nicosulfuron (E, sulfonylurea)	10.24 (0.08%)	**18.9**	**-**	-	-	*Highly toxic to aquatic organisms. Degrades in water to methomyl (insecticide).*
Oxadixyl (F, phenylamide), **withdrawn in 2003**	0.11 (0.001%)	**7.9**	**10.5**	*X*	-	-
Penconazole (F, triazole)	15.7 (0.12%)	**11.5**	**3.6**	-	*X*	*In soil, metabolites are very toxic to earthworms.*
Propamocarb (F, carbamate)	38.9 (0.3%)	**21.6**	**8.2**	*X*	*X*	*Neurotoxic to birds.*
Propiconazole (F, triazole)	12 (0.09%)	**13**	**-**	-	*X*	*Persistent and toxic substance. In soil, metabolites are very toxic to earthworms.*
Propoxur (I, carbamate), **withdrawn since 2003**	0.003 (<0.001%)	**15.5**	**18**	*X*	*X*	*It is very toxic to mammals and bees.*
Pyrimethanil (F)	18.6 (0.06%)	**10.2**	**6.8**	-	*X*	*It is very toxic to bees.*
Tebuconazole (F, triazole)	88.9 (0.67%)	**28.6**	**6.1**	*X*	*X*	*Toxic to aquatic organisms. Produces metabolites toxic to earthworms.*
Thiabendazole (F, benzimidazole)	0.1 (0.001%)	**31**	**10.6**	*X*	-	*Toxic to reproduction. Toxic to aquatic organisms.*
Thiametoxam (I, neonicotinoid)	6.75 (0.05%)	**30**	**10.7**	*X*	-	*Neurotoxic to birds. In mammals it causes reproductive disorders. Toxic to aquatic organisms.*
Thiphanate-methyl (F, benzimidazole)	46.75 (0.35%)	**-**	**-**	*X*	-	*Damages earthworms. Toxic to aquatic organisms. Metabolites include carbendazim.*
Thiabendazole (F, benzimidazole)	0.1 (0.001%)	**28.6**	**10.6**	*X*	*X*	*Very toxic to aquatic organisms.*

Notes: E = herbicide; I = insecticide; F = fungicide; N = nematicide.

This table, although incomplete, is proposed with the intention of highlighting some critical aspects:

1) Pesticides that have not been authorized for years have been found in large quantities in the waters.
2) There is no correlation between the quantities sold and the percentage of positive water samples. For example, acetamiprid (a neonicotinoid insecticide) is sold in small quantities (0.02% of total pesticide sales, i.e. about 2 tons) but is found in more than one tenth of the surface waters tested. The same applies to other pesticides such as boscalid, bentazone, carbaryl, diflubenzuron, dimethomorph, diuron, and fenazaquin. This inconsistency reinforces the suspicion that information on quantities sold is deficient and incomplete.
3) Between different pesticides there may be a difference in the level of acute toxicity that varies by as much as a factor of 100, 1,000 or more. The amounts used, if not referred to as toxic equivalents, do not allow adequate comparisons. Very toxic pesticides, such as

neonicotinoid insecticides, can generate the same adverse effects at significantly lower doses than organochlorine insecticides.

4) Derived molecules (e.g. metabolites and degradation products), which are equally or more dangerous than the starting products, may be found in large quantities. The metabolites of glyphosate are found in more than 70% of surface water samples and those of atrazine are found in more than 66% of them; it should be remembered that the latter herbicide has been banned since 1992.
5) Some molecules that are not authorized, because they are considered dangerous, are possible metabolites of authorized molecules. The fungicides carbendazim or benomyl are not authorized but are metabolites of thiophanate-methyl, which is authorized. It has to be pointed out that most of the metabolites are unknown and the known ones, in official monitoring, are usually not searched for. Some metabolites are even thousands of times more toxic than the starting compounds and are more persistent in the environment. In fact, molecules that are banned because they are dangerous can be used with this chemical strategy without the risk of penalties.
6) Pesticides registered for one type of action produce metabolites that act in other targets. For example, penconazole is a fungicide that generates metabolites in the soil that are very toxic to earthworms; some fungicides (e.g. carbamates such as propamocarb) are neurotoxic to birds. Methomyl which is registered as an insecticide is also a degradation product of the herbicide nicosulfuron. So distributing the herbicide also has effects as an insecticide.
7) Little information is available on effects in non-target organisms such as aquatic organisms. As an example of an active substance used in great quantities, aquatic invertebrates are vulnerable to the insecticide imidacloprid: some crustaceans (Mysidacea) may be damaged by concentrations below 1 µg/L; the larval stages of insects of the ephemeral group (genera *Baetis* and *Epeorus*) may be damaged by even lower concentrations of imidacloprid (0.1 µg/L).
8) Some pesticides that in Italy are among the most sold ones are not sought in water monitoring. For example: dazomet - of which at least 204 tons were sold (in 2012) - generates metabolites in the soil that are very dangerous for earthworms and aquatic organisms; folpet, which belongs to the category of fungicides and thiophthalmic fumigants, of which 316 tons were sold, is suspected of being carcinogenic and very toxic to aquatic invertebrates; mancozeb, which is a dithiocarbamate fungicide with 1,307 tons sold and is suspected of being a carcinogen and endocrine disruptor, is highly toxic to aquatic organisms and generates equally dangerous metabolites.

The results of pesticide monitoring carried out by the Regional Health Services in Italy show that few molecules are searched for and in a non-specific way: there is no prior assessment of what crops are present in the territory and therefore what are the most used active molecules. The analytical control of plant protection products in water, before designing the monitoring to be carried out annually, could:

-consider the spread of the dominant agricultural crops in the area and especially in the areas that influence the catchment points;

-provide for prior scrutiny of the field notebooks (compulsory recording of the use of herbicides, fungicides, insecticides, etc.) of farmers in the areas of influence of the catchment points;

-take into account sales data for plant protection products;

-consult agronomists to better finalize the research to be done.

It should be possible to search for a greater number of active molecules and in a selective manner, on the basis of the information available, so as to avoid wasting resources such as those for searching the active molecules that are definitely not used. In addition, the missed search for active substances that are certainly used should be avoided. [1247, 1248]

9) Some additives, adjuvants, and synergists may be as dangerous as the active molecules with which they are mixed in the commercial formulations. Little information is available on these molecules. We report the case of piperonyl butoxide, which increases the toxicity for bees of the active molecules with which it is mixed, and is toxic for aquatic organisms; moreover, it is found in olive oil and is a synergist suspected to be carcinogenic. Another example is that of adjuvants in commercial mixtures of glyphosate (an herbicide). Adjuvants are usually considered harmless, when in fact they may be more toxic than the active ingredient. Pre-marketing toxicological studies should evaluate the active substance alone, the whole commercial mixture and the main components of the commercial formulation in the absence of the active substance.

Information from water monitoring has many limitations as it is impossible to search for all the substances used, many of which are unknown (e.g. metabolites), and it is a very expensive approach. A systematic and punctual registration of the substances manufactured, marketed, sold, and used should be implemented in order to know the molecules used in each field and in other applications (greenhouses, railways, green areas). The agricultural sector, in Europe and in Italy, benefits from a lot of funding with public resources and other opportunities that should be tied to the collection of certain information that would allow a more effective planning of monitoring, such as that on water. In addition, agricultural development plans should stop providing incentives for the use of pesticides, as is the case for compulsory treatments of some crops (e.g. grapevine).

The hazard of pesticides, both to the environment (e.g. on pollinators) and to human health, is estimated using models that imply a certain linearity of response. The main characteristic of a non-linear system is that it does not satisfy the principle of superposition of effects. For a non-linear system it is therefore impossible to calculate the response to an external input given by the sum of two signals, calculating separately the response of the system to each signal and then summing the results thus obtained. The responses could be multiplicative or step responses. Therefore, the systematic poisoning of the biosphere associated with all the other factors of perturbation generated by man (e.g. climate change, deforestation, reduction of soil fertility, destruction of biodiversity, alteration of biochemical flows of nitrogen, phosphorus, and carbon, etc.) may produce effects unknown and unforeseen to date.

CENSUS OF PESTICIDE USE: AN ORGANIZED BLINDNESS

Approximately 1,400 active molecules (of pesticides) are used in the World accompanied by a huge number of metabolites, derivatives from environmental degradation, adjuvants and substances that increase their effectiveness and dispersion in the environment. [196] In 2016, almost 400,000 tons of pesticide active molecules used mainly in the agricultural sector were sold in Europe. [264] The European countries that sold the greatest quantities in 2016, in a descending order, were Spain, France, Italy, Germany, and Poland. Statistical data on the sales of plant protection products in Italy recorded the following quantities: 71,613 tons in 2010, 59,422 tons in 2014, and 63,322 tons in 2015. In the period 2010 - 2015, fungicides accounted for the largest fraction (about 60%) of the quantities sold, followed by herbicides (about 13%) and insecticides (between 9.4% and 11.4%). [194] At the same time, the number of active substances used in Italy increased from 280 in 2010 to 300 in 2015.

The data on the sale of pesticides are self-certified by sellers and the registration of their use by farmers, although mandatory, fails to collect the desired information. It is recalled that the legislation (EC Regulation No. 1107/2009 in Art. n 65 and Art. n 67) states:

> "*Producers, suppliers, distributors, importers and exporters of plant protection products shall keep, records of the plant protection products they produce, import, export, store or place on the market for at least five years* (too little time: the effects are measured even after 30 years, like those of DDT). *Professional users of plant protection products shall, for at least 3 years* (again too short a time), *keep records of the plant protection products they use, containing the name of the plant protection product, the time and the dose of application, the area and the crop where the plant protection product was used.*
> *They shall make the relevant information contained in these records available to the competent authority on request. Third parties such as the drinking water industry, retailers or residents may request access to this information by addressing the competent authority. Authorization holders shall provide the competent authorities of the Member States with all data relating to the volume of sales of plant protection products in accordance with Community legislation concerning statistics on plant protection products*.". [265]

Many years have passed and the legal obligation is still not being met. The information that would allow for greater control and better prevention is not being collected and catalogued. The availability of the internet and of computer systems would enable an easy and quick electronic registration of this information. Despite this, most European countries do not collect this information or consider it confidential, so even when it is collected it is aggregated, reducing the quality of the data (e.g. the quantity of insecticides sold in a country). [266]
Sales data should be collected, but also user data which, when cross-checked with water and food monitoring information, would give a more realistic picture of the situation (when and where single active substances are used). Transparency and accessibility of information is not ensured and no particular control measures are taken for the most dangerous pesticides such as those that are persistent, bioaccumulative, and have proven carcinogenic effects. [267] Since no specific information on individual molecules is collected, it is not possible to measure increases or decreases in the use on specific crops and in particular areas. The information collected is not categorised by level of hazard and more serious situations of impairment (e.g. highly contaminated water) are not highlighted. In Europe this level of information is lacking. [276] The result is an objective difficulty in monitoring an illegal behaviour: national authorities are not able to ensure compliance with regulations. The lack of information and registration of what is happening in the territory allows greater freedom of action to sellers and users. This strategy actually plans an organized blindness. To get an idea of how little information is available at a European level, one may consult the Eurostat website. [279]

CHOICES TO IMITATE

Awareness of the damage caused by the use of pesticides to bees, but also to humans and to the environment in general, is not enough to take the necessary action. Among the simplest solutions already adopted by various beekeepers, farmers, and entire geographical areas is the application of the principles of organic farming. This type of farming system involves the non-use of pesticides. Some communities have even decided through a referendum to abolish pesticides from their territories as it happened in the community of Malles of about

5,200 inhabitants in the Alps, in the Province of Bolzano. The community of Malles is located in an area of Trentino where about a half of the Italian apples are produced and where 45 kilos of pesticides are used per hectare per year. For reference, the Italian average pesticide use is between 5 and 6 kilograms per hectare per year, while the European average is around 4 kilograms per hectare per year. It follows that fruit (66%) and vegetables (40%) contain pesticides. [196]

The same decision (banning pesticides in the territory) was taken by the State of Sikkim in India, which was awarded by the UN for being the first to have made this choice. [196]

Superficiality in dealing with the pesticide problem manifests itself in many ways, including the organic farming sector which is an insufficient attempt - but worthing to be rewarded - towards a more sustainable agriculture. A 2018 document - on the requirements for the accreditation of Bodies certifying organic products among the high-risk factors due to the content of pesticides - lists (in Table 2) the products of the hive and in particular beekeepers with more than 500 hives. [77] The risk of contamination of bees by pesticides used in agriculture is independent of the number of hives managed by the individual beekeeper. Certainly the likelihood for an industrial beekeeper of using active molecules to treat bee diseases is greater.

COUNTERACTING AVOIDABLE CHEMICAL CONTAMINATION

As early as the 1990s, studies showed that it was possible to reduce pesticide use by more than 50% without reducing yields or compromising the aesthetic aspects of fruit or vegetables. Reducing pesticide use by 50% could have generated a very small increase in production costs, which would have been more than offset by the reduction in environmental and health damage. [538] Designing a food production system that is less destructive and able to function without the support of pesticides is probably one of the pivotal points on which the sustainability of the agricultural system is played out. The necessary change will have to be worked out gradually. Among the recommendations that could be suggested to safeguard pollinating insects (and others) are the following:

- Prohibiting the sale of pesticides for domestic and ornamental use, and in the urban environment: they should never be used in parks, public and private gardens, sports facilities, railways, roadsides.
- Prohibiting a preventive use, namely before assessing economic thresholds for intervention. The systematic distribution of certain active molecules, such as neonicotinoid insecticides with seeds, is madness. Pesticides should not be used until their urgent need has been confirmed by a qualified public service.
- Not using pesticides (e.g. insecticides) if the damage generated (e.g. on fruit) is mainly a cosmetic one.
- Prohibiting also the use of fungicides and herbicides on plants that are attractive to pollinators, i.e. those that produce pollen and nectar. Simply prohibiting the application during flowering (e.g. of insecticides) is not sufficient, especially for molecules that are distributed in the soil or with the seeds. We must not forget that many molecules persist in the soil for years.
- Recording all information relating to the production, sale and use of insecticides in a more reliable manner. Annually, information should be made public regarding the pesticides used in each crop, with a detail not exceeding 10,000 square metres.
- It should be compulsory to record all accidents such as increased animal mortality, occupational diseases and water contamination. Provision should be made for records to be kept and published annually.
- Making it compulsory to list the pesticides used on food labels.

- Prohibiting the use of pesticides in areas of naturalistic importance and otherwise in areas that are not predominantly agricultural.
- Increasing penalties for those who make illegal use of pesticides.
- Encouraging cultivation practices that hinder crop pests and favour pollinators, such as creating refuge zones (at least 10% of the cultivated areas), not turning over the soil (e.g. prohibiting ploughing), alternating annual crops, cultivating more than one crop on the same plot at the same time, using mechanical systems of control (e.g. weeding).
- Pesticides should only be purchased by registered users (properly informed, registered and monitored) after receiving written instructions from a qualified public plant protection service (agronomists and veterinarians). Records of prescriptions should be kept, surveyed, monitored and published.
- The health status of pesticide producers and users should be closely monitored. Pesticides are extremely potent poisons, a chemical arsenal more dangerous than conventional weapons, so they should only be sold and used by authorized, trained and monitored people, and only when their use is necessary for the public benefit.

Among the pollutants that should be banned, there are "fruit cosmetics" such as *citrus red* (No. 2 or E 121) which has been used in the United States since 1956 to colour the peel of some oranges (in Florida). In 2019, this colouring agent was allowed in Florida but was banned in Europe as it is considered to be a probable carcinogen. In Europe, citrus peel can be coated with waxes added with fungicides such as thiabendazole and imazalil: the latter is also used on the surface of bananas, legumes, potatoes and other tubers. On citrus fruit, in some cases, natural waxes are used, such as beeswax (E 901), carnauba wax (E 903), shellac polish (E 904) or oxidised polyethylene wax (E 914). [314] The outer coating not only improves the appearance but also seals the pores of the citrus fruit and does not allow the fruit to breathe, slowing down its metabolism and prolonging its shelf life. The USA Environmental Protection Agency or EPA has classified imazalil as a probable carcinogen. After imazalil was administered to some rats, adenomas and adenocarcinomas of the thyroid were observed. [316] Both the EPA and the European Commission considered that there is no increased risk for people directly applying imazalil to agricultural products. [315]

The spread of chemicals in agriculture has not solved the problem of hunger in the World and has made it impossible for many farmers to lead a decent life. As a result of the spread of chemical agriculture, farmers have become poorer and more dependent on other actors: pesticide and seed industries. Pesticides will have to be restricted to extraordinary and indispensable uses.

Many changes are necessary, but they cannot even be planned without first creating a new culture of agricultural production, one that is free of agro-chemical interests. A society that cares about the health of the next generation should not waste time.

BEES AS AMBASSADORS OF AN ENDANGERED WORLD

THE SUDDEN DEATH OF BEES

In many countries of the World, an increase in the frequency of bee colony mortality and a decrease in the number of bee colonies has been recorded for several years; for example, in Europe and North America between 1980 and 2008, or in Germany between 1952 and 2010. [193, 867, 869] Probably in 1881 the first bee death was recorded due to the use of an insecticide: it was based on arsenic and was used in plum fields. [974] In 1929, pyrethrum (*Pyrethrum*) was responsible for the death of honeybees; in 1930, copper-based compounds caused problems for beekeepers, and in 1935, rotenone used in raspberry cultivation was the cause of the recorded increase in bee mortality.

In the last 10 years, the increase in honey bee mortality has become more alarming. In the USA from 1985 to 1997, the number of honey bee colonies decreased by 57%. [196] This phenomenon has recently been called "*sudden bee death*" or "*colony collapse disorder*". This expression is used recurrently by some operators in the sector, by some experts and by the mass media, even though it indicates factual data and many uncertainties. The increase in bee mortality is sometimes described as a kind of curse due to dozens of causes (multifactorial origin) or, alternatively, as a havoc whose causes have yet to be identified. In reality, it is difficult to accept what should be very obvious.

One can try to summarize what is often presented under this acronym, which leads one to think of sudden disappearances of bees due to mysterious causes. *Hive depopulation syndrome* refers to the sudden and abrupt death of colonies of bees (*Apis mellifera*). *Hive collapse disorder* occurs when most of the worker bees leave the hive, leaving behind the queen, a few worker bees and abundant food supplies. In the literature there are many descriptions of this pestilence which are often discordant; a summary of some characteristics is given below: [172, 243, 482]

- Rapid loss of most worker bees: in a few days or overnight.
- Presence of an abundant brood.
- Presence of the queen bee.
- Abundance of food supplies (both honey and pollen).
- The stocks are not immediately stolen by other bees and the attack by other insects is considerably delayed. This signal could lead to the suspicion of the presence, inside the hive, of repellent factors for bees but also for pests.
- Absence or presence of few dead worker bees either outside or inside the hive.

These are characteristic signs but it is not said that they are always present. On the contrary, in some cases they manifest themselves differently, such as finding a large part of the colony dead near the hive. Normally, in fact, bees never abandon a hive in which there is an abundant brood to raise and feed. Probably the symptoms just presented may be attributed to a minority of the colonies experiencing increased mortality (perhaps less than a quarter). However, for the sake of the record it should be noted that according to some beekeepers the first case of this "*mysterious death*" dates back to the autumn of 2006, when it was reported in the USA. Also according to some interpretations, in February 2007, numerous other cases occurred against many nomadic beekeepers, some of whom lost up to 50-90% of their hives. In the same year, cases were reported among resident beekeepers in America, but also in Canada, Portugal, Italy, Spain, Greece, Germany, Poland, France and Switzerland. [172] In the USA, a survey carried out in 2007 estimated an average loss of 31.8% of colonies (these were those that were declared dead or too weak to survive); however, only 29% of beekeepers reported the presence of one of the characteristic signs of this mysterious syndrome, namely the absence of dead bees in the vicinity of the hive. [243] The following year, the American beekeepers interviewed declared a higher mortality rate: 35.8% (owners of 450,000 hives out of 2,440,000 in the USA were interviewed). In 2009, the mortality had dropped to 29%, still higher than the expected natural mortality, which should be less than 15% or 10%, and rose to 32.4% in 2010. Also in 2010, only 28% of the hives that registered an increase in mortality showed one of the characteristic signs, i.e. the absence of dead bees near the colony. In 2011, a winter mortality of 30% was recorded. In 2012, colony losses are reported at 29%, but in 2013 they reach 45%. According to some estimates, winter mortality of bees in the US averaged 30% between 2009 and 2016. [868] In the years 2015, 2016 and 2018 the annual losses were around 40%.

Between 1950 and 2010, the number of bee colonies in the US declined by 45%. [602] Many years ago, between the years 1966 and 1979, the colony loss was attributed to exposure to pesticides such as pyrethroids, organochlorines, organophosphates and carbamates. Between 1981 and 2005, the number of colonies declined from 4.2 million to 2.4 million. In these years new pests were introduced which aggravated the situation (*Acarapis woodi* in 1984, and *Varroa destructor* in 1987). Over the last 60-70 years, there has been a decrease in the number of bee colonies kept in the USA, which has led to a reduction in the pollination capacity of crops.

The census of winter mortality and overall bee mortality recorded in the USA, from 2006 to 2019, reveals up to 45% of colonies dead; mortality is always above the rate that is considered economically acceptable (15% or at most 20%). [379]

In Canada, bee colony mortality between 2007 and 2017 ranged from 15% to 35% (2007 29%, 2008 35%, 2009 34%, 2010 21%, 2011 29%, 2012 15%, 2013 28%, 2014 25%, 2015 16%, 2016 17%, 2017 25%). [243] These values indicate the constant exceeding of the threshold of mortality considered natural (i.e. below 10%) and also of the economically unsustainable one which is 15%.

In Europe, an increase in mortality among bee colonies has been recorded: since 1997 in France and since 1999 in Belgium; also in Germany, Switzerland, Austria, and England. [859] In England, mortality has fluctuated between 10% and 15% for years, but has risen to 30% since 2008. [260]

In France, honey production fell by 22% between 1991 and 2001. [859] Surveys carried out in France between 2007 and 2010 show a bee mortality rate between 23% and 30%. In France, however, in 2011, the year for which more information is available, the characteristic symptoms of "*mysterious disappearance*" are recorded in less than 12% of dead colonies (one of the characteristic symptoms that was little observed was the absence of worker bees). Most of the recorded mortality does not show the characteristic symptoms classified as "*colony collapse disorder*". However, a mortality rate above the economically unacceptable threshold is also recorded in France: a loss of more than 15% of colonies. Honey production in France also declined significantly, i.e. by 50%: from 32,000 t in 1995 to 16,000 t in 2003. [280] Opinions differ widely on acceptable mortality thresholds. Beekeepers consider mortality of up to 10% to be normal, but if it exceeds 15%, the economic damage is considerable.

In Italy, honey production is also steadily decreasing: from 2011 to 2016, production dropped by 70%. In some cases, as for acacia honey, losses up to 80% have been recorded. [196] In the years 2006 and 2007 a survey conducted by EFSA (the European Food Safety Authority) recorded a mortality of colonies in Italy between 40% and 50%. [243] For the majority of mortality cases recorded in Europe, it is difficult to know whether symptoms of *colony collapse disorder* have occurred. A survey conducted in Europe between 2008 and 2009 (involving 9,471 beekeepers owning 172,252 hives) recorded an average mortality of 12.3%, which in 2016 and 2017 became 20.9% (involving 14,813 beekeepers owning 425,762 hives). [243, 246]

In winter 2015/2016, the mortality recorded in Europe (421,238 colonies recorded at the beginning of winter) was 12%: 12.5% for Italy, 22.1% for Spain, 13.4% for France and 29.5% for Ireland. [479]

The increase in the mortality rate of farmed bees, despite the care provided by beekeepers, has led to a drastic reduction in the number of colonies in the World: in 2008 in many countries they were probably halved. It is not impossible to exclude a collapse of the pollination system in the coming years, with repercussions also on crops and on the ability to produce food. The increase in bee mortality is nothing more than a sign of a sick ecosystem that needs urgent change.

The numbers given above, if not accompanied by some essential information (as is often the case), are not very informative as they do not consider important factors such as beekeeper care, medicines, feed, queen replacement, purchase of swarms, and nomadism. It should be remembered that most honey, even that produced in Italy, comes from beekeepers who manage hundreds of hives. These are industrial farms where the bees live in an artificial nest and no longer have to worry about secreting wax, as it is supplied through recycling, they are fed with feed and are continually moved in wheeled vehicles in search of nectars, pollen and better climatic conditions. It should also be remembered that queen bees are artificially reproduced and replaced every three years at the most, by purchasing them from special breeders who can use sophisticated biotechnological techniques.

Data on changes in honey production or number of colonies over time are not, by themselves, an indication of the health of beekeeping, since beekeepers can artificially and rapidly increase the number of colonies. In addition, colonies can be artificially fed and cared for so it is possible to generate an increase in honey production even under adverse conditions. Many parameters should be evaluated in order to measure the quality of beekeeping, such as the energy input (in terms of oil equivalent) used by the supply chain per unit calorie of honey produced, the use of pesticides, medicines, and feed in beekeeping, the number of kilometres travelled by bees and the entire supply chain, the intensity of artificial colony renewal (e.g. purchase of queen bees), the frequency of nomadism, and recorded pathologies. In the absence of this information, the countless data, such as the graphs that can be analyzed in detail on the website of the National Honey Observatory, represent absolute values that are difficult to compare and not very informative. [201] A sudden and consistent collapse should certainly be a cause for concern, but exclusive indications on the quantity of honey or the number of dead colonies cannot provide the information needed to assess the seriousness and artificiality of the situation. Indicators that could be taken into consideration are those relating to energy spent compared with energy obtained, transport carried out (km/honey/year), quantities of feed per hive per year, quantities of antibiotics and pesticides per hive per year, frequency of pathogens present in the apiaries. These are data that would provide more useful information. Recording the times of the year when mortality occurs could also provide a useful confirmation.

The natural mortality of bees is estimated to be less than 10% of the colonies each year. However, there is another threshold, that beyond which economic damage becomes substantial. Some professional beekeepers consider a colony mortality of more than 15% as intolerable. Information on mortality alone is not sufficient to estimate the seriousness of the problem, since industrial beekeepers in particular purchase new queen bees (which may live more than 5 years, but in the USA and Canada they are replaced every 2 years; moreover, laboratory insemination and artificial selection can be used), purchase new entire colonies, and can reproduce existing ones in a few months. [230] Even if the honey production of the year is compromised, a beekeeper is able to obtain three swarms from one colony. As already mentioned, we must also consider other aspects which, if ignored, contribute to making real problems invisible, such as artificial feeding, the use of chemicals and pesticides in agriculture and beekeeping, and nomadism. To give a striking example, in the USA, during the winters of 2006-2007 and 2007-2008, mortality levels of 32% and 36% were recorded, respectively, but during the same period there was an increase in the number of colonies of 5%, in the winter of 2006-2007, and a reduction of 14% in the winter of 2007-2008. [484] So mortality figures are very insignificant if they are not accompanied by other information such as nomadism, purchase of whole colonies, purchase of bee packs without queens, purchase of queens, honey production, presence of diseases etc.

A study investigating winter mortality of bee colonies was conducted in Europe between 2012 and 2014. The project (EPILOBEE) was funded by the European Union. [382] The first year this study was conducted in 17 European countries and in 16 in the years 2012-2014. Winter mortality was recorded in 9,566 apiaries in 2013 and in 8,580 apiaries in 2014. In the 2 years 176,860 hives were inspected with three visits per year: one before winter, one at the end of winter and one at the end of summer. Mortality in the first winter ranged from 3.2% in Lithuania to 32.4% in Belgium (it was 5.5% in Italy). In 2014, winter mortality of colonies ranged between 2.4% in Lithuania and 15.4% in Sweden (it was 4.8% in Italy). [382] Northern European countries were found to be the most affected. Compared to the mortality recorded in the USA, according to this research, the mortality observed in Europe is much less worrying, being less than 15% in most countries in 2013 and across the Old Continent in 2014. The average mortality in winter was 15% in the first year and 8% in the second, while that during the summer was 4% in the first year and 3% in the second year. [383] So the mortality recorded was very low and, according to this study, not to worry about. Regarding this project, it should be noted that 117,269 laboratory tests were carried out which looked for some parasites. No information regarding pesticides was looked for and colonies were considered non-viable if they housed less than 500 bees. No information was recorded regarding honey production, which is another useful and simple indicator of colony health. A large proportion of hives were excluded from the initial sample (e.g. between 15% and 24% in Italy). In practice this study, although it has employed enormous resources, only investigates manifestations of infections and parasites (*Nosema*, *Varroa*, viruses etc.). Little information is available on the environment in which the hives are placed. Among the factors highlighted several times in the final document, regarding the causes of variability in mortality, is climate. [382] Overall, the study considers 36 variables (of the more than 100 initially considered) such as age of the beekeeper, size of the apiary, type of production (e.g. honey and pollen), queen bee problems and various parasites. Probably political choices decided to avoid having more details about the impact generated by pesticides. In this study, among the causes associated with winter mortality, the following are highlighted: the location in a broad sense as the State, the size of the apiaries, the type of beekeeper (industrial or amateur), the objective of beekeeping (e.g. only honey production) or the presence of plague (a parasitic bacterium). [383] Summer mortality is correlated with the level of beekeeper training or the presence of the *Varroa* mite. [358] There is no conclusive information on the influence of the environment around apiaries or on pesticide treatments carried out by beekeepers and farmers. Concerning pesticides, it is written that this study aims to provide information for future research investigating pesticides and the possible interaction between pests and pesticides: "*The outcomes of EPILOBEE were an essential prerequisite to the implementation of future explanatory studies investigating the potential causes of honeybee colony losses such as multiple and co-exposure to pesticides (e.g. insecticides, fungicides and acaricides) and their possible interactions with infectious agents.*". [383]

In addition to taking into account pesticides distributed in the environment and those used by beekeepers, surveys of winter or annual mortality should take into account many other variables that may conceal the seriousness of the situation, such as the multiplication of colonies: beekeepers can easily obtain two or three from one. Another aspect is the possibility of buying queens or entire colonies, which can come from very far away. Input in terms of pesticides (e.g. acaricides), antibiotics (which should be banned), feed and the intensity of nomadism is not assessed. Honey production is not measured and it is not considered that, in industrial beekeeping, bees are spared the effort of constructing the honeycombs, as the hive is made of recycled wax combs. This is quite an energy saving on the part of the colony and the beekeeper will get several kilos of extra honey. Another very relevant aspect is that beekeepers, if they notice that their colonies have certain types of diseases considered particularly dangerous and for which there is an obligation to report to the veterinary authorities, must destroy the colonies affected by parasites. So, without incentives there will probably be an underestimation of some problems that will be intentionally hidden. One has also to consider the illegal treatments that we have seen are relevant. Considering mortality for a few months without assessing critical fundamental aspects means excluding a priori the possibility of knowing the real weight of several factors. Perhaps it is possible to make a simple analogy to better understand this methodological error: it is difficult to hope to have information on the effects of tobacco smoke by recording only smokers who die over a few months. Or one can hope to free smoking by conducting a survey without recording who has smoked (for how long and with what intensity) and who has not. The conclusion would lead to the assertion that mortality from tobacco smoking is a very rare and negligible risk; alternatively, one could argue that one is unable to reach a conclusion.

Partial and scientifically incorrect investigations (e.g. from a statistical point of view) produce heterogeneous results, which are not comparable, not repeatable and therefore not conclusive. In practice, the data collected from these studies can be used to construct very different results and interpretations, which are not very objective and unfortunately often have a common weak point: underestimating the risk generated by pesticides. [372, 397] Mathematical tools can be skillfully managed to confirm or exclude hypotheses. Even applying the best and most objective methods, statistics, like any scientific discipline, has objective limits. For example, that of considering the hypothesis mathematically useful and necessary for the functioning of the statistical model. [570] The choice of the sample size and many other factors can play an important role in favour or against the initial hypothesis. In practice, the same data can lead to completely opposite results depending on the boundaries of the statistical and mathematical model used. These boundaries can be used to critique some results or to produce others in support of the preferred hypothesis. Any experimental model suffers from limitations such as being able to record false positives or false negatives. That is, the responses obtained do not depend on the object of the study. Therefore, the absence of disinterest and detachment, regarding the freedom of possible results, implies that research protocols can be manipulated in a subtle and inconspicuous way even from a statistical point of view. The limitations of the scientific method can be curbed by enhancing certain aspects such as: impartiality, accessibility, transparency, comparison, participation, communication, utility, and public funding.

COLONY COLLAPSE DISORDER, OR THE SYNDROME OF THE CONSCIOUS BLINDNESS OF A PLANNED DISASTER

Among the various positions there are those who believe that this new disease is present mainly or only in certain areas of the World, such as the USA. According to some, therefore, the "*sudden death of bees*", also known as *Colony Collapse Disorder*, is a phenomenon which exists in the USA but not in Europe. [252, 869] It should be pointed out that, fortunately, not everyone agrees on the existence of this syndrome, the causes of which have yet to be discovered. It is more realistic to think that the factors that damage bees are generated by man; there are many causes and each one has a different weight in different contexts, but the situation is so widespread that the final result is always the same: an increase in the mortality of pollinators such as bees. This mortality is partially and temporarily compensated for by an increase in the artificial production of new colonies by beekeepers: importing new queen bees from companies specialised in this type of market (they carry out assisted fertilization), providing more feed, distributing more medicines such as acaricides, moving colonies more often and over greater distances, providing an artificial nest complete with wax, buying new swarms.

Some factors that certainly contribute to make the life of bees very difficult and that are the cause of their death, which in some cases is defined as sudden but still predictable and preventable, are well described in an interesting investigation on the World of bees: the documentary "*More than Honey*"[I] which focuses on bees and explores the theme of the relationship with man. It shows the difficult relationship between bees and humans and, more generally, between man and nature. In the documentary several hives are shown around the World, in particular in California, China, Switzerland and Australia.

In reality, very different factors, each of which alone is capable of exterminating insects, in different contexts and situations weigh in a variable way with the same final result. In the USA, in 1947, at least 6 million hives were registered, which after sixty years have decreased to 2.6 million. [196] A drastic reduction in the number of hives was also recorded in Germany: between 1952 and 2010 more than one million colonies were lost. [869] Various scientists agree that there are different causes that, individually or together, depending on the conditions, generate this effect. As we have amply highlighted, bees are exposed by the main beneficiaries of their services in economic terms, farmers and beekeepers, to hundreds of toxic molecules. These operators voluntarily and systematically expose bees and the environment in which they live to poisonous substances. Thus, among the main causes of increased mortality there are pesticides such as insecticides.

Insecticides are designed to exterminate insects and may cause 100% mortality (by contact) of bees. Some toxicologists consider those molecules that generate 30% to 40% mortality, instead of 90-100%, to be less dangerous. [866] Why be surprised if bees die when exposed to the most powerful molecules we have designed specifically to exterminate insects? These molecules are synthesized with the goal of killing between 40% and 100% of insects (within minutes or hours) when they are contaminated with very low doses (millionths of a gram per insect). The farmers' expectations of the effects of insecticides and other pesticides are different from those of beekeepers, but both chemical monoculture and intensive beekeeping are often rooted in environmental illiteracy. Unfortunately, the consequences of ignoring the laws of nature are proving devastating: we have underestimated the negative effects for too long. One of the most applied precautionary measures by farmers is to warn beekeepers at least two days before distributing pesticides such as insecticides directly (with sprayers) or indirectly (with seeds). This is the protective measure proposed by the President of the *European Institute for Sustainable Development in Agriculture* or EISA. [869] In the same document it is written that pesticides are essential to provide safe food and that if used properly they do not generate unacceptable effects on non-target organisms, such as pollinating bees and birds (for the record this document is also signed by the *European Landowners' Organization* or ELO and by the *European Crop Protection Association* or ECPA). In the same document it is also recommended to eliminate the flowers (the wild ones, of course) in the vicinity of the fields to be treated, just before the distribution of pesticides. So it is recommended to destroy the only natural food known to honey bees. It is incredible to think of saving bees (and others) by continuing to use very powerful poisons (pesticides) and eliminating the only food they have: flowers. Instead of seriously tackling the problem, we circumvent it, without actually changing anything, by proposing insignificant or devastating actions, depending on the point of view: that

[I] *More than Honey* is a 2012 Swiss documentary film directed by Markus Imhoof.

of the beekeeper or that of Nature. The increase in mortality may certainly be linked to the reduction in biodiversity and the use of chemicals in agriculture.
Another factor influencing mortality figures is the size of the apiary. Big beekeepers, i.e. those who manage more than 25 colonies, are better able to counteract the increase in mortality. This is another reason why the number of small beekeepers in the US decreased by 70% between 1987 and 2002. [484] Big beekeepers can more easily increase colony numbers rapidly to counteract increased mortality, partly for economic reasons. They are also more adept at using pesticides and medicines.
Mortality statistics should also be accompanied by information on productivity, such as the quantity of honey per hive. For example, France has lost half of its honey production capacity in less than 20 years since 1990.
One mistake could be to hope to find, in the distant future, a new cause, hitherto unknown, of the increase in bee mortality, not considering that current knowledge is more than sufficient to explain this phenomenon. Thus, on the one hand we risk devoting resources to the search for phantoms and on the other we distract attention from the known, concrete problems that need to be urgently addressed, such as the chemical risk and the reduction of biodiversity. It should be remembered that some of the surveys that have tried to classify and catalogue the symptoms recorded by beekeepers, which have reported an abnormal increase in mortality, have highlighted the following factors: [247]

- Colony mortality at the same time as sunflower flowering and immediately thereafter.
- Decrease in honey production after sunflower flowering.
- Bees shivering after the sunflowers bloom.
- Disappearance of the queen.
- Decrease in larvae.
- Little or no honey production.
- Presence of shaking bees.
- Presence of apathetic bees near the hive and on the ground.

One of the effects recorded in bee colonies in France (it was 1979), following the use of deltamethrin, a newly marketed pyrethroid insecticide, was precisely that of not finding dead bees near the hive, as they died far away or were unable to return to the colony. [243]
The above list highlights some of the consequences of the exposure to sub-lethal, i.e. very low, doses of pesticides or generated by additive effects, always at concentrations that are difficult to measure instrumentally. Thus, pesticides alone may explain the symptoms of so-called "*sudden bee death*", both individually and, even more so, in complex, bioaccumulative and persistent mixtures. To this negative contribution to bee health, other factors must be added or rather multiplied: malnutrition, diseases, pollutants, reduction of biodiversity, and climate change.
The mortality of farmed honey bees is alarming, but in comparison the biodiversity of wild pollinating insects is coming under even harsher attack. To give a measure of the severity of the problem, wild pollinator biodiversity, recorded since 1980, has declined by 52% in Britain and 67% in the Netherlands. This damage is irreversible and invaluable. [243]

MULTIFACTORIAL DILUTION

As with global warming one of the themes used to distract attention from uncomfortable topics is that these changes have also happened in the past. The proposed message is that today there is an increase of a natural phenomenon that happened also in the past: in 950 in Ireland, in 1869 in the USA, Mexico and Australia, in 1900 in Utah (USA), in 1995 in Pennsylvania (in this American State 50% of the colonies died). [243] 1900, in Utah, the increased mortality had already been given the name of *bee disappearance disease*. In the 1960s, several cases of

sudden bee decline caused by pesticides were recorded in the USA: in Arizona, 48% of colonies were lost between 1963 and 1977. [484] In 1967, 70,000 bee colonies were lost in California due to an organochlorine insecticide: carbaryl. [859]
Another mysterious possibility proposed in the literature is that of a cyclic phenomenon occurring every 2-7 years, independent of insecticides (neonicotinoids) or diseases (*Varroa*). [859]
Among the incorrect messages reported when it comes to *sudden bee death*, there is to equate over 60 different factors such as: [35, 240, 243, 246, 252, 307, 481, 482, 484, 697, 1155]

- over 20 types of viruses,
- over 10 parasites including mites, insects, fungi and bacteria,
- climate change,
- the alteration of the nitrogen cycle,
- the disturbance generated by beekeeper inspections of the hive,
- the fragmentation of ecosystems,
- the flight of the planes,
- electromagnetic fields,
- the power lines,
- the misdistribution of seeds treated with pesticides,
- mobile phone antennas.

It basically supports the unrealistic assumption that bee colonies are rarely killed by pesticides, such as insecticides used in agriculture. [974]
This strategy is potentially very compelling and helps to dilute problems into a myriad of factors. Multi-factorial dilution easily translates into inaction and effectively helps distract attention from the main problems. Unfortunately, dozens of researchers all over the World have joined the search for these ghosts, a real waste of resources. [246, 253, 260, 280, 307, 479, 482, 597] At this point of the reflection we could open a long parenthesis on the most fanciful explanations that imply mysterious phenomena but it is better to cut and avoid wasting further comments. The attention is diluted among many factors and the excessive simplification puts them all on the same level of danger. [357, 358, 382, 482, 484, 678, 860] For example, the more than 18,000 commercial pesticide products containing one or more of at least 1,200 active molecules licensed in the US are put on the same level as more than 60 factors. [678] At least 32% of the crop acreage in the USA in 2010 is treated with neonicotinoids, and some crops such as corn record pesticide-treated seeds in 99.8% of the acreage planted with this grain (0.2% of corn acreage is organic and thus should not use this chemical treatment). In these cases, multifactorizing as an explanation is even more difficult, when it turns out that more than 50% of the pollen collected by bees comes from corn that contains these powerful insecticides, neonicotinoids (one hectare planted with corn may produce more than 150 kg of pollen). [678, 974] Pollen collected from untreated plants such as dandelion plants also contain neonicotinoids (e.g. clothianidin); the explanation is simple: neonicotinoids are persistent and may therefore move through the biosphere. [678] The colonies of bees in the vicinity of cornfields, which occupy millions of hectares in the USA, in the first ten days of life are exposed to doses of neonicotinoids equivalent to at least 50% of the LD_{50}. [678]
Multifactorism is a misleading message carried strategically in many different contexts, and has a long history: tobacco, asbestos, plasticizers. In the case of bees, the risk generated by pesticides is compared to the imbalance generated to the colony by continuous manipulation and intrusion by beekeepers (inspections using smoke), the use of feed lacking in nutritional value, the use of mobile telephony, climate change, the flight of aircrafts or the low quality of pollen. [243, 482] It is very crass to think of confusing these different causes by putting them on the same level. Another example of incorrect messaging is climate change and butterflies. Some species of butterflies, which are supposed to be favoured by climate change (warming), are in

fact also in decline because of pesticides and the reduction of biodiversity (herbicides, herbivores, monocultures, and also climate change such as droughts or floods).
The method of diluting attention between very different factors that actually contribute with different weights is a distraction strategy that has been used systematically in many sad stories of the past such as those of the cigarette industries or the asbestos factories. [260] The tobacco corporations systematically funded pseudo-science in the service of money. [307] The same can be said of the disbelievers in climate change generated by human actions, the so-called *climate skeptics*. Probably, in the history of science, there has never been such unanimous planetary agreement on the causes of climate change and actions to prevent disaster. Yet the industry of doubt manages to get very good results.
The doubt machine is fed in different ways such as unfounded, misleading statements or using unreliable sources. [722, 723] The creation of a message that highlights the uncertainty of knowledge, such as the disastrous effects of pesticides on insects, biodiversity, and the biosphere. The attacks, by the doubt industry, on climate science are also well known and documented. [680]
The misinformation machine manages to have an enormous influence by dangerously encouraging wrong decisions. Phantoms are generated that as such are untraceable so the desired effect is achieved: delaying action limiting entrepreneurial freedom and continuing to do business as best as possible. The disinformation machine creates confusion and ignorance, allowing to delay any necessary political and collective action.
By using the method of scientific investigation correctly, it is easy to see that the claims of disinformation campaigns are superficial and dictated by political and economic criteria of convenience. Unfortunately, they succeed in distracting attention and instilling doubt, delaying urgently needed action. The story of the *sudden death of bees,* presented as mysterious and frightening, risks being a means of exploiting science to distract attention from the real problems and focus it on inconsistent factors. The information available to us is more than sufficient to sound the alarm and at least to apply the precautionary principle in cases where there is still some reasonable and proven uncertainty. [571] A deleterious effect generated by this misinformation is that of wasting enormous economic resources in useless activities, since they are not based on the principles of the scientific method but on the principle of satisfying private interests. In the USA alone, millions of dollars are wasted trying to explain the sudden death of bees or "*colony collapse disorder*". [867] To give a European example, the project entitled *EPILOBEE* discussed above was financed with 3.3 million euros (public funds): overall the project cost more than 5 million euros and involved the use of considerable resources such as 1,350 inspectors. [382] In conclusion, the English acronym "*CCD*" which, as already mentioned, stands for the fanciful expression "*Colony Collapse Disorder*", could be responsibly used as a manifesto of conscious blindness of a planned disaster, that is, to represent unsustainable food production and practices.

INEFFECTIVE MANAGEMENT OF RISK PREVENTION FOR BEES: vested interests instead of the common good

The evident weakness of the control and regulation system in the face of large economic interests such as those of the companies marketing pesticides and cereal seeds made artificially resistant to high concentrations of herbicides (genetically modified maize and soya) is very worrying.
Information on the toxicity of pesticides to bees has many weaknesses. The analytical methods used to obtain information on toxicity focus on acute effects measured over a few hours or a few days. Long-term assessments such as chronic effects are not carried out. The toxicological

assessment is based on the measurement of the dose that kills 50% of the exposed individuals in the laboratory by ingestion or contact (LD_{50}) over one or at most three days. There is a risk of considering a substance as not dangerous when it is capable of killing 49% of bees in less than three days. That is intellectual and economic suicide. It has been known for more than a century that small doses of toxic substances, taken over a long period, can produce fatal effects equal to or greater than those produced by a high dose taken in a short period. It would be appropriate to name this threshold (LD_{50}) "*the value of acceptability according to political and economic criteria*". By becoming a commercial and political concept, the acceptable dose is being monetised. Even the toxicological assessment of risks to humans, through animal experimentation, may be conducted in such a way as to make the results more favourable to predetermined objectives. For example, the choice of laboratory animals more resistant to the effects of endocrine disruptors, or the appropriate choice of the diet administered to the tested animals, may favour the increase in doses necessary to measure adverse effects by thousands of times. [260]

The toxicological assessment for bees, in the case of pesticides, has many limitations.

- It is not carried out on non-target organisms such as other pollinating insects.

- It does not assess chronic, sub-lethal, synergistic and/or additive effects. The fact that intermittent, lifelong exposure to low doses may have equally dangerous effects is underestimated. [419] The methodological error is very gross. To make a comparison, it is like trying to measure the danger of smoking by considering only the single dose capable of killing 50% of smokers. Probably the cigarettes contained in more than 100 packets are necessary. The conclusion of such a study could only be positive for the industry: let's not worry because the probability of dying is negligible. Exposure to pesticides for bees is considered safe when it is below one-tenth of the lethal concentration, despite the fact that countless studies have long shown that chronic exposures, at doses below one-thousandth of the LD_{50}, are deadly, especially when combined with other molecules that enhance their toxicity. [378] It is very difficult to determine the possible sub-lethal effects, i.e. those generated by very low concentrations and exposure for long periods. For example, to generate the same mortality within 48 hours as recorded in bees from a single dose of imidacloprid (60 ng), a repeated exposure over ten days to a 100,000-fold lower dose is sufficient. Ten days can be considered the duration of a flowering, and the lowest concentrations are those measured in flowers, pollen and nectar. Often the nectar and pollen of plants treated with neonicotinoid insecticides (such as sunflower seeds) contain concentrations below 10 ppb (parts per billion). [260] This is 100,000 times lower than the dose that can kill 50% of bees after a single administration, but if this exposure occurs over two weeks it may generate the same lethal effect. The same problem is registered in the case of genetically modified plants whose toxicity, by food, is tested in the laboratory for less than 90 days (on laboratory animals). [557]

- It is carried out on experimental models which, in some cases, do not adequately reflect reality; for example, honey bees consisting of less than one fifth of the number of insects in a colony are used (artificial colonies of less than 10,000 insects are used). In some cases only a few dozen insects are used.

- The protocols for investigating adverse effects on insects and other living organisms do not consider that both the target organisms and all others are exposed to complex and time-varying mixtures, even throughout their life. It is very difficult to measure in advance the synergistic effects, i.e. those generated by different combinations of mixtures, because it is very expensive and often the combinations are so numerous that it is impossible to consider testing only a fraction of them. [419, 497] This is because it is very expensive and often there are so many combinations that it is impossible to think of testing only a fraction of them. In addition, there are hundreds of thousands of other pollutant molecules produced by human activities that interact with pesticides, the multiple synergies of which are virtually impossible to study.

- Sub-lethal effects such as chronic effects are generated by very low concentrations of the order of millionths of a gram per kilogram of matrix such as pollen, nectar or honey. The concentrations are so small that many analytical methods (e.g. high-pressure liquid chromatography with UV analyser for imidacloprid, which is a polar and thermo-labile molecule) are able to measure up to concentrations ten, twenty or more times higher than the residual concentrations in plants and beekeeping products (as already mentioned, these are concentrations capable of generating sub-lethal effects). [363] Therefore, the molecules searched for by some analytical methods, even when they are present at concentrations capable of generating negative effects, are invisible. This aspect must make us reflect on the ability to obtain information (both preventive and retrospectively), with analytical methods that may be unsuitable.
  It is laborious and expensive to use analytical research methods that have sufficient levels of sensitivity to measure low concentrations at which adverse biological effects can be measured. For example, measuring the concentration of neonicotinoids with methods that cannot detect below 1 mg/kg means that contamination levels that can kill bees and generate other adverse effects cannot be detected. [475] The biologically active concentrations of these substances are more than 1,000 times lower (than 1 mg/kg) and are also those that are most likely to be found in matrices such as pollen, nectar and beekeeping products. When neonicotinoid insecticides were first marketed in the 1990s, the best analytical detection limit was 10 ppb (0.01 mg/kg or ppm). [553] This instrumental sensitivity did not allow the detection of concentrations that are the most common in sunflower leaves, nectar and pollen and that are capable of generating effects, from chronic exposure, equivalent to those of single exposures at doses 100 or 1,000 times higher.
  Seeds treated with imidacloprid show concentrations of 3.3 ppb in pollen and 1.9 ppb in sunflower nectar, or 2-4 ppb in maize pollen. It must be remembered that pollen is the main nutrient of the juvenile stages of bees and therefore very low concentrations (less than 10 ppb) are in any case capable of generating problems for insects. Chronic exposure to 0.1 ppb, which in 10 days is equivalent to 12 pg/bee, generates the death of 50% of the individuals. [553] So concentrations 100 times lower than the detection limit of laboratory instruments are able to kill 50% of bees, when they are exposed to contaminants for 10 days. The alarming conclusion is that the effects from chronic exposures are equivalent to those from single dose exposures, but occur at concentrations over 1,000 times lower. In spite of these results, a safety threshold (the concentration below which no dangerous effects to the bee colony should have been recorded) of 20 ppb was suggested in 2002, when concentrations in the order of 0.3 ppb (e.g. in pollen) were already being measured and sub-lethal effects were already known to occur at these much lower concentrations. [553] This freely available scientific knowledge was ignored.

- The effects of the derivatives of the active molecules are not considered, such as the metabolites, which can be much more persistent and equally dangerous. In this regard, it is possible to remember that imidacloprid generates several metabolites, some of which are certainly dangerous also for bees, such as: 5-hydroxy-imidacloprid, 4-hydroxy-imidacloprid, 4-5 hydroxy-imidacloprid, olefin, imidacloprid-guanidine, imidacloprid-urea, and 6-

chloronicotinic acid (this last metabolite is found in 44.4% of the pollen samples examined, when 49.4% contained imidacloprid). [553] The products used in mixtures are also underestimated and often considered harmless without the necessary tests. [419]

- Experimental tests carried out in the field are hardly able to evaluate differences in witness colonies, i.e. in those not exposed to pesticides. The bees to be exposed to the pesticides under study are placed near a field treated with a single molecule (e.g. neonicotinoids in maize) of a size between 2,500 and 10,000 square metres; it must be remembered that bees can move within a radius of a few kilometres, therefore they may prefer to visit wild flowers that are very distant and more attractive, or different crops by exposing themselves to other pesticides. The control colonies, i.e. those theoretically not contaminated by the molecule under study, are not very representative because, even if the experimental hive is placed in an area dedicated to organic farming, bees can move up to 5-10 km away. Therefore, it is practically impossible that they do not come into contact with pesticides. The probability of measuring effects generated by a single molecule, in colonies located in different places, without unwanted and uncontrollable interference from other contaminants is nil. It is therefore very easy to produce results useful for chemical agriculture: no difference in mortality between exposed and unexposed colonies. [307] The methodological error can be easily understood with the following example: it is like trying to measure the effect of tobacco smoke on the respiratory tract by comparing a group of people who smoke a pack of cigarettes a day and live in the city, and a group who smoke with the same intensity but live in the countryside. The result is obvious: the difference in incidence should be attributed to other factors such as air pollution in urban areas. The experimental method should have made comparisons with non-smoking groups.

- The procedures adopted to implement pesticide risk analysis do not consider effects on the whole colony, as they focus on measuring acute effects, which may be recorded in a few hours on a small number of individuals. [1184]

- The risk analysis procedure has been entrusted almost entirely to the companies producing the pesticides themselves, with an obvious conflict of interest. [1184] It is not possible to know the possible position of conflict of interest among the participants in the decision-making tables for the authorization of pesticides. Some documented scandals report conflict of interest rates as high as 75% of the experts. [307]

Other factors that limit our knowledge are: [416]

- The lack of information about pesticides sold and used.
- The impossibility of measuring the concentration of any molecule on the different environmental matrices (e.g. plants, water and soil).
- The long-term effects are largely unknown and not easily investigated a priori. Some molecules remain active in the environment for years.
- Effects on non-target organisms are largely unknown and poorly studied (e.g. in soil).
- The effects of bioaccumulation are poorly understood.
- The interactions between disease, food shortages, eating habits and other factors such as pesticides are poorly understood.

Finally, it is important to point out that the benefits in terms of increased production and for farmers resulting from the use of pesticides are not very evident.

POLLUTION CONTAMINATES WILD FLOWERS, BIRDS AND SOIL INVERTEBRATES

In support of the difficulty of isolating desired factors from unmanageable ones in the field, it is useful to dwell on the fact that bee exposure to pesticides contained in wild plants may be higher than the exposure from crops. For bees, 97% of the exposure to neonicotinoids may result from wildflower pollen rather than from crop pollen. [369] In pollen collected from wild plant species, concentrations of neonocotinoids as high as 200 ng/g may be found (in pollen stored by bees in the hive). [369] The hives were placed in the vicinity of rape fields (seeds treated with neonicotinoids) and wheat fields (seeds treated with neonicotinoids). All soil samples, taken a few months after oilseed rape sowing, contained imidacloprid, thiamethoxam and its metabolite clothianidin; 42.9% of soil samples contained thiacloprid. It should be noted that imidacloprid and thiacloprid had not been used in the three years prior to sampling. These molecules were also found in neighbouring untreated fields (with lower concentrations). Soils cultivated with wheat, whose seeds have been treated with neonicotinoids, also contained these molecules. Clothianidin was found in 100% of the soil samples from wheat, imidacloprid in 75%, thiamethoxam in 50%, and thiacloprid in 25% of the samples. [369] In oilseed rape crops, 100% of pollen samples contained thiamethoxam given in the seeds and it was also contained in 54% of nectar samples (they also contained clothianidin which is its metabolite). The maximum concentration of thiamethoxam in rapeseed pollen was 11.1 ng/g while in nectar it was 13.3 ng/g. Of the pollen samples, 90.5% contained clothianidin and 85.7% contained thiacloprid. 58% of the 43 pollen samples from wild plants near oilseed rape fields contained the same neonicotinoids, such as thiamethoxam (*Heracleum sphondylium* 86 ng/g and *Papaver rhoeas* 64 ng/g). The alarming result of this research is that neonicotinoids are more concentrated in the pollen of wild plants grown near treated fields than in the pollen of treated plants such as oilseed rape. Even imidacloprid, which was not found in oilseed rape pollen, was present in the pollen of wild plants grown nearby (in 11.6% of the wild plant pollen samples). In addition, 20.8% of the wild plant nectar samples contained thiamethoxam, but at lower concentrations than rapeseed nectar. Bee colonies located close to oilseed rape fields contained only 9.9% of pollen from this crop, indicating that they probably visit these flowers to obtain mainly nectar. Most of the pollen collected by bees (62.5%), in this study, belonged to hawthorn (*Crataegus monogyna*) during the month of June and to other species during the month of August [*Epilobium hirsutum* (23.1%) and *Rubus fruticosus* or common bramble (13.5%)]. Of the neonicotinoids present in pollen collected by bees in June, only 3% comes from rapeseed: 97% comes from wild flowers. In general, an average concentration of neonicotinoids of 0.56 ng/g of pollen was recorded (the sub-lethal dose that could generate negative effects on the colony is considered to be around 5.31 ng/g for thiamethoxam and 2.05 ng/g for clothianidin). In pollen collected in August, the concentration of neonicotinoids was reduced to 0.2 ng/g. It wais estimated that 71.8 ng of neonicotinoids per day enter the hive in June and 11.1 ng per day in August. This research confirms that environmental contamination is widespread, that molecules move and concentrate in wild plants. Thus, the exposure to harmful substances at low concentrations and over a lifetime is underestimated, and it is difficult to assess effects on wild pollinators. Wildflowers, because of the high level of diffuse contamination, may pose a greater risk to bees than crops.

In 2014, in the USA, between 79% and 100% of the 36.6 million hectares of corn and between 34% and 44% of the 33.8 million hectares of soybeans had been cultivated with seeds containing neonicotinoids. [370] The sheer size of the agricultural areas where poisonous substances are distributed gives an idea of the importance of this widespread contamination: millions of hectares. Precisely because of the large quantities of pesticides distributed, even with seeds, wild plants are contaminated.

In the USA, pollen collected by bees in non-agricultural areas is contaminated by 29 of the 65 pesticides sought such as (in descending order of occurrence) fungicides (azoxystrobin was found in 93.3% of samples and trifloxystrobin in 63.3% of samples), herbicides (metolachlor was found in 83.3% of samples), pyrethroids (prallethrin and phenothrin), carbamates, neonicotinoids and organophosphates. [370] In pollen collected from wild plants grown in non-agricultural areas pesticides reached a concentration of 317 parts per billion. However, in hives placed in non-agricultural areas, 7.9% of the pollen was soybean and 0.4% corn. [370] In pollen collected from bees located near maize fields with untreated seeds, 31 pesticides were found, at a maximum concentration of 736 parts per billion. In pollen collected from bees located near maize fields, whose seeds had been treated with pesticides, 28 pesticides were found at a maximum concentration of 1,453 parts per billion. [370] In the latter case, the highest concentrations were found, even though most of the pollen was from non-cultivated plants (less than 21% of the pollen was from corn and less than 4.4% from soybeans). Some interesting conclusions from this research are as follows:

- complex mixtures and high concentrations in wild plant pollen are recorded;
- although the hives are placed near cultivated fields, bees prefer to collect most of their pollen from wild flowers.

The areas chosen for this study are representative of a geographical area of the USA, where maize and soya are mainly cultivated. Although bees were able to visit corn and soybean fields, they preferred to collect other types of pollen in all three study areas, including agricultural areas; in the latter, crops accounted for more than two-thirds of the area, within a 2 km radius. Among the pesticides found, most were fungicides that are used, like neonicotinoids, on seeds before sowing (azoxystrobin and trifloxystrobin). It can realistically be assumed that bees will ingest 65 mg of pollen in their lifetime and will come into contact with 1 g of pollen per day: the amount of neonicotinoids to which bees are exposed by ingestion and contact through pollen constitutes a risk. Synergistic and additive effects are also easily predictable and may increase acute and chronic effects. For example, the simultaneous presence of the fungicide propiconazole and neonicotinoids significantly increases the risk of mortality, due to additive and synergistic effects. Fungicides have also been associated with an increased probability of registering pests (e.g. *Nosema ceranea*). Unfortunately, this mixture of poisons will also harm wild insects and other living things, including humans.

Usually pesticides used in the same field in previous years and also those used in neighbouring fields are not considered. For example, imidacloprid moves through the soil, and can be found a year after treatment. Organochlorines and other insecticides are still found thousands of kilometres from where they were used and decades after their last recorded use. Once in the food chain and biochemical fluxes of the biosphere, persistent molecules move rapidly and mat bioconcentrate.

This research shows that field assessment can lead to erroneous results, as even untreated and non-agricultural areas may be a significant source of poisons, comparable to cultivated areas. Therefore, comparing results between colonies located near cultivated fields or areas with lower intensity of agriculture does not allow to discriminate the real risk. This assessment strategy, which in some cases is handled in an amateurish way, is not able to measure effects such as those on the immune system, the endocrine system, fertility or behaviour (i.e. the nervous system).

Some crops, treated with pesticides such as neonicotinoids, for which the agronomic rule of rotation, i.e. alternation with other crops, should be applied, are not alternated with other plants; crop alternation is a good agronomic and ecological practice, but when it is not carried out pesticides accumulate (e.g. maize is repeatedly sown in the same fields for years). After the cultivation of plants treated with imidacloprid, during the following 12 months, concentrations higher than 10 ppb may be found and, in the following year, over 4 ppb. [553]

Both wild and cultivated plants have different nectariferous capacities. Under the best conditions, phacelia (*Phacelia tanacaetifolia*), buckwheat (*Fagopyrum esculentum*) or rape can be grown to produce over 300 kg of honey per hectare. Some wild plants also produce abundant honey (*Taraxacum officinale*, *Centaurea cyanus*). Bees may be exposed to toxic substances present in dozens of different species, both cultivated and wild. To monitor the level of risk, a research conducted in Poland examined the presence of 142 insecticides in 41 samples of nectariferous plants, in the months between May and July 2017 (500 g of flowers were collected for each plant of rape, phacelia, buckwheat, common dandelion, and cornflower). [1222] Twelve insecticides were detected in 19 of 41 flower samples (acetamiprid, chlorantraniliprole, chlorpyrifos-ethyl, clothianidin, cypermethrin, deltamethrin, diazinon, dimethoate, fenpyroximate, imidacloprid, omethoate, and thiacloprid); 17% of flowers contained 2 or 3 insecticides simultaneously. Neonicotinoid insecticides were found in 7 samples and at the maximum concentration of 498 ng/g (thiacloprid). In wild flowers near maize fields, neonicotinoids may be recorded at a concentration of around 10 ng/g. The most frequent residues found in Poland (in 4 flower samples each) were deltamethrin, dimethoate and its metabolite omethoate (with the highest concentration of 49 ng/g). [1222] Dimethoate is found in rape flowers but its use in this crop is prohibited. Eight of the twelve active molecules measured in nectariferous flowers are classified as very dangerous (acute toxicity or LD_{50} orally or by contact below 2 µg/bee for deltamethrin, dimethoate, omethoate, chlorpyrifos-ethyl, cypermethrin, and thiacloprid). More than 47% of agricultural fields record the presence of hazardous insecticides in nectariferous plants (100% of rape flowers tested contained insecticides, while they were not detected in buckwheat). This study confirms that pollinators such as honey bees are exposed to numerous lethal chemicals through pollen and nectar.

In Switzerland (2015), 700 soil samples were analysed to quantify the concentrations of five neonicotinoid insecticides (imidacloprid, clothianidin, thiamethoxam, thiacloprid, acetamiprid). [366] The results are alarming: 93% of soil samples from organically farmed soils contained at least one neonicotinoid; 80% of soils from natural areas contained at least one of the 5 molecules sought (21% contained at least 2 neonicotinoids); 100% of soil samples occupied by conventional agriculture contained these poisons (imidacloprid at a maximum concentration of 29.72 parts per billion or ppb and clothianidin at a maximum concentration of 20.95 ppb or millionth of a gram per kilogram). [366] 46% of soils from conventional agriculture contained at least 2 neonicotinoid insecticides. The concentrations of pesticides found may generate negative effects on invertebrates. In fields cultivated according to traditional or integrated farming methods, it was found that between 5.3% and 8.6% of the 94 species of invertebrates (arthropods and annelids) in the soil are exposed to lethal doses of clothianidin, and between 31.6% and 41.2% of the 94 species are exposed to doses with sub-lethal effects. Even in organic farming, at least between 1.3% and 6.8% of useful soil invertebrates are exposed to sub-lethal doses. The concentrations recorded in the soil are not lethal to the 12 species that are considered harmful to farmers. The molecules were also found in plants present in these areas and in 14 of the 16 samples of seeds used in organic farming, at a maximum concentration of 81.9 ppb: clothianidin and imidacloprid were measured in 62.5% of the seeds used in organic farming. These poisons are also found in plants used in organic farming. The study shows that there is widespread contamination which can harm useful non-target species such as soil invertebrates and birds. [366] Species used in organic farming to control plant pests are also dangerously contaminated. In conclusion, soils and plants in Switzerland are contaminated by neonicotinoids, leading to a chronic exposure with lethal, sub-lethal, additive and synergistic effects. Chemical bombs with a delayed effect are systematically distributed in the environment; they can move in the food chain and in water, and accumulate. In order to protect biodiversity, it could be suggested, in addition to banning the use of these molecules, to leave a fraction of the agricultural area uncultivated, with hedges, meadows and wild plants. To achieve this goal, farmers could be encouraged, as it happens in Switzerland or Norway. [366]

The release of molecules such as neonicotinoid insecticides into the environment results in dangerous concentrations in water, sediments, and honey with toxic and sub-lethal effects also on birds and other wild organisms. Neonicotinoids may be found in the blood of insectivorous birds, diurnal birds of prey, owls; in the liver of granivorous birds such as the wild turkey (*Meleagris gallopavo*) and in the feathers of sparrows (*Passer domesticus*). [1214] The concentration of neonicotinoid insecticides in birds living in the vicinity of treated fields is higher, as is to be expected.

Research conducted in Canada between the years 2015 and 2018 detected insecticides in the cloacal fluid of some nectar-feeding bird species such as honeybees: the hummingbirds. [1214] Insecticides were measured in hummingbird birds, honey, water, and sediments at several Canadian sites where they were primarily used in the blueberry cultivation. Cloacal fluid was examined from the following bird species: red-throated hummingbird (*Selasphorus rufus*), Anna's hummingbird (*Calypte anna*), calliope hummingbird (*Selasphorus calliope*), black-throated hummingbird (*Archilocus alexandri*), and ruby-throated hummingbird (*Archilocus colubris*). The following insecticides were searched for: imidacloprid, clothianidin, acetamiprid, and flupyradifuron. Flupyradifuron is an insecticide (a butenolide) licensed in Canada in 2015 for berries, vegetables, and cereals. In 26.5% of the 49 hummingbird samples in 2017 and 11 in 2018, the molecules searched for were found to be: imidacloprid (up to 0.9 ng/mL), clothianidin (0.29 ng/mL), acetamiprid (1.2 ng/mL), and flupyradifuron (4.6 ng/mL). [1214] Honey was also contaminated with the insecticides searched: up to 3.1 ng/mL of imidacloprid, 3.2 ng/mL of clothianidin, and 2.2 ng/mL of flupyradifuron. Water was contaminated with imidacloprid (up to 1,459 ng/L) and clothianidin (up to 49 ng/L); sediments: between 0.36 and 5.66 ng/g (of dry matter) for imidacloprid, between 0.3 and 0.88 ng/g (of dry matter) for clothianidin, while acetamiprid is measured at a concentration of 0.73 ng/g (of dry matter) at one site. At four sites of the eleven monitored ones, the waters recorded concentrations of neonicotinoids (imidacloprid) considered hazardous (above 200 ng/L; concentrations of 130 ng/L can be easily measured in surface waters). [1214] These results confirm the diffusion of the dangerous molecules both in the abiotic component (water and sediments) and in the biotic component (birds), therefore unknown summative and multiplicative effects are foreseeable and that should worry us.

PESTICIDES ARE PERSISTENT

The persistence of pesticides generates a lot of adverse effects such as the lasting contamination of non-target species. A study carried out in India examined the persistence of the toxicity of some pesticides used in sunflower cultivation. The flowers of these plants can produce both pollen and nectar, which are used by many insects: hymenoptera (such as bees), diptera (flies), lepidoptera (butterflies) and beetles. [663] The use of insecticides and other pesticides exposes many insect species to harmful substances. To study the persistence of different molecules, bees (*Apis mellifera*) were exposed to six pesticides used at the maximum recommended doses (spray on the leaves of flowering sunflower plants). The insecticide molecules were distributed when at least 50% of the sunflower flowers were in full bloom: azadirachtin (used in organic farming and extracted from the seeds of the Neem tree or *Azadirachta indica*), dimethoate (a phosphoranic), cypermethrin (a pyrethroid), fipronil, imidacloprid (a neonicotinoid), and indoxicarb. [664] High mortality rates were recorded even nine days after the treatment, which was carried out under the conditions of use of the pesticides recommended by the manufacturers. In particular, the persistence of toxicity to bees was found to be, in decreasing order: fipronil > imidacloprid > cypermethrin > dimethoate > indoxacarb > azadirachtin. [663] This research shows that insects visiting sunflower flowers are exposed to lethal doses of insecticides even nine days after distribution. Other studies performed for longer times have shown that, for example, the toxicity of fipronil is high even 28 days after treatment. [663] Fipronil and imidacloprid were found to have the highest residual toxicity and therefore the most persistent adverse effects. All active molecules tested were found to be very toxic to farmed bees, including azadirachtin.

The detection of banned pesticides in many matrices and the recording of the presence of active substances in drinking water at high concentrations rarely lead to serious and effective measures. The reference legislation (EC Regulation No. 1107/2009) states that "*An active substance, an agronomic antidote or a synergist shall only be approved if it is not considered to be a persistent, bioaccumulating or toxic substance.*". [265] A substance, such as the active ingredient of a pesticide, is considered persistent based on the half-life, which indicates the days it takes for the molecule to break down, degrade, or turn into something else by reducing the initial concentration of 50% (degradation may be aided by the exposure to light, metabolism, or soil microorganisms). This concept still does not consider the well-known fact that many derivatives (e.g. some metabolites) are as dangerous or more dangerous than the starting products, they are more persistent than the starting products and may show adverse effects with new mechanisms of action and on non-target organisms. Returning to Community legislation, *a substance,* such as the active ingredient of a pesticide, *is considered persistent if*:

— *the half-life in seawater is higher than 60 days,*
— *the half-life in fresh or estuarine water is greater than 40 days,*
— *the half-life in marine sediments is higher than 180 days,*
— *the half-life in freshwater or estuarine sediments is higher than 120 days,*
— *the half-life in soil is higher than 120 days.*

According to the legislation, if a substance or its equally dangerous metabolites remain in the environment such as soil (with a half-life of more than three months) they should not be authorized. [553] With the intention of highlighting the inability to enforce the rules developed for our protection, it is useful to examine the half-life in the soil of some active molecules of pesticides: [447]

- For imidacloprid the half-life of the soil concentration is up to 1,230 days, i.e. more than 3 years (between 40 and 997 according to other estimates). [678] In the official documents accompanying imidacloprid, half-lives between 188±25 and 249±40 days are stated, calculated in two different soil types, which are in any case longer than the 120 days foreseen by the legislation. [359, 553]
- For dinotefuran it is about 138 days. [678]
- For clothianidin it is over 500 days in water and between one and three years in soil (between 148 and 1,155 days). [368, 442, 678]
- For aldrin it is at least 365 days.
- For chlordane it is at least 350 days.
- For DDT it is at least 2,000 days. Some studies report that DDT and its metabolites (DDE and DDD) can remain in the soil for more than 40 years. [541]
- For dieldrin or hexachlorobenzene it is at least 1,000 days.
- For fenarimol it is at least 360 days.
- For heptachlor it is at least 250 days.
- For imazalil it is at least 150 days.
- For monuron it is 170 days.
- For paraquat it is 1,000 days.
- For the propazine it is 135 days.

- For tebuthiuron it is 360 days.
- For thiabendazole it is 403 days.
- For benomyl, it is 240 days. [448]

This knowledge and common sense would suggest, for many pesticides, to exclude their registration and use because they are persistent. The facts confirm that the economic strength inherent in the sale of pesticides is much greater than that of States and supranational Governments (e.g. in Europe) to defend health and the environment.

PESTICIDE RESISTANCE

Resistance to the lethal effects of pesticides has been known for a long time: since 1940 more than 1,000 species have recorded the appearance of resistance to at least one active ingredient. The increase in this phenomenon affects biodiversity and creates additional problems for food production and health.

Already in the 1980s, at least 520 species of insect and mite pests were identified as resistant to pesticides, as well as at least 150 weeds. [502] By 1970, resistance to the herbicide atrazine had been recorded at least in one weed. One of the first reports of plants becoming resistant to the herbicide glyphosate dates back to 1996; in 2011 at least 19 weed species were reported to be able to tolerate the presence of this dangerous molecule. [761] Worldwide in 2014, 432 plants were recorded as having become resistant to at least one herbicide. This tolerance generates enormous expenses: in the case of cotton, in the 1980s, this cost was estimated between $45 and $120 per hectare per year. The appearance of pesticide-resistant pathogens increases costs by 10% and 25% of those generated by the use of pesticides themselves. [502]

Some of the most commonly used acaricides by beekeepers have recorded the development of resistant mites. [859] For more than 10 years it has been observed that the pyrethroid tau-fluvalinate has become ineffective against the *Varroa* mite in several places on the Planet. Populations of mites resistant to another acaricide used by beekeepers, coumaphos, have been known for at least 20 years (since 2001). [602] Another molecule, amitraz, is also known to have selected resistant mites, although in many countries its use in beekeeping has been banned for years (in the USA since 1994).

MISINFORMATION IS MORALLY UNACCEPTABLE

The economic and financial rules that have temporarily overwhelmed the biological and physical ones produce environmental disasters and diseases. Science plays a key role in this process as it is partly instrumentalized, manipulated or designed to maintain conditions favourable to industry. [334] The scientific World should find the strength to detach itself completely from deleterious influences that undermine its foundations. Over time, entrepreneurs in the agro-chemical industry have implemented many unfair, incorrect but successful strategies with the aim of achieving economic results. In this regard, there is an interesting literature that exposes some of the problems. [260, 280, 307]

The chemical risk generated by pollution and in particular by the use of poisons such as pesticides is underestimated both in terms of its effects on ecosystems (e.g. pollinators) and on human health. [682] The lack of attention paid to risks is facilitated by misinformation. The distorted process of misinformation is worse than not informing at all. Manipulation of the information has become a new weapon of mass disinformation greatly enhanced by ubiquitous vehicle tools such as television and the internet. The industries that produce and market pesticides apply various communication strategies to deny the evidence, distract attention and instil doubt. [261, 722] These are real investments in disinformation campaigns which, by exploiting certain themes, which are the workhorses of advertising campaigns, have told distorted tales. Several topics could be suggested to be developed in order to favour business interests. Some of them are proposed.

- *Pesticides are needed to feed the increasingly hungry Planet*. In the US, insecticide use, in the 50 years since World War II, has increased by a factor of 10 and crop losses have more than doubled. The corn crop may be used as a model to dispel this myth. In the USA, the use of organophosphate insecticides has increased more than 1,000-fold but losses due to insects have increased from 3.5% to 12%.

- *Farmers have no alternative to the use of pesticides*. In truth, there are many alternatives to the use of pesticides, such as crop rotation, mechanical weed eradication systems, biological control, intercropping, sustainable or less polluting farming methods (e.g. organic farming). The great challenge of this era is to build a new agronomic culture that is independent of the chemical multinationals.

- *Pesticides are not dangerous*. This topic has been widely discussed previously, but it can be further highlighted that in many countries of the World the complaints made by beekeepers to the Authorities should be sufficient to activate a concrete action.

- *Bans on the use of certain pesticides such as neonicotinoids are not based on scientific evidence*. Unfortunately, it would have been enough to listen to the complaints made by beekeepers all over the World to justify banning certain unsustainable agricultural practices, instead of waiting for scientific confirmation as well. Because of this waiting, actions were taken late, such as the ban on the use of DDT or neonicotinoids. In spite of the numerous reports of insect poisoning by beekeepers (such as those reported due to the damage caused by neonicotinoids used in maize and sunflower fields in France in 1994, in Italy in 2008 or in Germany in 2009), the scientific evaluation has been awaited, which has been long and expensive.

- *The effects of pesticides belonging to the same category, such as the different active molecules of neonicotinoids, should not be considered similar.* This strategy tends to underestimate additive, sub-lethal and synergistic effects.

- *New molecules are also better because smaller quantities are used.* Banned molecules can be replaced by others with a similar chemical structure and equivalent mechanism of action. History has taught us that the active molecules of pesticides have always, over a period of 60 years, been replaced by others with greater toxicity, so smaller quantities can be used. However, over time, there has been a steady trend towards increasing rather than reducing the use of these poisonous substances and the overall equivalent toxicity has certainly increased.

- *The concentrations recorded in the environment are not dangerous*. This statement does not consider the multiple information and cumulative effects, metabolites or substances that can move within the biosphere and within an organism. The risk analysis underestimates the damage to children, women, weaker people, and the long-term effects. [570] The same applies to animals such as pollinating insects. In many cases, history has taught us that the estimated hazard has been underestimated. It may be recalled that the doses of active molecules found in pollen and nectar are significant and dangerous. The exposure to pesticides used in seeds has also been underestimated, despite the fact that many beekeepers were quick to point out their devastating effects.

- *Using best practices in pesticide distribution reduces the risk so much that we no longer have to care*. This message is repeated in different ways and strategies. For example, in order to diminish the reports of bee mortality due to the sowing of seeds treated with systemic and long-lasting pesticides, the use of sowing machines that produce less dust has been proposed. This is an ineffective solution given that these molecules are persistent, bioaccumulative, mobile, and very toxic. These molecules are distributed without assessing economic thresholds for intervention. Therefore, a sort of *preventive pasteurization of the biosphere* is carried out: in this case, plants will be poisonous for their whole life and some substances will continue to carry out negative biological actions in the soil and in the food chain for years (fortunately, in an ironic sense, no chemical weapons capable of *sterilizing the biosphere*, i.e. capable of exterminating all forms of life, are used; we limit ourselves to damaging most of them, by carrying out a pasteurization).

- If *the correct way of use is* followed, the risks are reduced to below unacceptable thresholds, i.e. the substances become harmless. This shifts the responsibility to the users.

- *The scientific assessments made by the authorities are based on unreliable or unconfirmed data*. This message is supported by some stakeholders with an obvious conflict of interest. Unfortunately, the chronicles constantly record the opposite. The toxicological assessment studies submitted by companies, in the preliminary phase of marketing authorization, are secret and scientific publications are used which are run by researchers with an obvious conflict of interest.

- *It is the dose that makes the poison*. This vision starts from the assumption that nothing is poison but everything can become poison if the dose is sufficient. Below a threshold you can be safe. In reality, some substances show effects that cannot be measured at higher concentrations, such as endocrine disruptors and carcinogens. Partly because of this misinterpretation, the concept of the Acceptable Daily Intake (ADI) was born. The ADI is the amount of substance that can theoretically be taken throughout life without any risk to health. As you can guess, this is a political assessment and a twist. Zero risk does not exist, especially when you are exposed to poisons such as insecticides or other neurotoxic, carcinogenic, and endocrine disrupting molecules. Only by not taking substances created to kill living beings can we be sure of not generating negative effects. This way of dealing with the problem is part of the strategy of leaving the task of demonstrating the chronic danger (especially in small doses taken occasionally or constantly over time), the sub-lethal, additive and synergistic effects to subjects outside the business interests. In this regard, it is useful to recall the case of parathion for which an acceptable daily intake of 0.004 mg per kilogram of body weight was assessed. According to this risk assessment, a 70 kg man could theoretically have ingested 0.28 mg every day for life without experiencing health problems. [280] In order to estimate the acceptable daily intake, the average intake of a food in the population within a State is calculated and the concentrations of the toxic substance found in food are examined (e.g. parathion in olive oil). Finally, the toxicological assessment refers to a 70 kg man. This method is questionable as it is not very protective of health and offers the possibility of varying the toxicological assessment in relation to the eating habits and consequently safety concentrations in food change. Precisely because this methodology lends itself to easy interpretations, it follows that even though all the same risk assessment protocols are applied, each State decides on a maximum concentration limit allowed in different foods that is different from the others (in some cases there are also differences between territories within the same Nation).

Unfortunately, living beings are exposed throughout their lives to small doses of poisons present in complex and unpredictable mixtures in the air, water, food, clothing, medicines, and cosmetics. The combination of many molecules in small doses produces multiplicative effects that are often unknown and, in any case, difficult to predict in advance. Many substances such as some pesticides are endocrine disruptors, so they can have devastating effects at very low doses. A study conducted in the USA (*Center for Disease Control and Prevention*) determined the presence in the bodies of Americans of most of the 212 substances tested, including 44 pesticides: they were found in amniotic fluids, urine, blood, and breast milk. One insecticide, DDT, which has been banned in the USA since 1972, was found in 85% of people tested.

In conclusion, the principle according to which it is the dose that turns a substance to a poison certainly does not apply to pesticides, which are endocrine disruptors and even mutagenic, i.e. potentially carcinogenic pesticides. In fact, molecules that interfere with the more than 50 hormones in the human body may exert their action at very low doses: parts per million or parts per billion. This negative action is also expressed on the hormones of other animals such as insects. Hormones and their interferents do not follow the rules of toxicology used to examine the hazard of pesticides, which is therefore ineffective in measuring these effects.

- The *authorities protect us sufficiently*. A weak point in the relationship between the public and private sectors is well represented by the expression "*revolving doors*". Politicians (but also many public managers such as those in the health sector) are allowed to work simultaneously or alternately in both the public and private sectors, creating blatant conflicts of interest. In this way, real crimes are favoured to the detriment of the community and of the indispensable services of nature. This situation of conflict also expresses another deviation: the law is interpreted for friends and applied to enemies.

- *Genetically modified plants and other genetically modified organisms will reduce dependence on pesticides*. Most of the companies that sell genetically modified plants are those that produce pesticides. More than 80% of the genetically modified plants grown in the World are designed to tolerate an increased use of pesticides. It follows that some pesticides, those subject to the modification that involves greater tolerance to phytotoxic effects on cultivation (e.g. corn, soy, cotton), are used in greater quantities (e.g. herbicides). Plants that produce insecticides independently have also revealed many criticalities. As an example, the cotton cultivated in India, which supports 50% of the World production of this raw material, in spite of the genetic modification that allows to produce in the tissues the toxins of the soil microorganism (*Bacillus thuringiensis*) with insecticidal action, has registered a reduction in productivity and the cost of pesticides has increased. In India, these facts have generated an increase in suicides of farmers due to debt.

  It is also important to reflect on another critical issue. In 2009, in the USA, 93% of soybean plants and 80% of the corn plants grown were genetically modified to be resistant to the herbicide glyphosate. Weeds have become resistant to this herbicide so genetically modified plants do not have the proposed benefits [insects have also been reported to have become tolerant to insecticidal toxins (*Bacillus thuringiensis)* as has happened in corn]. To overcome this obstacle, the companies producing transgenic plants have proposed seeds with a simultaneous resistance to glyphosate and another herbicide: dicamba. Dicamba is mainly absorbed through the leaves, but also by the roots, and is transferred throughout the plant via the lymphatic circulation. [262] This molecule is very dangerous and has also caused damage to untreated fields. The agribusiness giants are faced with thousands of complaints from damaged farmers, a number of lawsuits brought by various States and a series of legislative restrictions which we hope will reduce its spread.

To counter the spread of weeds resistant to herbicides such as glyphosate, another category of genetically modified plants has also been produced, those resistant to a different herbicide: 2,4-D. In the USA, maize with this new resistance was approved in 2014 and therefore an increase in the use of this herbicide can be expected. Maize resistant to the 2,4-D herbicide is simultaneously resistant to glyphosate as well. Resistance to 2,4-D has also been conferred on soya. Consequently, genetically modified plants lead to an increase in the use of the pesticide marketed by the same companies that created the transgenic plants.

It must be remembered that glyphosate has been classified as a probable human carcinogen while 2,4-D is suspected of being an endocrine disruptor and has been associated with cancer and reproductive problems. A sad piece of information in this regard is the use in warfare of herbicides such as 2,4-D or 2,4-dichlorophenoxyacetic acid to destroy crops and thus starve the enemy (e.g. by the British in Malaysia in the 1950s and later by the USA in Vietnam). [280] A concluding message from this story is that plants made resistant to herbicides through genetic modification have generated an increase in the use of the chemical arsenal that is harmful to our health. For example, in fields of cotton or soybeans resistant to herbicides such as glyphosate, the total amount of pesticides used has increased up to three times. [175] A foreseeable negative consequence has been the artificial selection of herbicide-resistant plants. For example, sorghum has become a plant considered a super weed, as it has become resistant to glyphosate and is infesting vast areas. The risk is to fall into the temptation of fighting super weeds with new super pesticides. This strategy leads to a disproportionate and ineffective increase in the use of poisons. In conclusion, genetically modified plants have been presented as an option for reducing pesticide use, but the information available to us shows exactly the opposite: the cultivation of herbicide-resistant genetically modified plants has seen an increase in the quantities distributed. [175]

- *We are getting rid of pesticides*. In fact, the pesticide market is growing all the time and at least 20% is not used for agricultural purposes.

- *Pesticides and/or genetically modified plants are a solution to climate change*. Technological applications of plants that can resist drought, salinity or other climate changes are not currently widespread.

- *Other factors are equally or more responsible for the problems recorded*. With this strategy one tries to divert attention and distribute it over many different factors, often of lesser importance but which are presented with the same or higher hierarchy. For example, the cause of the tumour is not the substance but genetic factors that are therefore more important. Alternatively, attention is shifted to individual behaviour (e.g. farmers who do not operate according to best practices) or lifestyles.

- *New and more in-depth scientific research is needed.* Lack of knowledge is a reason that is used all too often, that is, even when there is actually sufficient evidence. [869] This strategy was used by the tobacco industry, by the asbestos industry and is still being systematically applied today. [260, 280] Unfortunately, even public organisations with authority in this area are attracting attention and resources with this kind of message and at the same time delaying the necessary action. Regarding pollinators and in particular the causes that generate the decline of insects, recorded on a planetary scale, the Entomological Society of America (ESA) officially pronounces itself with precisely this type of message (12 February 2019): we do not have sufficient evidence to justify action. [374] Unfortunately, even authoritative organizations benefit from (legal) funding from large corporations, such as those that produce pesticides for the benefit, in this example, of the American Entomological Society. [307]

- *The investment required to design a new pesticide requires safeguards such as secrecy of eco-toxicological test results.* This claim is often used to prevent access to information and to promote a lack of transparency in authorization and decision-making processes. It is true that the investments may be substantial but the possible gains are enormous. It may have cost €50 million to develop imidacloprid, but as early as 1998, sales of this insecticide were already bringing in over €409 million per year (€556 million in 2007). [553] Any restriction of access to information on risks, when it comes to the health of everyone, should be prohibited.

- *The toxicity measured in animals allows us to assume that there is no risk to humans.* Many aspects, such as the possible increased susceptibility of humans to exposure to certain hazardous substances, are superficially underestimated.

The agro-chemical industry is well supported by a propaganda arsenal capable of orchestrating diabolically designed disinformation campaigns. Special media battles are waged by hiring experts against researchers who have published work that is troublesome or damaging to the business. Independent researchers may be approached in different ways: they are offered advice and funding, or they are attacked directly with publications criticising their results. [307]

THE ROLE OF SCIENCE FOR DEMOCRACY

Unfortunately, science supported by strong economic interests, which in many cases is more correct to call *pseudo-science* or better *servile science*, continues to reinforce the thesis that it is not yet proven that pesticides may be the cause of increased mortality of bees and insects. In the case of farmed bee mortality the focus is shifted to a myriad of causes such as parasites, climate, or phantom enemies (in the latter case the causes of "sudden" death should still be discovered); this becomes an opportunity to solicit funding to delay the obvious and urgent action. The use of science as a tool to support and defend commercial interests has been well documented. Industry dictates the law and finances scientific research used to defend economic interests. The literature is full of documentations that describe this sad and shameful chronicle that is very current. [260,295, 372, 397, 569, 570, 680] The art lies in creating and spreading doubt and distributing attention to a myriad of factors that are much less important but presented with arbitrary hierarchies. These are campaigns of deliberate distortion and deception of information. In some cases we can speak of real mercenaries of science, i.e. of a science lacking conscience and impartiality: therefore it cannot be considered as such. Tricks can be employed in the

experimental method or in the statistical interpretation that can hardly be detected by non-experts. [557] So these are diabolical strategies, in some cases discovered and denounced. [280, 372, 397] Indeed, research may be conducted in a cunning manner with the intent to conceal and not to reveal. Laboratories may have links with industry as a result of research project funding, consultancy or other more or less transparent pressures. A formidable machine for organized disinformation is set in motion. Industry funds hundreds of studies and research projects that aim to support their cause while at the same time confusing, distracting, and diverting attention to other factors. The weight of independent publications is reduced by occupying the field of scientific literature with servile literature. A disinformation campaign is implemented and doubt is cultivated, one of the pillars of pseudo-science in the service of commercial interests. Moreover, a link is created with internationally renowned experts with the consequent advantages, especially in case of need. In some cases, those who are supposed to be experts are actually experts in other fields and are therefore unable to carry out the evaluations required from them. [553]
Strategies to misinform are very powerful, in fact they have been used by the tobacco industry or to cultivate climate-skepticism. Some important strategies on which disinformation is played are given below. [260, 280, 307, 553]

- Funding scientific research in a way that fuels uncertainty and disagreement in the scientific literature. Scientific production financed by the industrial world can be very substantial; the case of the tobacco industry is famous in the news: more than 6,000 studies financed between 1950 and 1998, for an investment of more than 300 million dollars. The invasion of the scientific literature by privately funded studies, which are not necessarily uninterested, affects independent researchers themselves. A culture driven by specific entrepreneurial goals is generated. The opposite of what the scientific method should produce: creating knowledge above the parties and in a free way. One of the most refined strategies is to mix private and public financial resources with the intention of driving public research. In this way one obtains the advantage of saving economic resources to direct and condition the results of scientific research which, apparently, is more independent than that sponsored exclusively by private companies. Another result is to affix the logo, the image of public research on that which is actually orchestrated by private companies. Public research is privatised, in an inconspicuous way, delaying the production of inconvenient results, and we obtain the amplification of the number of scientific articles published on a subject (e.g. the sudden and unexpected death of bees generated by unknown factors or caused by dozens of equivalent factors) rather than another (e.g. insecticides). So you get a super-representation of less important aspects that influence the researchers themselves and anyone who has to deal with the work of bibliographic research. It becomes easier to cite the most numerous works. In many areas, investments by the private sector have overtaken those of the public sector, as has happened in the USA in agricultural research. In areas where there is a large gap between the public and private sectors it becomes difficult to contrast or otherwise investigate very technical aspects when the difference, in terms of knowledge and skills, becomes enormous. It is difficult to keep the pursuit balanced and without impropriety when the controllers travel by bicycle and the controlled ones by supersonic aircraft. In many areas of science having a strong public research is a necessity for the good of all.

  Private companies can finance public research and make very big donations. In a sometimes invisible way, it is possible to influence apparently independent parties. In addition, as already mentioned, there is another advantage: research is financed that is also partly supported by public funds, so it becomes a profitable investment too. Public resources, which can be more substantial than private ones, are directed towards results useful to private individuals thanks to donations. [749]

- Funding the popular press and mass media. This distracts attention from more important issues. In the non-scientific press it is also easier to publish incorrect and biased information and, at the same time, to ensure its wider dissemination. This strategy includes filling the internet and the non-scientific press ("grey" press) with pseudo-scientific arguments and legends. The continuous hammering on the multifactorial nature of mortality in bees and pollinators, or on the need to discover a new factor are effective examples.

- Making the results of public research not accessible for free to everyone. Among the many inconsistencies of the current system of public research it is important to highlight a particularly painful note: researchers can choose whether to publish their results in scientific journals available on the internet for free, i.e. read by anyone without charge (*open access*) or in journals with a subscription to pay for. Research groups that receive even a small amount of public support should be prohibited from publishing their results in journals that charge readers. Publishing in journals to which readers have to pay for access is a further obstacle to the dissemination of information. Publicly funded research should be accessible to all and also published in the official languages of the funding countries.
Another critical aspect concerns the use of results obtained with public funds. All over the World there are laws authorizing Universities to register the results of public research with patents shared with private companies. [845] In this way, the social benefits of a common intellectual property are privatized, turning into a great source of profit for a few entrepreneurs.

- Use weak and unsuitable scientific methods and protocols to prove what has been published. Unfortunately, only very specialised experts in the field are able to unravel this type of pseudo-scientific information. Experiments carried out in the field to demonstrate that a treatment is not dangerous (e.g. neonicotinoids in rape) require the positioning of few beehives in the vicinity of a treated plot of land of few thousand square metres. [475] Bees, however, have a range of action of several kilometres, i.e. at least 10,000 times greater. It is now unthinkable to hope to find in Europe, but not only there, such vast areas uncontaminated by herbicides or insecticides. Therefore, bees at a distance from the treated field will have the same mortality rate as those found in hives located nearby, since the area in which they move is the same and they are all equally exposed to complex mixtures. This confirms what was intended: bees exposed to pesticides do not record different damage or problems from those considered unexposed.
The probability of detecting pathogens or problems in the colony depends on several factors. In general, the sampling method and sample size influences the level of accuracy and reliability of the results that can be obtained. For example, if the objective is to detect the presence of infected bees, when only 5% show signs of the parasite, with 95% probability, at least 59 bees should be sampled. But if it is necessary to try to detect signs of disease with 99% probability, when signs are evident in only 1% of the insects, it will be necessary to sample at least 459 bees. [480] Thus, sample size may make detection of a problem statistically likely or unlikely and thus make the difference between a positive or negative result. The conclusion is that the application of simple

measures, which have a statistical significance, may or may not facilitate the concealment of certain results.

- Writing scientific articles by reviewing only those publications that report conclusions favourable to the proposed thesis and distracting attention by diluting it to other causes (e.g. other equally dangerous pesticides) are issues that have already been discussed. [475]

- Recruit pseudo-experts who will take the floor in newspapers or on television programmes. They could be ironically termed *differently qualified consultants* or rather presumptuous ignoramuses like those in the ranks of climate change or environmental disaster deniers. [680] Unfortunately the incompetent propaganda of the doubt industry has enormous economic resources at its disposal and this is undoubtedly a great waste.

- Attack people with press or legal threats. The strategy is most effective when attacking people rather than the organisations they belong to, if they hold views that are bothersome and contrary to commercial interests. [553] Independent researchers can find many obstacles in supporting results contrary to the interests of big companies that result in much more difficult careers (e.g. reduced funding). [557] We report the now famous case in which the discoverer of the effects of lead on the brain was persecuted on the basis of unfounded accusations of dishonesty. [570]

- Harmful secrets. The analyses necessary to establish bans, maximum permitted concentrations, and crops on which to use the various pesticides in the World are the result of research conducted directly or indirectly by the manufacturing companies. [682] When industry finances science and, at the same time, independent science does not have sufficient funds and resources, a very dangerous imbalance is generated. The documents needed to decide whether a product is useful or not, and how dangerous it is, are designed by the stakeholders, namely the entrepreneurs who market them. The studies used to document the risks to human health and the eco-toxicological studies carried out before a substance is placed on the market are often not published in scientific journals. [280] Strangely, this information is classified as a trade secret and therefore confidential. The secrecy of this information effectively puts it outside the scientific domain, as one of its main aspects is missing: comparison and validation. Only the results that companies decide to make public are published. This way of doing things reinforces the power of agro-chemicals. Secrecy is only useful to entrepreneurs and harms public health, yet it is so throughout the Planet. The names of the experts in the institutional decision-making tables that will evaluate, at the European level and in every single country, are considered confidential and secret. Therefore, it is not possible to know the names of those who protect public health. In truth, these names are not secret to everyone, for example they are known to agro-chemical companies and politicians, so they may be subject to pressure.

The strategies just presented are just a few of the possibilities available to those with enormous economic resources.

AN ALARMING GAP BETWEEN THE RESOURCES IN THE FIELD

The independent scientific community (as it works in public research centres and does not accept funding from agro-chemical companies) that deals with bee toxicology in the World is tiny. It is probably a few hundred researchers at most. In 12 months (December 2018-December 2019) there have been 162 scientific articles published (on *PubMed*) that have "*Apis mellifera*" in the title and 14 that have the words "*Apis mellifera*" and "*pesticide*". To get an idea of the difference in resources involved, a company like *Monsanto* that is a manufacturer of pesticides and genetically modified seeds has at least 20,700 employees and reported sales of over $14 billion (2017). [377] The *Du Pont* company, which is in the same field, probably had at least 60,000 employees (2005), the *Syngenta* company at least 27,000 employees (2017) and sales of over $73 billion. [380, 381] The agro-chemical company *Bayer*, in 2017, had over 99,000 employees and sales of at least $35 billion. [384] These numbers are an indicator of the strength of the agro-chemical industry that produces pesticides, seeds, and genetically modified plants. Each of them is a giant with representatives in every corner of the Planet. In comparison, the World of independent scientific research and the public bodies responsible for national controls are very small and less aggressive organisations. When it comes to defending their interests, the various agro-chemical companies form organisations with common goals and are therefore even stronger and more fearsome.

Another aspect in favour of big companies is the following: external experts (e.g. university researchers), in many cases, are not paid to carry out the review of toxicological risk documentation, so those sponsored by the industries will have many fewer obstacles.

We must always remember that it only takes a few seconds to destroy a soil that takes hundreds of years to regenerate and that an extinct species means the definitive cancellation of a delicate balance that is the result of millions of years of work: that of natural selection.

THE ROLE OF PUBLIC AUTHORITIES IN PROTECTING COLLECTIVE INTERESTS IS CONSTANTLY BEING UNDERMINED

The weakening of the function of the State to protect collective interests is a very alarming aspect. The public bodies responsible for protecting the environment, health, and democracy are being corroded by economic and financial rules designed to benefit a few entrepreneurs in the short term. The strategies applied to weaken the public system are multiple and could be the subject of hundreds of pages of in-depth analysis. [260, 280, 307, 741] In some countries of the World the agencies in charge of authorizing pesticides are financed, in a transparent and legal way, by the chemical companies that produce the molecules to be marketed. The group of experts, which from time to time has to evaluate whether to authorize the marketing of a pesticide in a Continent or in a Country, is composed of few people, who mostly represent the interests of the producing companies. External experts (e.g. university researchers) may have received funding or have been consultants for the companies producing the pesticides. Below are some aspects that should make us reflect.

- Private stakeholders may occupy positions of power in the public bodies responsible for authorizing, influencing or supporting the use of pesticides (e.g. politicians). In the USA, where the economic resources used by private individuals to finance politicians must in part be declared, it turns out that we are talking about investments of tens of millions of euros per year supported by each agro-chemical company. This type of conditioning is called "lobbying" and is aimed at influencing political choices. At the same time, numerous scandals have been recorded concerning managers and experts who, alternately over time, have occupied crucial roles in relation to control in public bodies and private companies. In this way, the controlled becomes one's own controller. The external experts, in the course of their career, may have been alternately employees of chemical companies (for which they compiled the documentation necessary to request marketing) and employees of the bodies responsible for issuing authorizations for the same molecules. Being able to sit alternately on both sides of the decision-making table is unacceptable. To occupy the working tables organized to evaluate the safety and danger of pesticides and to be numerically and economically advantaged is a scandalous critical point. In such cases, the institutions indulge in ethical laxity which allows private companies to control themselves. The private companies prepare the documentation on the toxicological assessment and their experts assess them in the guise of the institutions. So the decision-making methodology is undermined at the outset and can only favour selfish decisions. Agro-chemical companies design, on their own, the rules that will have to guarantee the protection of the environment and health. The dream of any industrial entrepreneur is thus fully realized.

- Influence the drafting of regulations on pesticide use and the design and implementation of deterrent measures such as sanctions. It is possible to influence policy choices that should protect the collective interest, such as that of environmental protection. If the choice were based only on health and ecological criteria, there would be no choice and selfish behaviour would be crushed, but the focus is shifted to political and economic criteria. In fact, the costs are only partially evaluated, since the short-term loss of earnings for private individuals is calculated, while the social, environmental, and health costs are systematically forgotten, as these usually become evident over a much longer period of time. Indirect costs, such as environmental and health costs, are much higher than the loss of earnings for private individuals, but are tacitly distributed to the whole community and to future generations. As an example, it is possible to recall a fitting event: in order to combat a beetle (*Diabrotica virgifera*) whose larvae affect the roots of maize, in Germany it was obligatory to use the neonicotinoid clothianidin. [484] Clothianidin was subsequently withdrawn from Germany, but the compulsory use of neonicotinoids in maize seed against this insect has also been regulated in other countries (Austria).

- Centralizing the production of rules and regulations in such a way as not to leave freedom of action to the inhabitants of the territory. This has several advantages: it reduces the number of subjects with whom we have to interact in order to achieve objectives, and it reduces the possibility of communication between those who officially make the decisions and the territory that will suffer the consequences. The application of this method and its implementation is simply undemocratic and decreases the options for self-defence.

- Funding external consultants, experts in the field. In this way, companies come into direct contact with important people who could be of help when the time comes. This is an unobtrusive way to the extent that the advisors themselves may not realise that by accepting funding they are losing their freedom. In many countries of the World, such as the USA, funding bodies that are also responsible for toxicological evaluation is allowed and is legal. So, instead of donating secretly or indirectly generous resources, they can do so publicly. Private funding of the control bodies is no longer an intolerable scandal, but is welcome and gratefully publicised. [307]

- Waivers. One of the mechanisms exploited by the agro-chemical powerhouses when things go wrong is the derogation mechanism. European legislation on pesticides provides for the possibility of derogating (e.g. for 120 days) from the ban on the use of a pesticide. In practice, each State may allow, temporarily, the use of banned molecules. In Europe, in 2007, 59 derogations were granted which, in 2010, had become 321, of which 170 for France (e.g. for the use of rotenone on apples, peaches, grepevines, and potatoes). [280] The derogations in some cases are based on a truly amazing and at the same time incredible concept: the sustainable use of pesticides. This concept is taken up by European (EU Directive 2009/128/EC) and national (in Italy the Decree of 22 January 2014) regulations to allow in derogation the sustainable use of products known to be poisons: an obvious oxymoron. Even not aligning with international agreements that seek to limit the use of very dangerous pesticides basically constitutes another bureaucratic tool to delay action. For example, the Stockholm Convention, which provides for limiting the use of poisonous substances including at least 15 pesticides, has not been ratified by several countries including Italy, India and USA.
  In Europe, in 2013, four pesticides considered very dangerous to insects (three neonicotinoids and fipronil) were banned by European regulations. Several European nations, between 2013 and 2016, issued at least 1,100 authorizations for the use of banned substances, of which at least 62 involved four pesticides that are very toxic to bees. [1170] This is a clear abuse of the application of procedures that should only be activated in cases of proven urgency with no possible alternatives. The Member States are in fact managing to disregard the bans and the European Commission is unable to stop these national actions contrary to environmental protection. In most of the emergency procedures (derogations) neither the dangerousness of the circumstances nor the exceptional nature are evident and, at the same time, no alternative measures to the use of the most poisonous substances at our disposal to exterminate insects (and not only) are evaluated. Requests for derogations are very often made by industries such as those that produce pesticides or seeds. Only 6% of requests for derogations come from public bodies. [1170]

- The burden of proof. In the field of environmental health there are very questionable safety management methods. Industry has succeeded in shifting the burden of proof of the hazardousness of substances to the public authorities or the community. [465] It should be the other way round, i.e. industry should prove that the substance is harmless. Therefore, as long as the danger is not proven in a clear and resounding manner, time and money are saved. Legislation is often designed to avoid restrictions and reduce the legal liability for deaths and ill health caused. [303] In general, a popular policy approach is to consider a product safe if it does not kill more than one in a million people each year. Unfortunately, however, this information, i.e. the number of deaths caused, will have to wait for tens of years of use of the product and the simultaneous costly epidemiological monitoring, carried out by public bodies independent of private economic interests. In this regard, the example of lindane (an organochlorine insecticide) can be taken: a dangerous neurotoxic, persistent pollutant, and probable carcinogen that was marketed in 1938 and banned in Europe in 2006. The action needed to contain the devastating effects of this molecule came almost 70 years later.
  A substance is considered unacceptable if it causes one new cancer per million people exposed. It must be remembered that IARC (the International Agency for Research on

Cancer) has evaluated the carcinogenic risk for only about 1,000 substances including some pesticides. Considering that only pesticides, their metabolites and substances added in commercial products (e.g. dispersants, solvents, adjuvants) are several thousands, it can be understood how the current system is not able to protect health. The acceptable daily intake and other indicators such as maximum permitted concentrations are not very reassuring.
We should have the intelligence to ban thousands of dangerous products, instead of blindly continuing to consider harmless substances that will certainly prove to be extremely harmful tomorrow. The result of this way of dealing with the problem is that mankind and the entire biosphere act as guinea pigs in the laboratory, pesticides are first massively distributed for tens of years and then, if the dead and the poisoned are somehow registered, perhaps more intelligent decisions will be taken. The irreversible degradation of the biosphere recorded in these times should lead us to apply the precautionary principle, even when the risk management is based on scientific uncertainties. [466]

- Unclear budgets. We are all forced to be exposed to toxic substances and for this reason we should have the right to know, to be informed about the toxicity of pesticides and their use. Not only should big pesticide companies be more transparent, but also farmers, food companies, supermarket chains. Their budgets should disclose how much money they invest in chemical agriculture, and food items should advertise information about the chemical molecules used to produce them.

- Non-transparent industrial processes. It is very useful to conceal the extreme toxicity of some industrial processes that in some cases have generated serious and numerous health problems for workers or serious accidents.

- Overcoming bans. Among the strategies that have been used successfully for more than 50 years, there is the marketing of new molecules (e.g. insecticides) with a chemical structure, mechanism of action, and toxicity similar to the one that has just been banned (e.g. organochlorines and neonicotinoids). This way of solving problems by replacing a substance with a new one that is completely equivalent in terms of danger should be opposed. Another possibility used is to ban the molecule in one crop and authorize it in others. Imidacloprid was introduced in France in 1991 on turnips, in 1993 it was also authorized for maize and sunflowers, and in 1999 it was banned in sunflowers but not in maize. [307] Later, in 2004, it was also banned in maize. On the other hand, between 2004 and 2007, three other neonicotinoids (e.g. thiamethoxam and clothianidin) were authorized, i.e. molecules that are equivalent in terms of chemical structure, mechanism of action, and toxicity. The expected benefit of the bans was immediately cancelled out by the introduction of new molecules that are just as toxic. This strategy allows agro-chemical companies to gain time. Bans can also be overcome with other strategies, for example by allowing the distribution of the same pesticides in a different way: with treatments on leaves instead of seeds. [583]

The limits on maximum permitted concentrations, for example in drinking water, are the result of political rather than toxicological choices. One example is the indication of a single threshold concentration valid for any molecule, independent of the assessment of hazardousness: the limit for maximum permitted concentrations of pesticides in drinking water is 0.1 micrograms/litre, despite the fact that many molecules are biologically active at concentrations 10 or 100 times lower. Even the maximum concentrations allowed in food are the result of political decisions

and economic evaluations, rather than risk analysis for the environment and human health. For this reason, each country regulates the use of pesticides in a different way and, in some cases, there are also regional differences, i.e. within nations.
The enormous economic resources available to agro-chemical companies allow them to use many weapons to conceal the evidence and delay any action contrary to their economic interests. Treacherous media strategies are planned that can easily influence politicians and convince non-experts in the field, who are more numerous and often more influential than independent researchers.
It is easy for anyone to observe that industrialists put an appalling amount of resources at stake to achieve economic goals, without the necessary concern for the repercussions generated by such doggedness. Short-term profits are above all other considerations. Science, increasingly, becomes subservient to the economic interests of agro-chemical companies and politics, which is also subordinate to business objectives.
The documents, the results of the toxicological assessments, the names of the experts, the situations of conflict of interest: everything plays in favour of private interests. It should be possible, for both citizens and their representatives (e.g. committees, nature associations) and for university researchers, to consult all the toxicological documentation produced by the agro-chemical industries to certify the harmlessness of their products before marketing.
Also because of all these factors, completely different decisions are taken in different countries of the World: the nations regulate the use of pesticides on the same crops in different ways. The result is the existence of thousands of different rules that indicate tens of thousands of maximum concentrations of pesticides allowed in food, in continuous evolution: at least 170,000 regulations exist in Europe. [280] In Europe, since 2008, an attempt has been made to unify the different maximum permitted concentrations regulated in the various countries: unfortunately, the game has been played down, adapting them to the most permissive limits. The more permissive the limits, the easier it is to comply with them. However, the international trade in foodstuffs makes checking compliance with these limits an almost impossible task.

UNDEMOCRATIC TRADE AGREEMENTS

A confirmation of the power of the big multinational companies, not only those in agro-chemicals, over national governments is given by economic treaties. Some international treaties for global trade propose the design of special and unappealable tribunals, before which entrepreneurs can sue national governments if they believe that state regulations harm private interests (e.g. the *Transatlantic Trade and Investment Partnership*, TTIP). [453] Disputes would not be judged by ordinary courts that would reason on the basis of all the existing legislation, as is already possible today, but by lawyers specialized in the application of trade rules, who would judge only on the basis of the treaty itself. According to this approach aimed exclusively at protecting private interests, if a State, perhaps by introducing a rule to protect the climate or health, causes economic damage to a company, it can be forced to backtrack and compensate the entrepreneur. If found guilty, that State or municipality or region could be forced to withdraw the measure or compensate the company.
Some organisations (such as the WTO) openly declare that commercial activities take priority over social justice or environmental protection. [980] Among the impositions resulting from market rules, which have been recorded for years, there is, for example, that of forcing a state to consume food with higher pesticide residues than required by national health protection regulations. Not accepting the freedom sought by market rules leads in retaliation to the application of high duties on the products one wishes to export. Such retaliatory measures have been officially regulated to counter the ban on imports of meat products with high hormone

concentrations from the USA to the EU. In Europe, this has resulted in increased duties for the export of various products to the USA. In practice, supranational rules, i.e. those established by multinationals, finance, and a few very powerful players, prevail over national rules. Unregulated trade effectively empowers business actors to neglect or ignore justice, the environment, ethics, and human rights. Local interests and collective health are overwhelmed by the artificial economic law of the strongest. The environment becomes subordinate to the economic interests of the few. In order to hope for benefits, the rules have to be changed, especially those for the richest who do not care about the wild exploitation of natural resources. Other alarming objectives that companies are setting themselves with the application of these trade agreements are the removal of non-tariff barriers, i.e. differences between technical regulations, between standards applied to products, between sanitary and phytosanitary rules. On the pretext of reducing obstacles to the free movement of products, an attempt is being made to standardise, throughout the Planet and downwards, the rules which protect the environment and health in order to satisfy commercial objectives.
Another sore point is that these treaties are negotiated in secret. [454] Some treaties establish rules that will promote injustice and a reduction in the possibility of defending the environment and health. Anti-democratic choices that privatize justice and harm the environment are supported. The promotion of these strategies generates several anti-democratic side effects that will promote inequalities: for example, public sectors are likely to be further privatized (e.g. drinking water, waste management, transport, education, and health). [455] Small producers and entrepreneurs will be easily overwhelmed. States risk losing sovereignty over environmental and health protection. The system designed by these rules pushes towards less sustainability and less attention to socio-environmental balances: very dangerous monopolies are favoured. More than 20 years ago, multinationals already controlled 40% of the World food trade, 20 companies controlled the global coffee trade, one company controlled almost the entire packaged tea market. [749] The formation of oligopolies and monopolies may be seen in many key sectors of food production, such as cereals (e.g. wheat). The concentration of the market in the hands of a few private organisations is very dangerous. The top ten seed companies control more than half of the World's seed sources. The same is true for veterinary medicines and the commercial power of pesticides is even more concentrated. These figures, probably inaccurate and subject to continuous fluctuations, do however allow us to record a trend in the concentration of power and wealth. The advertising strategy for this trend in trade contains a major paradox: the free market, which is often presented as a system capable of ensuring healthy competition, instead generates an increase in monopolies or oligopolies. As a result, consumers are less and less free and informed, and producers such as farmers are becoming poorer and poorer. Even scientific research in many sectors has become predominantly private, with the consequent obvious situations of conflicts of interest and unacceptable compromises. The power to negatively influence the rules for democracy, justice, fairness, transparency, and environmental protection is increasingly concentrated in a few entrepreneurs.

REDUCTIONISM GENERATED BY HYPER-SPECIALIZATION

A not insignificant aspect, which emerges when reading hundreds of publications, is that scientific research, when it is so specialized, has no longer the ability to critically see the context in which the results obtained are placed. One completely loses sight of the reality in which phenomena occur and limits the examination to a few variables, chosen to achieve predetermined objectives. Excessive specialization has reduced the ability to critically examine even domains other than those in which one works. Therefore, the perception of the gravity of the risks is undermined and involuntarily theories and results that have little scientific merit are

not opposed. The inability to see critically from a distance implies conforming to the practices in force, which are those designed and wanted by private interests. A critical spirit is lacking just as common sense is lacking.
Sometimes, however, we are surprised by the apathy, negligence, and inaction of the independent and free scientific world, which fortunately exists, and of other categories such as those for sustainable agriculture, environmental associations, and State Authorities such as the Health Service. To think that everyone is somehow involved in the private interests of big businesses is simplistic and incorrect. Many factors come into play such as hyper-specialization. Professionals and specialists are increasingly educated, trained, and accustomed to thinking in strictly mono-disciplinary terms. Super-specialization offers undoubted advantages for the progress of science but in this case, it limits vision and common sense. Among the deleterious effects, there is a sort of blindness, a scientific indifference that is epidemic and nullifies itself when it comes to dealing with crucial aspects for our very survival. The *reductionism* that limits itself to trying to solve problems with mono-disciplinary approaches, when applied to major issues such as health and protection of the biosphere is wrong and even counterproductive, as it can generate results opposite to those obtained by a systematic approach. The assessment of risks such as those to pollinators suffers from this simplistic way of dealing with hazards, which are now so clear and obvious that they do not need scientists to identify and analyse them. Moreover, the models used in the laboratory do not produce the predictable results in ecosystems. Pesticides replace mortality from natural predation with mortality from artificial chemical poisoning, as they also damage ecosystems by extinguishing predators. Resistance phenomena are underestimated. In this regard, it is useful to remember that pesticides on average have an effectiveness of a few years (about six) because the target organisms, in this short period of time, are able to become resistant: it is the selection for survival, in this case to the destructive action of the human species. Therefore, pesticides become ineffective, as happens with antibiotics. We enter a dead end as we try to overcome this problem by increasing the doses, the number of active molecules, and by searching for ever more poisonous molecules. The history of pesticides confirms this thesis in that the acute toxicity of insecticides has increased thousands of times in 60 years and the number of species that have become tolerant is increasing.
In the laboratory, it is almost impossible to test metabolites that can be generated in nature and within organisms, such as non-target metabolites. In many cases, history has taught us that the metabolites are as toxic or more toxic than the starting molecules (e.g. heptachlor).
Educational paths should provide alternatives and free themselves, at least in part, from the radicalism of reductionist thinking. The concept of sustainability must become a pillar of all disciplines because it means reconnecting with reality and the changes that are now inevitable.

PART TWO

REFLECTIONS ON ECOLOGICAL UNCONSCIOUSNESS AND CHOICES AGAINST NATURE

BEYOND ECOLOGICAL LIMITS

BANKRUPT NATURE AND OPERATIONAL SPACE FOR HUMANITY

Improving the culture regarding environmental issues may contribute to give a value to what has no price: the health of the Earth ecosystem. Education, information, and training may be more effective than other strategies such as financial measures (conditional aid), taxation (those who pollute more should pay more, at least in theory), enforcement through legislation. Investment in a new and better culture may be more profitable than other investments, such as financing environmental certification strategies (e.g. EMAS and ISO 14001). [12] Thus, investments in "culture" may be less expensive, more effective, and more profitable than others.

Human civilization risks a serious environmental, social, and economic crisis unless there is a timely reversal of the trend. However, before any decision on how to remedy, it is necessary to understand the mechanisms of the origin of our current system based on consumerism and capitalism, incapable of a vision of the future. The destructive practices, which are now obvious to everyone, are neither ecologically nor economically sustainable.

In the last twenty centuries, the planetary population has increased exponentially. The global density of *Homo sapiens* has increased: from about a little more than one inhabitant per square kilometre to over 45 inhabitants per square kilometre. This rapid growth has not been steady or equally distributed. Our ecological footprint has thus far exceeded the Planet's capacity to regenerate the resources it uses. Most of the problems humanity faces are a result of the desire for unlimited growth in a system that is limited. At least 4 billion people live in poverty or are threatened by wars and environmental disasters. Unfortunately, many indicators suggest that the number of people who will see their current living conditions worsen is increasing.

Deforestation is probably the most obvious manifestation of the process of anthropogenic transformation of the Earth. The modification of vegetation structures may be considered the mother of all human ecological footprints. An ironic but significant vision of the forest richness present in Europe in the past (12000 years ago) could be that of imagining that a monkey could have moved from Spain to Greece without climbing down from the trees. At that time, the Planet was inhabited by about 2 million human hunter-gatherers. In a few thousand years we have passed from a World controlled by nature to one dominated by men, generating a real ecocide.

Some causes of biodiversity reduction are hunting, fishing, fires, urbanization, intensive agriculture (e.g. herbicides, insecticides, tillage), and pollution. Since 2000 at least 300 million hectares of forests have been altered or destroyed, and the reduction of biodiversity is now proceeding at a rate at least 100 times faster than previously recorded (from studies of fossils). [736] At least a quarter of the Planet's natural areas have been altered or used for commercial purposes. The introduction of alien species also generates much damage, as beekeepers are well aware of.

In addition to the destruction of biodiversity, another obvious indicator of the human ecological footprint is the excess of carbon released into the atmosphere with the consequent change in the heat balance of the Planet and the acidification of the oceans. These changes have no analogues in the eras in which the human species has evolved. We have therefore entered a zone of insecurity, outside the norm. The ecological experiment underway is unique and is self-managed by the guinea pig in lab that voluntarily launched itself into a mad rush towards

unknown conditions. It is certain that climate change is the result of ecological disasters caused by man, and consequently the choices that will be implemented in the coming years will determine the intensity and dramatic nature of the environmental consequences. Unfortunately, even if climate-altering gas emissions were reduced, greenhouse gas concentrations would still remain higher than in the pre-industrial era for centuries. In any case, a substantial general warming of the Planet is estimated. Different scenarios are envisaged (e.g. IPPC), but even the most optimistic ones foresee an increase in temperature, an increase in the melting of glaciers (the water banks), an increase in the levels and acidification of the oceans. [775] Unfortunately, this global warming trend would not stop even if we were able to stop all carbon dioxide emissions. The power of the ongoing changes is manifested in the alteration of the most remote and pristine parts of the Planet such as the glaciers of the North Pole.
In 1700, more than half of the entire biosphere was in a wild state, while 45% was in a semi-natural state. [680] Currently, almost three quarters of the Planet's surface has been transformed by human activities and less than one quarter may be considered in a semi-natural or wild state. Unfortunately, the artificial rules of economics and finance do not consider a simple evidence: it is not possible to exceed the limits of the natural systems of our Planet. It is frankly worrying to continue to witness political debates or listen to university economists who wish for continuous and constant economic growth. This is a manifestation of the rampant environmental illiteracy. Economists do not consider the biophysical limits of the Planet and the fact that many planetary limits have been exceeded: we have entered a very worrying zone of uncertainty regarding climate change, the loss of biodiversity, the alteration of the nitrogen and phosphorus cycles. Ice is melting, insects are disappearing, seas are being plundered and the speed of negative change is increasing. For some limits of the biosphere we have already touched the points of no return, i.e. we are outside a safe operating space for humankind. Mankind derives from and depends on nature so it cannot do without it. The wealth of economies depends on natural capital but, naively, the rules of the markets consider them infinite resources. It is necessary to correct our relationship with the biosphere because we have become the most important geological force on the Planet. The biosphere cannot bear further unsustainable appropriations of natural capital without compromising balances that are indispensable for the safety of humanity. The thoughtlessness with which we are irreversibly eroding natural capital is reckless and self-defeating. Especially industrialized countries and richer economies are living far beyond the possibilities offered by the biosphere. The debacle may paralyze, but we have to imagine a different society trying to get out of consumerism and the free market, which are pathological and generate horrors. Infinite economic growth, the free market, consumerism (based on disposability and wastes) destroy the natural life-support systems.

The knowledge available to us is lacking and limited, such as that on the interactions between the atmosphere and the biosphere, on the effects of deforestation and pollution. The lack of knowledge should worry us more than what we know about the effects of ongoing change. The predictions and analyses presented in the following pages do not consider the surprise effect, such as that generated by threshold effects. Resilience, that is the ability of a system to absorb a disturbance before changing its structure and processes, in the case of the Earth ecosystem, is largely unknown. We can reasonably believe that the resilience to collapse of the Earth ecosystem is seriously compromised by the destruction of natural capital.

For the first time in the short history of mankind, a handful of entrepreneurs are able to irreversibly transform the biosphere and control the destiny of the majority of the World's population, for example with regard to their eating habits. A few armies of workers, managed by ruthless entrepreneurs, are able to conquer and subjugate entire nations, expropriating them of sovereignty and food security, destroying forests, polluting the air, soil and water, exterminating biodiversity for commercial purposes. The protection of the biosphere, like a real

war played out on a planetary scale, requires the creation of organized, aggressive, vigilant, far-sighted, and altruistic defence systems: the time has come to organize the self-protection of the environment and to safeguard the interests of future generations.
With soil degradation, biodiversity destruction and climate change, agriculture is set to change. The distressing question is: how long will society and ecosystems be able to resist these changes without seriously undermining peace and well-being? Implementing the necessary epochal change requires a strong public sector, a community that is better informed and more active in influencing the choices that affect the community and the future. What is needed is a public sector that does not bow to the interests of the few.

The idea of planetary boundaries that must not be crossed is very effective in understanding the size of the operational space in which it is possible to move safely. Some of the factors that constantly undermine the ability of ecosystems to provide essential services for our survival are the following:

- The destruction of biodiversity (deforestation, fires, spread of alien species, artificial selection, etc.).
- Climate change.
- Soil degradation.
- Chemical pollution.
- The alteration of the water cycle and its unsustainable use.
- The alteration of natural nitrogen and phosphorus fluxes.
- Ocean acidification.
- The reduction of atmospheric ozone.

Other sources of concern that are mentioned are as follows:

- Weapons of mass destruction (nuclear, chemical, and biological weapons).
- Artificial intelligence that allows to increase the possibility of control and management of data flows, information, and enhances the ability to predict and influence behaviour.
- Biotechnology is making it possible for the first time to modify and alter the human genome and that of all species. New technologies increase the ability to intervene on the lottery of evolution and may reserve many surprises. For example, small groups of people possess sufficient tools to promote the extinction of entire ecosystems, uncontrollably by the rest of the population.
- Capitalism fosters disproportionate inequalities and allows, to few individuals, to accumulate such enormous riches to be able to condition the rest of the World. The ability to intervene on the biosphere of a few entrepreneurs exceeds that of entire nations, in an alarming way and, also in this case, uncontrollable by the rest of the community.

Some indicators of the unsustainable trend are in continuous growth: the great acceleration. [981]
The increase in the speed of resource exploitation cannot be explained simply by population growth. In fact, the use of fossil fuels and the extraction of fish resources have grown much faster than population growth.
It is not necessary to have a particular intelligence to imagine what changes could be generated by a continuous increase of pressure by humanity on the biosphere. It is necessary to design an emergency plan because we do not have another Planet to consume. The challenge is cyclopean and it will not be the laws of the market or economic and financial liberalism that will propose the solutions necessary to protect the community.

THE SKY BELONGS TO EVERYONE

The astounding image of the Earth taken from the Moon in the 1960s may be our starting point. The Blue Planet floating in the void in a hostile, cold, radiation-crossed, largely empty environment provides the dimension of our smallness and at the same time our good fortune. As in a spaceship, a crew and the operation of essential services are required for survival. The death of even part of the crew can jeopardize the mission, just as the modules needed to provide air and water cannot be dispensed with. We are irreversibly compromising the journey through the extinction of a large part of the crew and through unsustainable processes such as those that generate waste.

Fifty-two years after the first photograph was taken from the Moon, things have changed considerably. Even in space, junk is increasing. Every year dangerous accidental events occur in the orbit. The large number of satellites amplifies the possibility of collisions with chain effects that are worrying to say the least. In the event of a collision, a cloud of debris is formed that will continue to travel in the same orbit as the original satellite, multiplying the probability of other impacts.

Light pollution is an insidious product of our civilization that fears the night. The problem is global: one third of the Planet's population lives in regions so brightly lit that they cannot see the Milky Way. We are talking about more than 2 billion people. In fact, Italy enjoys the unenviable record of being one of the industrialized countries with the highest light pollution: among the G20 countries we are second only to South Korea. [927]

Astronomers have also raised the alarm: light pollution and thousands of satellites (most of them inactive) are capable of hindering astronomical observation. It is estimated that about 1/3 of the images from large field telescopes will be crossed by a satellite leaving a smear that is very difficult to eliminate with cleaning algorithms because it is too bright. [927] In conclusion, we have managed to contaminate even the space outside the air we breathe (the troposphere).

The pollution of the high atmosphere leads us to a general reflection: is it reasonable that the authorization to launch satellites is given by an entity that represents a Nation (e.g. USA), when their orbits fly over all the other Countries of the World that have not been minimally involved in the decision? The environmental issue concerns everyone and cannot be solved without the involvement of everyone. In fact, everyone can contribute to limit light pollution, for example by choosing carefully the lamps for courtyards and gardens. The difference between an excellent lamp and a bad one also depends on the ability to direct radiation downwards and not towards the horizon or upwards. This is what the regional ordinances that have been approved almost everywhere in Italy ask for, just to limit light pollution. Moreover, it is an enormous waste of energy, and chemical pollution, due to emissions, generates irreversible and worrying changes.

The vault of heaven is a heritage of mankind that we can all enjoy because the starry sky is an integral part of the lives of human beings, animals and plants. Besides enjoying the beauty of the starry sky, we should all contribute to its preservation to try to keep it intact for future generations.

Starting from afar it has been possible to raise some reflections such as the privatisation of the high atmosphere, the production of waste and the unsustainability of our actions. We must recognize and accept limits. We cannot continue to do everything that technology allows us to do. We must plan for a sustainable future.

THE FINAL CENTURY: PLANETARY LIMITS AND FOOD SECURITY

Humanity has come to consume more renewable resources annually than the Earth can regenerate and more non-renewable resources than are found in new deposits. We produce far greater quantities of waste substances than the biosphere is able to metabolize and we distribute increasing quantities of non-biodegradable pollutants into the environment, irreversibly altering the biosphere. We must all reflect starting from the following question: for how much longer can we resist not caring about this self-destructive process?

The human species consumes a large portion of the Earth's natural photosynthetic productivity, and the percentage of biomass we consume that ends up in our stomachs is increasing. What should alarm us is that the remaining part of the Planet's productivity, which we can selfishly hope to take from nature, is less than that which we have already appropriated. Therefore, the economic forecasts of doubling consumption and, consequently, food production, in the next 20 or 30 years are unrealistic. Unfortunately, if we do not succeed in carrying out a green revolution, which is not realistically conceivable at the moment, we will realize the ruthlessness of the laws of nature when it is too late, that is, when we have destroyed most of the Earth's biodiversity. We have only one Planet and we are allowed only one such experiment. We will not have a chance to go back. We should ask ourselves why we continue to rush towards catastrophe.

The changes that have taken place in the last 50-60 years in the ability to produce food are an achievement but at the same time pose challenges. In 50 years, the number of malnourished people has halved while that of overweight people has doubled: probably less than 3% of the World's population has a caloric intake of less than 2000 kcal a day but more than 39%, that is at least 2.1 billion people, are overweight or obese (in Italy 36% of males and 34% of females between 5 and 17 years of age are overweight or obese). [755, 985, 1178] At least 820 million people suffer from hunger and a much greater number due to inadequate nutrition have high mortality and morbidity rates. For example, at least 2 billion people suffer from an insufficient intake of micronutrients (vitamins and minerals) and the frequency of diabetes has doubled in 30 years. [1178] It would probably be possible today to ensure a better diet to all the inhabitants of the Planet if there were fewer inequalities. [1178]

Agriculture uses at least 70% of the water consumed, occupies at least 40% of the land, is responsible for the emission of at least 30% of climate-altering gases, and is one of the main causes of the reduction in biodiversity: agriculture is the main cause of the extinction of birds and mammals. Sixty percent of the energy consumed by mankind comes from just three crops: rice, maize and wheat; however, in 2010, at least 34% of cereals were used to feed animals and by 2050 this fraction might increase. [1178] Other food supply systems are also unsustainable and can only decline, such as fisheries. How can we ensure adequate nutrition for the more than 10 billion people likely to inhabit the Planet before 2050 and safeguard the natural resources essential to our survival? How can we increase food production without increasing the irreversible consumption of non-renewable resources such as soil? The availability of fertile soil is decreasing and climatic conditions and degradation by the food industry itself will not allow for more resources than those available today. Food production is one of the major causes of negative changes such as climate change. There are no easily applicable solution recipes but some indications may be useful, such as: [1178]

- Reduce consumption of animal products (e.g. red meat).
- Prefer unsaturated fats of vegetable origin instead of saturated fats.
- Increase consumption of plant-based proteins such as legumes to replace animal-based proteins. An increased consumption of plant-based foods could contribute significantly to reducing climate-altering gas emissions.

- Increase consumption of vegetables such as fruit (e.g. consume between 100 and 300 g of fruit daily).
- Reduce consumption of simple sugars and highly processed foods (simple sugars should provide less than 5% of total calories).
- Use organic fertilizers instead of inorganic ones.
- Recycle phosphorus and nutrients in general.
- Reduce biodiversity destruction by halting the felling of primary forests: between 2000 and 2014, Brazil lost an average of 2.7 million hectares of forest per year, Indonesia 1.3 million hectares per year, and the Democratic Republic of Congo 0.6 million hectares per year.
- Reduce fishing catches. For more than 3 billion people, fishery products account for at least 20% of their total daily protein intake.
- Reduce waste production.
- Increase food production sustainably and without the use of pesticides. Transforming the traditional agricultural system to make it sustainable (organic regenerative agriculture) would allow most climate-altering gas emissions to be sequestered in the soil. [1181]
- Reduce the consumption of fossil fuels for food production and not only.
- Reduce population growth (this is not a problem in many countries such as Italy where the birth rate allows for the possible halving of the population in one generation; a reduced birth rate, in the absence of compensations such as immigration, can be another problem due to the excessive increase in the fraction of elderly people in relation to the labour force).

Ensuring food security for an increasing number of people is becoming a very difficult challenge. Exploitation demands are increasing but the capacity of natural services to support our expectations is shrinking. Ensuring a safe operating space for humanity by continuing to increase natural resource exploitation, degradation of ecosystem services, and irreversible changes in the biosphere is an unrealistic expectation. We must remember that we live on a Planet that is not infinite.

Since 1961, grain production has increased over 400%, pig production 450%, cattle 230%, and chicken thirteen times. Since 1960, *per capita* calorie consumption has increased by one third, meat consumption has at least doubled, and fertilizer use has increased more than nine-fold. This growth cannot continue indefinitely and the choices we make will determine our health, that of the Planet and above all that of future generations.

We have domesticated nature. To get a measure of humanity's dominance over nature, only 30% of the Planet's bird biomass is made up of wild species, the remaining 70% being farmed poultry. Among mammals, the proportions are even more impressive: 60% are farm animals (cattle and pigs), 36% are humans, and only 4% are wild mammals. [799] The Planet cannot support billions of carnivorous humans. In nature, for every carnivorous mammal (e.g. canids or felids) there are at least 100 prey items. More than 50% of the agricultural surface of the Planet is used to produce food of animal origin. If all the inhabitants of the Planet were to adopt a vegetarian diet, we would double the space available, thus being able to re-naturalize at least part of it.

The supply of food has grown faster than population growth, generating heavy and irreversible impacts on natural balances. On the positive side, food production today could support the energy needs of the entire human population, but the supply of some nutrients essential for health is insufficient. For example, the *per capita* availability of fruit and vegetables is insufficient to ensure good health for all. [755] At the same time, the consumption of less healthy foods, such as products derived from meat processing and preservation and sugar-sweetened beverages, increased by 35% and 50%, respectively, between 1990 and 2015. Production systems and food habits have created new risks such as that of the excessive consumption of

simple sugars, the use of antibiotics (e.g. in animal husbandry), and the use of pesticides. Unfortunately, the biosphere is seriously threatened and some limits in the capacity of self-regeneration of energy and matter flows of the Planet have been exceeded, so we are operating in an unsustainable zone of insecurity.
It has been ascertained that at least four "planetary limits" have been exceeded: emissions of climate-altering gases, loss of biodiversity, modification of the nitrogen and phosphorus cycle, and soil degradation. For these four aspects mankind has certainly entered a zone of insecurity, crossing the limits beyond which devastating effects are triggered. It means that the changes already made to the biosphere will generate irreversible and negative effects to humanity, even if the degradation perpetuated until today were to stop completely. In practice, the system of physical, chemical and biological balances on which we depend has been modified with the consequent disturbances to the capacity to maintain in health and peace an increasing number of human beings, whose ecological pressure increases even more rapidly.
It is important to draw our attention on some of the challenges that humanity faces and will have to face differently than we have done in the last three generations. [36] Accepting the limits and taking action to prevent irreversible changes is equivalent to adopting the recommendations for degrowth and better democracy. For many planetary limits, we do not know exactly what are the ceilings not to be exceeded, that is, the level of degradation not to be trespassed to avoid the point of no return. We do not know how far we can continue not to care about the irreversible destruction of ecological diversity, before some natural systems necessary for the survival of humanity collapse catastrophically. The scientific literature also shows weaknesses. For example, among the planetary limits, that of pollution is underestimated because, in addition to destroying life forms through acute intoxication, it undermines some of the basic mechanisms of life, e.g. through the alteration of genetic expression and transmission (e.g. mutagenic substances or fertility-altering molecules) or the deterioration of hormonal regulation (endocrine disruptors) in all living beings. We release thousands of tons of persistent and bioaccumulative substances into the environment which have significant and fundamental biological effects at doses of millionths of a gram (e.g. herbicides that block photosynthesis). The consequences for human health are recorded with epidemics from chemicals that are not given due prominence. Even less importance is given to the effects of massive contamination of water, soil, air, and food chains. Pollution, among the planetary limits, probably does not receive due attention and does not arouse the necessary concern to activate information and divulgation movements. The chemical contamination of the environment often remains overshadowed in debates between scientists and politicians and is sorely lacking in mass dissemination.

There can be no health, no prosperity, and no democracy in a society that is unable to set limits for itself. Capitalism is based on the illusion of the non-existence of physical and biological limits. We have replaced ecological rules with the "*forbidden to forbid*" principle that underlies artificial human rules such as economic, financial, political, and market rules. This freedom implies the acceptance of inhumanity, as it allows the commodification of anything like the future and human beings. The administrators, unreasonably, betray their mandate by becoming totally subordinate to market and finance, but they behave omnipotently towards the people. We have been very clever in creating the conditions that undermine the survival of morality and ethics. The culture is also homologated, cancelling also in this case the limits of action: it has been estimated that 6,000 languages still exist (of the 20,000 spoken in the recent history of humanity) and that at least 50% will become extinct in less than 100 years; curiously an Amazon parrot was the last to pronounce about 40 words in a language of South America whose human speakers had all been extinct. [836] The parasitic development of human society is enabled by the illusion that limits do not exist and as a result the biosphere and social identity are destroyed. Exceeding the limits does not destroy the Planet but the ecosystems on which we depend, therefore we condemn ourselves to collapse.

UNSUSTAINABLE APPROPRIATION OF PRIMARY PRODUCTION

To understand how an ecosystem works we need to introduce the concept of primary production, which is the amount of energy that is incorporated by an ecosystem in terms of carbon through photosynthesis. The energy of the Earth's ecosystem depends on the physical characteristics of the Planet itself and on the energy that the Sun sends from about 150 million kilometres away: every hour from the Sun, more energy reaches the Earth than is consumed by man in one year. Less than 1% of the solar energy radiated on Earth is captured by plants and converted into primary production, i.e. the construction of biomass. Photosynthesis converts light energy into chemical energy stored in organic molecules: carbohydrates such as starch and cellulose. One molecule of sugar and six molecules of oxygen are produced from six molecules of carbon dioxide (CO_2) and six molecules of water. The carbon in atmospheric carbon dioxide (CO_2) is incorporated into plant tissues. Photosynthesis can be referred to as *carbon organication*, as it is transformed from an inorganic compound into an organic molecule. Primary production is measured in grams of carbon (g C) per unit area (m^2) in the unit of time (year).

The primary producers, i.e. plants, use part of the carbohydrates produced for their own metabolism, in a process similar to that of animal respiration.

Simplifying greatly, under natural conditions, carbon dioxide is exchanged continuously between the atmosphere and living beings in the biosphere, through two opposing processes: photosynthesis and respiration. The two reactions:

Photosynthesis: $6CO_2 + 6H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6O_2$

Respiration: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy}$

Photosynthesis removes carbon dioxide from the atmosphere, while respiration releases it as a by-product of the oxidation of organic matter (e.g. carbohydrates) in order to produce energy. The Sun and plants have contributed, over millions of years, to the formation of non-renewable fossil fuels; theoretically they are renewable, but over a period of time not of interest to the human species.

The amount of energy that the Sun radiates every second on one square centimetre of the Earth's surface is called the *solar constant* and is about 1.97 cal/cm^2 per minute (calories per square centimetre per minute). This is an approximate value as it assumes that the atmosphere is transparent and increases on the inclined plane to the south. In reality the energy available may be lower and varies with the changing of seasons and conditions (e.g. presence or absence of clouds). In Italy the average irradiation in the winter months is 3-4 kWh/m^2/day and 6-7 kWh/m^2/day in the summer months. [935] Probably the Sun provides a huge amount of energy for free, at least the equivalent of about 133,000 Gtep/year (billion tons of oil equivalent).

In the temperate zones of the Planet it can be considered that between 15 and 40 million kcal per day per hectare (1 hectare corresponds to 10,000 square metres) arrive in the form of solar energy. In one year, this energy is equivalent to 1.7 million litres of petrol per hectare. In temperate zones the net energy transformed into biomass corresponds on average to 10 million kcal per hectare per year. This energy in dry matter corresponds to about 2,400 kg/ha per year, with a minimum in desert and ice areas of zero and a maximum in the most productive ecosystems of 10,000 kg/ha per year.

In agricultural ecosystems, a net quantity of solar energy of about 15 million kcal per ha may be considered. Even if this quantity of energy corresponds to about 0.1% of that which reaches the surface, it theoretically allows obtaining 3,500 kg of dry matter biomass (i.e. biomass weight excluding water) per year and per hectare (it is possible to hope to obtain about 4,200 kcal per kilogram of biomass). Some of the energy captured by the plants will be used for metabolism, respiration, pest defense and more; therefore, only a fraction can be used to build biomass. Some crops such as corn and sugarcane can fix higher amounts of solar energy as they can get up to 5% of it, but it is necessary also to invest energy to irrigate, produce, and distribute fertilizers and pesticides.

Approximately 25-30 t of maize dry matter per year per hectare, produced with energy-intensive agriculture (i.e. the fossil energy used is greater than that obtained in the form of food), are able to feed 2 to 4 dairy cattle per year (between 200 kg and 500 kg each), 12-15 pigs (from 80-100 kg each) or at least 12-15 humans, simplistically considering the caloric intake of 12 t of grain dry matter. [741] If maize is used for human consumption it can supply about 980 kcal/kg (at 70.3% humidity), corresponding to about 3.3 kcal per gram of dry matter of the seeds, with the following chemical composition: 19.5% starch, 3.4% proteins, 1.3% lipids. Whole maize chopping, that is the harvest which, besides the caryopses, includes also other parts of the plant, has an energetic content suitable for animal feeding equal to 2,600 kcal/kg, with a concentration of dry substance equal to 35%. About 45% of the dry matter of the maize plant is contained in the parts that have a lower digestibility and nutritional value than the grain, such as the leaves and the stalk. Therefore, if parts of the plant other than the grain are used, the energy yield per unit weight is reduced, but increases per unit area.

According to rough theoretical calculations, one hectare planted with potatoes or one hectare planted with rice can feed a maximum of 10-12 people per year. One hectare of grassland can support one steer for a year. [938] If the grassland is not very productive, one hectare is not enough to feed a bovine animal for one year. [939]

Assuming the following conditions:

- a man who lives as a hunter-gatherer, i.e. leading a lifestyle that we now consider primitive, might need 2,500 kcal/day;
- 1 kg of plant foods can provide 3,000 kcal;
- in temperate regions the natural production of plant biomass is about 2,400 kg/ha per year (dry matter);

to ensure the survival of a person leading a hunter-gatherer lifestyle, 40 ha of average productive land are needed. From this area it can be estimated to harvest 7.5 kg of plant food per hectare per year; in this example it is considered that less than 90 kcal per day are derived from animal proteins. A family of 5 persons could require an area of more than 200 ha to survive. In some regions of the Planet much larger areas may be required. [425]

In primitive agricultural systems, where practically no fossil energy is used, the ratio between energy invested and energy obtained can be very positive, but it requires a considerable commitment in terms of man-hours and the yield per hectare is much lower than in intensive systems. The use of machines and chemicals (pesticides, fertilizers, etc.) to produce vegetables increases productivity considerably and frees man from manual work, but increases the amount of energy needed. For example, a small tractor can do the work of ploughing in 100-fold less time than manual work (with a hoe), but requires at least 6 times more energy.

The average productivity of agricultural land in the USA can be considered as 5 t/ha per year. The most productive maize crops can provide 18,000 kg of biomass per ha (of dry matter), equivalent to about 9,000 kg/ha of seed. Considering a caloric content of 4,000 kcal/kg, this energy corresponds to 72 million kcal/ha per year, or 0.5% of the solar energy received in a year. This can be considered as the maximum productivity of a hectare cultivated with cereals, but obtained after consuming energy, for example, with irrigation, cultivation practices, use of pesticides, fertilizers and transport.

In marine ecosystems only 0.03% of sunlight reaches aquatic plants. [425] It means that in the marine ecosystem the food chain, from phytoplankton to fish, can hardly sustain more than 4 levels; each hectare of marine surface can produce 400 kcal per year from fishery by-products (about 150 g of fish meat) for human food. It must be remembered that a large proportion of fishery products are used to produce feed.

Primary production is the generation of organic compounds from the carbon dioxide present in the atmosphere or in the oceans; this process occurs mainly through photosynthetic and, to a lesser extent, chemosynthetic processes. [623] Primary production is the amount of organic matter necessary for life on our Planet produced by photosynthetic organisms such as plants, algae, and some microorganisms. The amount of carbon fixed in photosynthesis, minus the amount of carbon re-emitted in respiration, constitutes the net primary production. Net primary production is measured by satellite and compared with the local consumption of food, fiber, and plant materials. The intensity of human appropriation of this quantity is a measure of the human pressure on the rest of nature. Satellite-supported calculations of the net primary productivity of the Earth's surface estimate a total of 54 Gt (billion tons), with an annual variability of about 1 Gt. Photosynthesis by plants (terrestrial and marine) produces at least 230 Gt of plant mass per year. Other estimates put the net primary production of the Planet Earth at around 105 Gt per year. More than a half is produced in terrestrial ecosystems and the remainder in the oceans. Different calculations lead to different estimates that give a measure of the uncertainty but also of the order of magnitude. This amount of organic matter, called primary production, should be shared with several million of other species. The share we leave to non-humans is getting smaller and smaller. On a planetary level, the alterations generated in the biosphere indicate a reduction in this availability.

The terrestrial vegetation consists of 40% forests and covers about 23% of the total surface of the Planet. The leaf surface of plants, if it were distributed in a single thin layer of photo-synthesizing surface, would be able to cover at least 2.5 times the entire surface of the globe. [624]

The human species appropriates more than one third of the Planet's net primary production, generating irreversible and unsustainable changes. [587] The appropriation of the net primary production by humanity, in some regions of the Planet, exceeds 60% or even approaches 100% (e.g. intensive cultivation in the plains). [587] Human appropriation of net primary productivity, in addition to subtracting organic matter from the rest of the life on Earth, alters the composition of the atmosphere, biodiversity levels, energy flows through food chains, and important ecosystem services.

A primitive society that relies on gathering and hunting consumes less than 0.1% of the net primary production of the territory in which it lives. Pastoralism involves the appropriation of at least 8% and agriculture more than 48% of the net primary production of a territory. [703]

We have appropriated the majority of the energy obtainable through photosynthesis, consequently reducing the number of living beings, as energy is used to sustain a single species: man. This necessarily leads to a reduction in the diversity that can be sustained by a limited and confined system. The more energy available in an ecosystem, the more flourishing and complex the trophic network will be.

One question that is difficult to answer is: what is the percentage of primary production that humanity can afford to consume without generating the collapse of ecosystems? We could reasonably assume that we have already exceeded this threshold. The recorded reduction in the services offered by nature due to the appropriation of net primary production also puts the human species in difficulty. This constitutes another underestimated planetary limit as we have entered a zone of insecurity and instability. Very deleterious amplification mechanisms could

easily be triggered such as increasing the rate of loss of fertile soil, biodiversity, and climate change.
It is important to remember that the human species, in the year 2000, had already appropriated at least 35% of the land area free of ice, irreversibly transforming it into agricultural land. [587] In 40 years, agricultural production has doubled but the area occupied by agriculture has increased by 12%. [587] This difference between the two indicators is possible thanks to the use of fossil energies and agronomic technologies (fertilizers, pesticides, irrigation, mechanization) that generate many negative effects in the environment.
The exponential growth of the human population, with the even more rapid increase in the consumption of finite resources, suggests impossible challenges for humanity. During the 50 years prior to 2011, the human population grew by 128% (from 3 to 6.9 billion), cultivated areas by 33% and agricultural production by 57%. The rapid growth in the exploitation of the Planet's resources has also produced a lot of negative impacts on pollinators and plant biodiversity. To sustain the growth in consumption that is foreseen, it will not be sufficient to invest more energy and resources to obtain more food, but it will be necessary to cultivate new areas. By increasing the amount of resources used per unit area, with the aim of producing agricultural products, the increase in harvests decreases per unit of resources provided (e.g. energy, fertilizers, pesticides). [615] It follows that to obtain significant increases it is necessary to have new areas to cultivate, since the increase in productivity of a crop decreases as the use of resources increases. It is difficult to imagine doubling food production before 2050 without irreversibly compromising the biosphere and our chances of survival.

THE CONSUMPTION OF ENERGY GENERATED OVER MILLIONS OF YEARS: THE SUN BURIED

Solar energy, thanks to chlorophyll photosynthesis and processes that have lasted millions of years, has been stored in solid, liquid, and gaseous carbon deposits that can be extracted from the subsoil. Solar energy is deposited underground in the form of potential energy, i.e. chemical bonds, mainly between carbon atoms and carbon atoms with hydrogen atoms. The amount of energy available therefore depends on the carbon content, the bonds between carbon molecules and with hydrogen. In reality, the energy contained is not always easy to use, as it must be extracted from the subsoil and, during the transformation, a large part may be dispersed in the form of heat; other consumption factors such as transport must also be considered.

Fossil fuels are derived from chlorophyll photosynthesis (carbon organification) and require a period of time to reform themselves one million times longer than the current rate of consumption: at least 170,000 kilograms per second (1,110 barrels of 159 kg each per second). [936]
The industrial revolution was possible thanks to the very rapid consumption of large quantities of hydrocarbons formed by solar radiation: we are burning the energy stored over millions of years. As a result, we are releasing carbon dioxide into the atmosphere at a rate far greater than the capacity of plants to reabsorb it. The use of fossil fuels, already in 1997, had produced an amount of carbon dioxide 400 times greater than the capacity of the Earth's primary productivity to convert it into plant biomass through chlorophyll photosynthesis. [937]
Between 1970 and 1995, the energy consumption increased by 2.5% per year. [97, 425] Overall, the World energy consumption almost doubled between 1973 and 2009: it rose from 4.6 to 8.4 billion tons of oil equivalent. [5]

Fossil fuels provide 88% of the World's energy and enable an inefficient food production system: it takes at least 10-15 calories of energy to produce one calorie in food. [5] It

can be argued that agriculture today is an industry that inefficiently converts fossil fuels into food. Agriculture has been developed since about 10,000 years ago and, even today, there are peoples who practice very primitive methods that do not use the energy of fossil fuels. On a planetary scale, 77% of potentially renewable energy comes from the combustion of plants, wood and waste. [15] In most developing countries, the most common way to heat homes and cook is by burning woody biomass from the surrounding environment. In Africa, more than 80% of the energy in rural areas comes from wood fuel harvested within a few kilometres. [19] A sustainably harvested forest can provide three tons of dry woody biomass per hectare per year (some energy will be spent in cutting and transport). Biomass can be considered renewable if it is exploited at a rate below its natural regeneration capacity. Furthermore, a distinction must be made between biomasses obtained from completely artificial systems, such as agricultural crops, and those obtained from natural ecosystems (e.g. primary forests). In the latter case the concept of renewability is certainly much more fragile, and in the former case the energy consumption may be higher than the energy obtained (e.g. from maize cultivation).
Perhaps current levels of consumption can be assured, in the case of oil, for another 40 years, natural gas and coal for much longer (at current levels of consumption). The environmental cost generated by the consumption of fossil fuels must be of much greater concern than their demise. The environmental disaster resulting from combustion will generate limiting effects before the very end of the fossil fuels. At current rates of consumption, non-renewable energies such as oil, natural gas (methane), and nuclear energy will sooner or later no longer be cheap. Solutions must therefore be sought as soon as possible, so that the change is gradual and managed peacefully.

INDUSTRIAL AGRICULTURE AND SOME PLANETARY LIMITS: CLIMATE, WATER AND SOIL

Agriculture has never been natural, in fact it is the primary cause of deforestation. It can in no way be considered a form of land protection. Industrial agriculture is based on the use of poisons, fertilizers and seeds that must be purchased every year. Intensive monoculture has simplified the biodiversity of agrarian landscapes and generated the cancellation of centuries-old knowledge that has always provided nourishment and sustenance. The extinction of this knowledge means becoming completely dependent on large companies, impoverishing ecosystems or destroying them: for a large part of the World's agricultural population it has meant poverty and hunger. [175]
The agricultural environment has become so hostile to bees that the urban spaces of cities provide a safer and more productive environment. [206] Paradoxically, bees in cities have a greater availability of nectar and pollen than in many agricultural settings where intensive farming takes place. They are also less exposed to pesticides. To give an example, in the city of London there are about 8.3 million trees, 30,000 gardens, 13,000 species of wild flora and fauna; it is estimated that the city of Turin has a melliferous potential of 440,000 kg. [35, 196] Urban beekeeping is spreading not only for economic reasons but also for social and educational reasons.
Practices in the agricultural sector that also harm pollinators include fertilizing, plowing, tillage, pesticide distribution, burning, destruction of fallow and semi-natural areas, and breeding. Genetically modified plants can produce pollen and nectars in quantities and qualities that are negatively altered for bees. Simple and banal principles, such as returning the fertility taken from the soil with harvests, are no longer respected. The natural capacity to feed life is compromised. The alternative could be agro-ecology, which to a large extent has yet to be

designed, but which is the only alternative to irreversible processes such as the decrease in the capacity to produce food due to the loss of soil fertility and the destruction of biodiversity.

Man's massive modification of the environment also generates changes that may be negative for beekeepers. For example, migratory insectivorous birds, in the absence of other food sources, may focus their attention on bees (e.g. bee-eaters, *Merops apiaster*, which are much feared by beekeepers but are a protected species).

Modern industrial beekeeping depends, like intensive agriculture, on a significant input of fossil energy. This aspect, i.e. the relationship between fossil energy input and energy output in the form of food, is currently not given due consideration. Agricultural production consumes much more energy (in terms of fossil energy) than that obtained (in terms of carbohydrates, proteins and food fats), and fossil energy will no longer be cheap in the next few years: oil could become rare and very precious in less than 40 years. This is another fundamental reason to accelerate the necessary change also in beekeeping management.

Agriculture is responsible for about 44% of methane emissions and 82% of nitrous oxide emissions. Overall, between a quarter and a third of all climate-altering gas emissions come from the agricultural sector, which is also one of the sectors of the economy most affected by climate change. [680] Reconciling the projected demand for increased food production of at least 60% - 70% before 2050 with the need to reduce the progression of climate change, biodiversity destruction, water cycle degradation, and land degradation is an impossible challenge. It is true that over time, in more than 50 years, the production of plants and food of animal origin has increased but *per capita* availability, for most of the World's population, it has decreased. [749] It is much more likely that food production will decline due to climate change and biosphere degradation.

Agriculture is also the sector that consumes most of the water (70% of fresh water withdrawn is returned contaminated) and at the same time it is the most sensitive to alterations in the water cycle and phenomena such as droughts, floods, and overflows. In this regard, it is useful to focus our attention on the fact that, for some months, about a quarter of the great rivers of the Planet no longer reach the sea. [680] The water cycle in turn is adversely affected by climate change. We have created interdependent mechanisms that amplify themselves negatively and degrade irreversibly.

Agriculture is also the main cause of soil degradation: more than 35% of the earth's surface is occupied by agriculture. Agriculture has generated the alteration of the nitrogen and phosphorus cycle. It is important to point out that phosphorus is a non-renewable resource and is extracted from the subsoil mainly by three nations: USA, China, and Morocco. China, in 2016, produced about 50% of the phosphorus traded on the Planet and Morocco probably holds three-quarters of the known reserves, in the form of rocks. Phosphorus recycling, through the use of manure, could reduce dependence on this non-renewable source. In the USA, using a third of the manure produced could probably meet the phosphorus needs of cereals: less than 5% of USA cropland receives organic matter in the form of manure. [761] Most of the phosphorus extracted from the soil ends up in rivers, lakes and seas, creating major problems such as eutrophication.

In order to reduce climate change, the destruction of the biosphere, and re-enter a safe operating space, agriculture should consume much less nitrogen and phosphorus, much less water, much less fossil energy, increase soil fertility, and protect biodiversity. Achieving these results is difficult because current population growth and projected increases in consumption require the exact opposite. Practices that go in the opposite direction of sustainability are spreading, such as intensification of monocultures, use of fossil fuels, use of pesticides, irrigation, chemical fertilization, deforestation, and soil tillage that make food production more vulnerable and very harmful to the biosphere. The green revolution is necessary and cannot be achieved or even assumed at the current rate of growth in market demands. Reducing consumer demand and reducing population are related, and are priority goals to achieve the decreases in negative pressures needed to save the biosphere. To hypothesize sustainable development without consumption reduction and without demographic reduction is unrealistic. Overpopulation and consumerism are the two key aspects on which the success of any strategy depends, if aimed at safeguarding the natural systems on which we depend. The overriding aim could be summed up as ensuring prosperity and social security without increasing consumption and pressures on the environment.

AGRICULTURE IS AN INDUSTRY THAT CONVERTS OIL INTO FOOD

In the agricultural sector, from 1970 to 2010, the average productivity increased, thanks to chemistry and technology, from 2.4 tons to 4.6 tons per hectare. [104] The World average of calories available in the form of food is about 2,800 kcal per day *per capita*, while in the USA it rises to 3,800 kcal. In the USA, food production ensures about 1,500 kg/year per person, while in China this availability is about the half. [263]

In 2003, on average, each inhabitant of the Planet theoretically had at one's disposal 158 kg of wheat, 167 kg of vegetables, 60 kg of fruit, 60 kg of meat (including fish). [263] In reality, we know that this distribution is not homogeneous and that the current estimated average availability of about 2,800 kcal per day per inhabitant is not sustainable, as it generates irreversible changes. [5]

During the twentieth century the extent of cultivated land grew by 30% but the amount of harvest increased sixfold due to a 150-fold increase in energy used in agriculture. [6] According to other more optimistic estimates, in the twentieth century, agricultural production increased sixfold thanks to an 80-fold increase in energy consumption. [37] For this reason it can be argued that the current agricultural system is nothing more than a large industry that destroys ecosystems in order to transform oil into food. The result is an irreversible and unsustainable change in ecosystems. The amount of energy obtained is always less than that used. Agricultural practices are probably responsible for more than a third of all climate-altering gas emissions.

The Planet's crops today can theoretically feed an average of about four people per hectare. In 1900 they fed three people every two hectares. Agriculture has become oil-dependent and its result is the production of food with an energy content much lower than the amount of energy invested to produce it. It can be estimated that 1,000 litres of oil are needed to breed a 500-kilo dairy cow and 7 litres of oil are needed to produce 1 kg of veal. [6] Vegetables produced in greenhouses may have an energy content 50 times lower than the energy used to produce them.

In order to dry (reduce the water content) a food product an amount of energy equal to that contained in the food itself can be consumed. It may be roughly considered that it is necessary to use 3,542 kcal/kg to dehydrate a foodstuff and that to dry corn more than 1,500 kcal/L may be necessary. [425] Theoretically, 620 kcal are required to evaporate a litre of water from an open container, but the energy actually required to remove 1 L of water from a food is higher than this reference value.

To produce an alufoil tin containing 455 g of sweet corn providing approximately 375 kcal may require over 3,000 kcal, of which 450 kcal is for production, 158 kcal for transport, and over 1,000 kcal for packaging. [425] Producing the food containers alone may require more energy than the food itself, not to mention waste management and environmental consequences.

One of the achievements for humanity generated by the use of concentrated fossil energy is the possibility of being employed in jobs other than food production. Currently, at least 50% of the World's population is predominantly employed in agriculture, but in industrialized countries it is sufficient to engage a small fraction of the population in this sector through the use of non-renewable energy: 4% or less in the USA. [147] Intensive agriculture, by devouring fossil fuels, has produced several advantages such as the possibility for a large part of the population not to worry about producing the food they need. However, it has generated many problems such as the irreversible reduction of biodiversity, water pollution, soil erosion and the selection of organisms resistant to pesticides. In spite of the annual use of more than 3 million tons of pesticides per year, the adversities that we would like to limit continue to generate a loss of about 40% of all vegetable production. [263]

If the agricultural system were to adopt more strongly the rules applied to organic production methods, some negative impacts could be reduced, such as climate-altering emissions. These benefits, according to some studies, could be generated by simple practices such as reducing the use of chemical fertilizers that can be replaced by organic fertilizers such as livestock manure. [103] The yields of the organic production system per unit area are lower than those of traditional agriculture but the use of energy is much lower. So an environmental benefit is generated in terms of energy savings. In 2017, the organic agricultural area in the World was about 70 million hectares of which 13 million hectares in Europe (2017) and 2 million hectares in Italy (2018). [98, 99, 102]

Some production systems are experiencing very fast and, therefore, at unsustainable growth rates: in the last 40 years, World egg production has increased by 350% and poultry trade by 3,200% (China is the World's greatest egg producer). [8] The current agricultural system, in Italy and in all the most industrialized countries, generates many negative impacts:

- Genetic depletion, extinction of ecosystems and wild species.
- The chemical destruction of the micro fauna of the soil, and of living beings in general, due to the voluntary use of thousands of molecules that are certainly dangerous for man (fertilizers, pesticides, waste, combustion products).
- The physical destruction of soil by irrigation, plowing, and other agricultural practices.
- Water pollution and the reduction of freshwater reserves (groundwater and surface water).
- Deforestation which is also one of the important causes of the change in the composition of the atmosphere.
- Air pollution, for example, due to livestock farming and the use of non-renewable energy.

Ultimately, the current agricultural system has many advantages but it is not sustainable and continues to be possible only thanks to demanding and enormous contributions in terms of energy and technology; it is an ecological disaster: unfortunately, the history of mankind has already recorded the extinction of communities due to bad agriculture. [91, 208] As a result, more and more energy will have to be invested in order to obtain the same quantity of food, which will contain much less energy than that invested in producing it.

MORE THAN 50% OF THE VEGETABLES GROWN WILL FEED NO ONE

One result achieved by the application of the principles of industrial chemical agriculture, from which many benefits, is the decrease in the number of people who have to take care of producing food: less than 5% in the richest countries. To better understand the extent of the change, between the 1960s and 2016, in Canada (agricultural area of over 64 million hectares, in 2016), the average size of a farm increased from 163 hectares to 315 ha and in the USA (agricultural area of about 386 million hectares, in 2007) from 142 to 169 hectares. [444] In Canada, 70% of wetlands and 75% of prairies have been destroyed. The extension of

monocultures has increased dramatically, the bio-diversity of the countryside has diminished, and the use of pesticides has increased. At the same time, between the 1960s and the 1990s, the number of people who are maintained by a single farmer increased 4 times: from 1 farmer for every 28 inhabitants to 1 for every 100 inhabitants. [444]
When our ancestors were hunter-gatherers they needed, depending on the ecosystem, at least 100 productive hectares *per capita* to survive. Today, with intensive agriculture, one hectare could be enough to sustain at least two people on a vegetarian diet for a year.
Intensive agriculture generates benefits mainly for 1.5 billion people, i.e. for a minority of the World's population: the richest part of society. Eighty percent of the World's food is produced by farming families and three quarters of all farms are less than one hectare in size. At the opposite extreme, there are some American communities where less than 2% of the population is involved in agriculture. Crucial aspects that distinguish these two types of agriculture, the industrial one from the family one (implemented mainly for self-sustenance), are very evident: the former consumes more than 10 calories of fossil energy to obtain one in food and produces less than half per unit of area. Family farmers grow several species on the same farm so they are more efficient, more resilient and have much higher yields per hectare. Despite the increased use of pesticides in chemical agriculture, between 30% and 40% of crops are lost to diseases and pests, and at least another quarter of food ends up in the trash (between production and consumption). So it is realistically possible to say that even more than 50% of the vegetables grown will not serve to feed anyone. [761] These are estimates and although approximate, they give an idea of the waste. We should favour systems that use less external inputs (energy, pesticides, fertilizers) and that reduce waste.

THE ECOLOGICAL FOOTPRINT OF THE HUMAN SPECIES: WE SHOULD BECOME MORE VEGETARIAN

Trying to communicate the immensity of the impact of the human species on the biosphere is not easy, while the inequalities and waste in our modernity are well summarized in a few numbers: overweight and obese people (in total at least 1.9 billion) are more than the double of the number of malnourished people. [36]
Already in 2011, at least 38% of the World's ice-free land, or about 5 billion hectares, was used for crop and livestock farming. Globally, the livestock sector is probably the greatest user of land: directly or indirectly, modern animal husbandry uses at least 30% of the entire land area not covered by ice and 70% of all agricultural land. [114] In Europe, 75% of agricultural land is used to produce feed.
Probably 40%-50% of all grain production on Earth goes to feed livestock to get 2% of all the calories we use. [96] In the US and Europe, more than half of all the grains is consumed by livestock: 59% and 56%, respectively. In Asia and Africa, however, less than a quarter of the grain produced is used as feed, 22% and 13%, respectively, and most is used for human consumption.
To produce 50 kg of animal protein one needs to consume up to 800 kg in vegetables, and one hectare of spinach provides 25 times more proteins than one hectare of cereals for meat. It is safe to say that livestock farms have entered into competition for land consumption with much of the World's population. This information should give pause for thought about the waste of resources needed to maintain a diet rich in livestock by-products for a fraction of the World's population who get ill from eating too much.
The production of animal proteins requires 2.5 to 50 times more energy than the production of vegetable proteins and, therefore, many more climate-altering gases are released. [114] To make 1 kilocalorie (kcal) in beef steak requires 100 kilocalories in feed. To make 1 kilocalorie of feed

you need up to 40 kilocalories of fossil fuels. The yield is very low and if all consumptions are taken into account it may be less than 1%: genetic selection of seeds, purchase of plants, cultivation, transport, breeding, processing, cold chain, packaging, waste disposal. Energy consumption may be increased by certain practices such as transport over long distances or the use of the cold chain: even 66% of the energy required to obtain meat is needed for the production and transport of feed; to maintain 1 kg of meat or fish at -18°C requires at least 265 kcal per month. [114]

Livestock farming is responsible for the following emissions on the Planet: [114]

- 9% of carbon dioxide emissions;
- 35-40% of methane emissions, which has an effect 23 times greater than that of carbon dioxide on global warming;
- 64% of ammonia emissions, a gas that contributes significantly to acid rain and the acidification of ecosystems;
- 65% of emissions of nitrogen oxide, a gas that has an effect 296 times greater than carbon dioxide on global warming.

The production of foodstuffs of animal origin generates more climate-altering gases than the transport sector. [27] Raising an adult cow in one year produces the same amount of climate-altering gases as a car that travels 70,000 km.

Other issues to think about include the following:

- The increase in livestock production encourages deforestation: probably 70% of the deforested land in the Amazon has been turned into cattle pastures.
- Farms use at least 8% of all available fresh water. One kilogram of beef may require over 15,500 litres of water that is returned contaminated to the environment. One kilogram of chicken meat can require over 3,900 litres.
- Livestock-borne diseases affected at least 2 billion people in 2012, mainly in poorer countries. [5]
- Antibiotics are used to prevent certain bacterial zoonoses. According to the World Health Organization, more than 50% of the antibiotics produced are for livestock. [114]
- The work of the farmer presents many health risks. The Report on the reconstruction of fatal accidents in the Piedmont Region for the years 2009 - 2010 *(Rapporto sulla ricostruzione degli infortuni mortali in Regione Piemonte anni 2009 – 2010)* indicates that 22% of all fatal accidents are related to animal breeding.

It is necessary to reduce meat consumption in rich countries (and therefore the number of animals bred) and to reduce the agricultural area involved in feed production. The market allows economic valuations to be distorted by financial aid that allows prices to be lower than the costs incurred. Environmental costs are not accounted for, as they are left to future generations or are silently distributed throughout the community (e.g. eutrophication, deforestation, water pollution).

It takes about seven pounds of grain to produce one pound of red meat, while it takes at least two pounds to produce one pound of chicken. [104] Changing the protein diet can lead to a reduction in the amount of energy used and the amount of land devoted to feed. Reducing meat consumption, especially in countries where more than 100 g per day is consumed, could have direct health benefits: [53]

- reducing the risk of heart disease,
- reducing obesity (an obese person has a reduced life expectancy of more than 10 years),
- reducing the chances of getting cancer in the gut or type 2 diabetes.

Reducing red meat consumption, to less than once a week for decades, can help increase life expectancy by up to 3.6 years. [873]

It is estimated that the total living beings on the Planet consist of 550 gigatons of carbon (1 gigaton is equivalent to 1 billion tons). The majority, about 450 Gt, are plants. The rest is made up of bacteria (70 Gt), fungi (12 Gt), single-celled organisms (7 Gt), algae (4 Gt), and animals (2 Gt). The human species weighs 0.06 Gt, or 0.01% of total carbon.

Our impact on the Planet is devastating: since the dawn of civilization, humans have caused the loss of 83% of all wild mammals, 80% of marine animals, and 50% of plants. [800, 801] Farmed animals represent a biomass at least 14 times greater than that of wild mammals, and at least 50 times greater than that of all wild birds. [799, 801] More than 70 billion farmed animals a year are killed for human food, most of them are chickens. [802] To get an idea of the ecological footprint generated by intensive livestock farming in Europe, it can be reasonably estimated that it is necessary to cultivate an area 7 times larger than that of the Old Continent in other countries in order to produce feed. [836] At least 20% of all the terrestrial biomass is made up of farmed animals requiring the exploitation of more than 30% of the land area that was once occupied by wild species. Humans, together with farmed animals, make up at least a quarter of all the terrestrial animal biomass. [36]

The terrestrial surface not covered by oceans is equal to about 149 million square kilometres (corresponding to 29% of the entire surface). Only 71% of this area (104 square kilometres) is usable by man, as the remaining part is occupied by glaciers, deserts and arid zones. At a planetary level, 50% of the usable terrestrial surface is used to feed livestock and 37% (39 million square kilometres) is covered by woods and forests. Thus, of the 51 million square kilometres used by agriculture, 77% (40 million square kilometres) is used directly or indirectly to produce feed. [803] Only 11 million square kilometres of the Earth's surface are used for agricultural purposes other than feeding farm animals. Most of the Planet's agricultural land is occupied by species that, in addition to being alien, are often unnatural because they are generated by human talent, such as genetically modified cereals and soybeans.

The use of more than 50% of the solar energy captured by plants to meet the needs of humanity favours the reduction of biodiversity. Therefore, the human species has appropriated most of the natural resources of the Planet to the detriment of all the others, irreversibly altering the natural balance. Technological innovation has allowed us to become dangerously efficient parasites.

It can be estimated that there are 11.4-11.6 billion biologically productive hectares available on the Planet, distributed between land and sea; dividing this number by the number of living people, about 7.8 billion, we have an average of 1.4-1.5 hectares of bio-productive surface per person. [5] Therefore, the bio-capacity (ecological footprint) that the Earth is able to bear is less than 1.5 hectares per inhabitant. [6] In reality, the distribution of the ecological footprint on the Planet is not homogeneous, since some countries consume far more resources *per capita*: it can exceed 8 hectares per person in the economically richest areas of the World. In Italy, the ecological footprint is estimated to be 4.5 ha *per capita* per year and in Europe 4.7 ha *per capita* per year. [19] The bio-capacity available in Italy is less than 1 ha *per capita*, so we generate soil consumption in other States and we mortgage our future. [27] The soil consumption to which a fraction of the World population is happily accustomed cannot be spread to all the inhabitants of the Planet. Those just described are averages, but a great part of the World's population, at least a billion people, die because they do not have access to enough food to survive.

The most relevant changes were generated with the beginning of the industrial era, which is conventionally established in 1750, when technology became established. At that time, less than 9 million km^2 of the Planet's surface area was probably used for agriculture and livestock breeding, which is less than Italy's current agricultural area. In 2011, 38% of the emerged land free from ice was used for cultivation and breeding, equal to about 5 billion hectares (50 million km^2). For reference purposes, Europe covers a total area of about 10.4 million km^2, with a surface area of about 7% of the land (amounting to a total of 149 million km^2; the utilized agricultural area in Europe varies around 175 million hectares). Thus, on the Planet, the total area devoted to crops and livestock is 5 times the entire surface area of Europe.

In 2050, the Planet will probably be home to another 2 or 3 billion human beings and at the current rate of growth in World demand for animal-derived foods, theoretically several tens of billions of farmed animals per year will be needed, in addition to those already being farmed. Today, the area of land cultivated *per capita* on the Planet is around 0.23 hectares per year: this is an average, in fact high-income countries have a larger area *per capita*. [804] To support the further increase in consumption expected due to the current rate of population growth and the increase in demand for farmed animals, one Planet is not enough. It is likely that if in the future at least 40% of the area currently devoted to feeding farmed animals is devoted to producing food for humans, 9 billion humans could be fed. Therefore it becomes crucial to reduce the consumption of meat, eggs, milk, and derivatives to avoid the collapse of the agro-food system. [805]

The data just summarized are approximate, but they give a good idea of how man, although small in terms of biomass and young compared to the Planet, has managed to profoundly modify it in a self-destructive way. A great obstacle to change is constituted by structural constraints, deriving from biochemistry and physiology, which are the same as 200,000 years ago: genetic, cultural, and social adaptations are much slower than the rhythms of technological innovation. The brain is the same as our primitive ancestors so it is unprepared for self-induced changes.

THE OCEANS ARE IN DANGER

The Planet's seas perform essential functions such as absorbing heat, producing most of the oxygen we breathe, absorbing at least a third of carbon dioxide emissions, stabilizing the climate, and providing food and economic support for billions of people. Despite their vastness they are in serious danger. The oceans are contaminated by plastics, petroleum derivatives, metals, agricultural products, compounds from the chemical industry, pharmaceuticals, and civil and industrial wastewater (sewage). Most of the pollutants reach the seas through rivers (e.g. plastics, pesticides, pharmaceuticals, fertilizers) while others come from the atmosphere (carbon dioxide).

Over 10,000 chemical compounds are used in the production of pharmaceuticals, cleaning products, and cosmetics. Some of these products contain micro-plastics. The effects of these substances on aquatic organisms are partly unknown: some compounds in sunscreens (benzophenone-3) are suspected to be toxic to the larvae of some coral species (they become immobile and DNA damage is recorded). [1271]

At least 10 million tons of plastic enter the seas every year: overall, at least 420 million tons of plastic are produced each year, most will be used once and become waste in less than a year after production, even though they are hardly biodegradable. [1271] At least 6% of fishing nets, 9% of catch systems, and 29% of the fishing lines used to fish marine organisms remain in the sea. One of the negative consequences is that 20 micro-particles of plastic may be found for every 10g of human faeces. [1271]

Another pollution of great concern is mercury, which originates from various sources such as coal combustion and gold mining. Methyl-mercury is a persistent pollutant that accumulates in the food chain: in marine mammals and some fish (e.g. tuna) it may reach concentrations of 10 million times higher than in water. It is known that exposure to methyl-mercury in the uterus reduces motor skills, attention span, verbal skills, and memory. [1271] In children as young as 11 years old, it reduces the IQ (intellectual quotient), reasoning ability, memory, and other brain skills. Even in adults some brain functions can be severely impaired due to mercury. High concentrations in the blood damage the cardiovascular system. In the USA, up to 600,000 children are probably born each year with cognitive problems due to their mothers' exposure during pregnancy to mercury in fishery products (they account for 3.2% of all recorded cases of mental retardation in the USA). [1271]

The massive use of chemical fertilizers promotes the growth of algae (eutrophication) in many areas of the Planet. Petroleum derivatives reduce photosynthesis causing a dangerous decrease in the amount of oxygen. The increase in the absorption of carbon dioxide from the atmosphere favours the acidification of the oceans, the extinction of coral reefs and damages molluscs.

Accidental events such as hydrocarbon spills in the sea are a problem: we remember the accidents in 1974 in Chile (over 200,000 t), in 1978 in France, in 1979 in Mexico (over 500,000 t), in 1989 in Alaska (37,000 t), in 1991 in the Persian Gulf (1,100,000 t), in 2010 in Texas in the USA (508,000 t), in 2019 in Chile (40,000 L of diesel oil). These are mixtures of hydrocarbons with substances that are very dangerous both for the health of aquatic organisms and for the human species.

The mechanisms by which chemical pollution damages marine ecosystems are largely unknown but the effects are very evident and alarming: fish stocks are declining. It is foreseeable that the artificial conditions created by various alterations may favour microorganisms that are pathogenic to humans, such as the cholera agent (*Vibrio cholerae*) and the toxins produced by certain algae that may be ingested through the consumption of crustaceans (saxitoxin, a domoic acid produced by diatoms) and fish (ciguatoxin produced by dinoflagellates that are part of the plankton).

Ocean protection must be addressed on a global scale and as soon as possible. Mitigation and prevention actions are very simple, some of which are suggested as follows: [1271]

- Do not use coal as an energy source.
- Do not use mercury.
- Do not use plastic and manage the waste in a way that reduces the poisoning of the Planet's waters.
- Do not use persistent pollutants.
- Do not use chemical fertilizers.
- Reduce the fishing activity.
- Protect most of the seas by establishing protected areas.

Unless this course is reversed, the degradation of the oceans and the resulting risks to our health and safety will increase.

THE DILEMMA BETWEEN INFINITE GROWTH AND THE NON-MATERIAL DIMENSION OF PROSPERITY

One of the evident failures of humanity is that of having relied on artificial rules such as economic and financial ones, which are unsustainable. The evident epochal error lies in considering the economy independent from nature. The current model of society is inadequate, unsustainable and full of uncertainties regarding future developments. Our ability to foresee is very insecure: although the forecasts are very worrying, they could turn out to be optimistic. On the one hand, we sin on the side of presumption when we believe we can predict the effects of the change we are generating, and on the other hand we are irresponsible in not implementing the necessary preventive measures. Many changes such as those generated on biodiversity, on climate or with pollution may surprise us with non-linear mechanisms but, for example, with thresholds and rapid and unpredictable effects. A far-sighted society, in addition to trying to reverse the process of destruction of natural balances, should put in place strategies to enhance resilience to destructive changes that will surely be recorded in the coming years. The economic wealth of the human society and the health of the Planet are closely related, just as health is to the environment. Probably at least a quarter of the diseases, and a third of those affecting children, are influenced by environmental factors that we are deteriorating, so they will only increase. We must stress this concept: the degradation of the environment precedes the worsening of the state of health (e.g. increase in zoonotic diseases and cancers) and the deterioration of social security. The current population growth and the even faster increase in the exploitation of the limited resources of the Planet are unsustainable: we can no longer be sure of maintaining social peace. Some planetary limits have been exceeded and we have entered a zone of uncertainty and high risk: climate change, the destruction of biodiversity, the bio-chemical flows of phosphorus and nitrogen, the change of land use (deforestation, reduction of fertility, etc.) are some of the destructive pressures on the environment that we should absolutely reduce. Other issues such as ocean acidification, freshwater exploitation and pollution, waste generation, air pollution have probably entered a phase of unacceptable uncertainty. The forecasts expected and hoped for by economists regarding the growth of wealth (of the GDP or Gross Domestic Product), even if only by a few percentage points each year, are a true utopia. The Planet we have does not allow us to do so and the results we will surely obtain are the destabilization of social security and the worsening of the quality of life. GDP indicates the monetary value of goods for final consumption, public and private investment, net exports (total exports minus total imports) exchanged for money in a given period of time. World GDP, in 2015, was valued at $77,302 billion and World debt was at least 2.3 times this value (two-thirds of debt is generated by private individuals); the 2018 World GDP was about $91,000 bilion, 200 times higher than in 1750. At the same time indicators of the existence of alarming speculation that should call our attention are ignored: [844, 979]

- In 2008 (the year of the severe economic crisis) the value of financial products was at least 14 times greater than global GDP;
- In 2011, 45 of the top 50 multinationals did not produce goods, they were banks or insurance companies;
- In financial markets, for every $100 that move between countries, only two are used to pay for goods and services;
- In 2012 between $21,000 bilion and $32,000 bilion were hidden in tax havens (companies avoid paying taxes in the country where they generate profits). [980]

An alarming fact are the incentives for fossil energies: at least six hundred billion dollars a year. The financial system is indifferent to the health of the population and of nature, although the limits are evident everywhere. The market does not care about children and reasons by quarters.

GDP cannot be considered a suitable tool for assessing the change in the well-being of a society. Among the weaknesses of this economic indicator, there is the failure to consider natural capital, environmental damage, waste, health effects, and also the omission of other, apparently less complicated aspects such as volunteering, unemployment, cultural level or domestic work. These items, such as pollution, are not accounted for as they should be (negative items include pollution and destruction of biodiversity). In any case, it is risky to try to put a price on all things in order to commodify them, such as pollution, human health or the pollinators that we are exterminating. The identification of the right price does not allow us to perceive the real immaterial value and generates us the illusion into thinking that we can buy anything, even life. Attributing an economic value to everything is an unintelligent vision and not at all far-sighted.

GDP can grow if weapons are sold, if wars are waged, if pollution increases (it will have to be cleaned up), if more cigarettes and drugs are sold, if thousand-year-old trees are sold, if roads and hotels are built in natural paradises, if new goods are designed using the strategy of planned obsolescence, if more medicines are sold, if diseases increase (expenses for diagnoses and treatments increase), and if waste production increases. At the same time, GDP *per capita* decreases if the population grows faster than the economies. Goods can be bought in the form of commodities but they can also be self-produced for one's own needs or exchanged (barter or gift). In the latter cases they do not influence GDP positively. In the industrial society goods are not produced to satisfy the needs of the buyers, but to make more money than is spent to produce them.

It must be remembered that GDP cannot provide a measure of inequality: at least three billion people live on less than €5 a day. The increase in GDP could benefit only those who are already rich, that is, the increase could not affect the economic wealth of the majority of humanity at all or could even worsen it, as is happening. Among the distorted effects of this vision of consumer capitalism is the generation of enormous debts that are left as an inheritance to the children (quite a gift).

One indicator of the suicidal circle represented by the myth of infinite growth is advertising, which, after the arms industry, has the second largest budget in the World. [836] Advertising has become a sophisticated and systematic tool for lobotomizing the brain and persuading people to indulge in the unbridled consumerism that invades our lives from birth to death. Thanks to advertising it has been possible to create a culture of being able to own anything by buying on credit, with more devastating effects than those generated by drugs. Among the measures that have been requested by supranational organizations (Central Bank of Europe, European Union, International Monetary Fund) to revive economic growth in countries where the GDP is shrinking, it is useful to highlight some of them in order to reflect on the future we desire: [844]

- Reduction in spending on social services: pensions, schools, health, assistance.
- Increased workloads and reduced salaries for public employees.
- Postponing the retirement age.
- Privatisation of local public services: aqueducts, waste management, transport, health service, education.
- Introduction of private management criteria in social services such as schools and health service.
- Selling off important public assets.
- Waste of resources to finance and support useless activities and works with unacceptable consequences. The idea of "too big to fail" is part of this vision: it is a collective suicide programmed to favour a few (banks and big companies).
- Subsidising, directly or indirectly, the consumption of resources in inefficient, unsustainable and undemocratic ways such as chemical agriculture, agro-fuels and fossil fuels.

These measures do not raise GDP, they increase the crisis and they do not alleviate environmental and social problems that should be addressed differently: the main benefit is the increase in the flow of money from the many to the few.

The public sector (the State) should decide the rules of the private one (the market) and not the other way around. Dominant policies distribute debts and failures of both the public and private sectors on the middle class, they promote unemployment, exploitation and, therefore, migrations. They weaken freedom and democracy. The use of GDP as a reference to measure the welfare of a State and to plan economic policies should be definitively abandoned.

Unfortunately, the economy is considered unrelated to the environment, committing a serious error that should be corrected in many ways, such as modifying the formative and cultural paths that generated it. There are probably sufficient resources for everyone, but certainly not enough for everyone's greed.

To continue to use economic rules to manage societies is irresponsible. We are compromising freedom and security, especially of the poorest and the next generation. Those who believe in the infinite growth of the World economy are trusting in the impossible, so they are as ignorant

as well as the economists who profess this faith are either insane or in bad faith. Sustainability and, therefore, the reduction of consumerism does not express a nostalgic desire for a life in harmony with nature, but is the result of the awareness of the non-substitutability of the functions of the ecosystem. Economists who support infinite growth should therefore understand the importance of the biosphere, also for finances.
A growth of the World economy at current levels means doubling every 20-25 years: theoretically, before 2100, it should get between fifteen and twenty times greater than today. An increase in GDP of 3.5% per year *per capita* (equivalent to that recorded by France between 1949 and 1959) generates an increase of 31 times in one century, 972 times in two centuries and 30,000 times in three centuries. To hope to achieve this, it is necessary first to secure the conquest of several other planets. Unfortunately, important institutions, such as the World Bank, foresee the possibility of sustaining these growth rates to ensure the well-being of humanity: increasing the wealth of the Planet fourfold by 2050. [836] The limits of a finite Planet have been completely disregarded and the capacities of the Kingdom of living beings have been considered infinite. Yet the signs evident throughout the World should shake these wizards of finance: GDP is shrinking inexorably. Attempts to revive growth show themselves as the irrational behaviour of a terminally ill person in the grip of madness.
The current rate of growth will be definitively stopped by the destruction of the biosphere and by social insecurity: a crisis is looming, which is the prerequisite for war. [681] In short, the social recession becomes more and more concrete and real. A melancholic degrowth is looming, leading to a sudden collapse. The myth of economic growth as a remedy for the World's problems is quite disturbing. And it is also an alarming signal to believe that we can not take care of environmental problems because technology will save us. [846]
At this point we should only ask ourselves which of the planetary limits, if we continue to espouse the faith of growth, will affect first and most the collapse of the human race. The crisis of depletion of non-renewable resources, the inability to absorb pollutants or the reduction of food production? Simulations of various future scenarios always lead to the same conclusion: the collapse of social security and of the biosphere before the end of this century. In the worst case between 2030 and 2070. [836]
The combination of negative factors generates considerable psychological pressure. Therefore, both among those who will be directly affected by the disasters favoured by human activities and those who will be fortunately less affected, an increase in phenomena such as anxiety and depression is easily foreseeable.
Unfortunately, some believe that humankind should accept the ecological disaster because we have created it as collateral damage to a beautiful destiny. This is a vision that implies environmental illiteracy and is egocentric, as we promote ourselves to the rank of gods. According to this unrealistic vision, the human species takes control of everything: of the ecosystems of the Planet Earth and the flows of matter and energy that regulate the health of the biosphere. The mere suggestion of this ingenuity and power, until now attributed only to supernatural forces, highlights our short-sightedness and greed. The biosphere, through a long evolutionary process, has originated the human brain which in turn has produced culture. The latter has succeeded in producing a frightening technological innovation, because it is able to destroy the biosphere. So we must ask ourselves if the human brain through culture will be able to save the biosphere and that is to defend itself from itself.

The great challenge that awaits us is to design a new society that consumes much less, that generates less negative pressure on the environment, and that, at the same time, is able to ensure water, food, health care, education, personal safety, freedom of expression, employment, equality, equal opportunities, and political participation for all. The society of the future will therefore have to seek to guarantee fundamental rights for the collective wellbeing by making much less use of the Planet's resources. Someone has called this (necessary) transition "happy

degrowth" or "peaceful revolution", probably to distinguish it from the unmanageable collapse and recession that lie ahead. [844] Degrowth must be a managed, programmed, controlled, shared, and gradual process with specific objectives, such as the reduction of the production of goods and the promotion of sustainable practices (reducing the use of fossil fuels, increasing soil fertility and biodiversity, etc.).
Among the actions proposed to oppose unjust laws, such as those that favour monopolies and inequality, there is civil disobedience:

- the self-production of goods;
- exchanges based on gift or barter (without using money);
- not using products from companies that are not able to guarantee transparency on environmental protection and worker safety;
- the purchase of locally produced food directly from farmers;
- the reduction in the consumption of animal products;
- not using bottled water.

The boycott of big brands that adopt opaque or even destructive policies towards human and natural resources should be a new form of expression of freedom and responsibility towards future generations. Among the many examples of peaceful protest, it is interesting to remember the distribution of seeds of plants (wild amaranth in South America, which is an edible plant) that have become naturally resistant to the herbicide glyphosate, in order to oppose projects that foresaw the diffusion of genetically modified plants tolerant to the same herbicide. [975]
The whole biosphere is so interconnected and influenced by human activities that personal, local, or minority solutions cannot be an answer, even if it is true that somewhere we have to start intervening. However local actions are necessary to organize resilience that is to cushion the blows that are coming.

Unfortunately, the current economic rules positively consider investments in weapons or for useless and harmful concrete works. Activities or services that have negative consequences for the community may increase some indicators that signal an increase in wealth. Paradoxically, even the increase in the number of illnesses is a positive indicator, if put in relation with an increase in expenses for treatments. Instead, wealth indicators should also take into account parameters that measure the quality of life (social well-being and health), and of the environment, such as:

- life expectancy;
- the average level of education and the possibility of access to quality educational pathways; the skills and educational level of students should be measured, rather than the public resources spent on schools, especially if monetary investments are not followed by an accurate assessment of the corresponding benefits; education may be considered a contract between society and the future and there is an increasing need to update it in order not to repeat the mistakes of the past;
- the average and minimum wage;
- initiatives to combat poverty;
- the possibility of access to a quality health service;
- the health of water and soil;
- air quality;
- the extent and quality of natural areas (biodiversity);
- the availability of green areas close to homes;
- independence from fossil energy sources;
- independence from resources outside the territory (e.g. food);
- the availability of free time;
- the possibility to access recreational (e.g. sports) and cultural (e.g. music) activities;
- the presence and prevention of crime;

- the availability of public transport and alternatives to private cars.

The indicators mentioned should be taken into account by economic and financial rules, which is practically not the case. Unfortunately, even in Italy, the environmental good is often sold out and given in concession to private individuals for little money, even if it earns billions of euros, such as concessions for beaches, quarries, mineral waters or authorizations to cementing. There are exaggerated incentives for private schools, spending on armaments, "fake" renewable energies (such as methane from corn), and private health facilities. Reducing these expenditures could provide resources for more important activities and services. In 2012, Italy's GDP (Gross Domestic Product) fell by 2.4% and as a result social spending was halved. [875] At least one family out of three is unable to meet unexpected expenses of a few hundred euros. Meanwhile, all over Europe, private banks are being bailed out using public funds.

We are reminded of a phrase that was formulated some time ago, but which is still valid, and which we can rewrite: *at one time we sought wisdom, then we were satisfied with knowledge, now we are left with information, filtered by political and religious rules, governed by economic logic, which cultivates ignorance in a systematic and programmed way*. There are many studies, conducted for example by sociologists, which show a significant increase in the likelihood of civil wars in societies where young people are educated and unemployed. Unrequited expectations may be an impetus for very dangerous social clashes. Perhaps some might consider this a valid reason for cultivating ignorance.

ECOCIDE: ECONOMY ACCEPTS NO LIMITS

The economic and financial growth revered by consumerism cannot be sustained; consumption growth is outpacing population growth: from 1950 to 2012 the population doubled but the World economy increased sevenfold and annual energy consumption increased fivefold.[37] The speed of energy consumption is unfortunately growing further and faster than population growth. Economic and financial rules do not consider the fact that population, production of goods and food, use of resources, waste production, and pollution cannot grow forever. There are physical limits to the sources of matter and energy and the capacity of reservoirs that receive pollutants and waste products. In fact, many impacts, such as that generated by the production of pollutants, have already exceeded the limit constituted by the capacity of natural depollution. It should give pause for thought and concern that politics and society are completely inattentive to these limits. Many pollutants have never existed in nature, and their effects, often mostly little known, are negative. The control of wildlife and its (conscious) humanization are irreversibly destroying the biodiversity necessary for our species to thrive.

The environmental degradation in progress makes it easy to foresee that, for the majority of the inhabitants of the Planet, the basic level of social security will not be assured. If we do not intervene with adequate policies, which necessarily require a decrease in consumption and in the use of natural resources, we risk the collapse. A new vision is needed to avoid barbarization. In this regard it is useful to remember that in 3,358 years (from 1496 b.C. to 1861) there have been 3,130 years of war and 277 of peace, that is thirteen years of war for every year of peace. Another indicator is also very alarming: the average duration of the more than 8,400 peace treaties, stipulated between 1550 b.C. and 1860, was only two years. [847]

It is urgent to design an ecological economy to avoid recession with the possible wars that follow: if less than 1% of the at least 14,000 nuclear weapons registered in the arsenals of nine countries (in 2018) were to be used, we would witness an implacable destabilization, like the end of agriculture and civilization, everywhere (the crazy strategy of mutual assured destruction, up to now, has avoided the worst). [980]

Degrowth must be planned and designed by supporting the non-material dimension of prosperity: family, friendships, sense of belonging, leisure, identity, life purpose, and altruism. Consumerism has addicted us to the idea that happiness lies in owning things, when we should begin to appreciate the freedom of non-consumption. Consumerism cultivates social traps that encourage selfishness, loneliness, and the commodification of every aspect of life: the standard of living of the wealthy is turning against the future of our children.

It is necessary to be realistic, at present a large part of the World population finds itself in very sad conditions of life. The truth is that for a large part of the population it is a false hope that there will be a scenario of growth in income and social security in a Planet that is increasingly crowded and despoiled. Another major obstacle is that it is very unlikely that individual actions will be sufficient to bring about widespread social change, especially in a social environment that is hostile to change. Leaving the market totally free cannot be the solution either.

More and more often we are witnessing a morally questionable phenomenon: groups of rich and powerful people try to persuade other, much more numerous groups (lacking prestige and power) to renounce material wealth; the subliminal message is the following: let's arm ourselves together but leave for the front without us because we are not renouncing anything. [841, 842] This kind of media communication, which reaches large swathes of the population, is ineffective and deprived of the necessary sharing, although it has positive aspects. The sense of common initiative is dismantled and the feeling of urgency is diminished.

Among the fundamental principles of the ecological economy we should include the concept that there are natural goods and services that are common to all humanity and necessary for future generations. These common goods must not be privatized but must be managed according to principles of preservation, sustainability, and equity. Private profit tends to socialize losses and hand over (environmental) debts to future generations. Prosperity must be ensured for all and must be decoupled from economic growth. The fairy tale of unlimited economic growth has become a lethal weapon in the face of the ecological, social and financial challenges that we must seek to resolve as quickly as possible.

It is necessary to accept planetary limits, that is, to limit the freedom of destruction and appropriation of natural resources. It is indispensable to protect, maintain and improve the ecological capital on which the economy depends too and apparently does not care. Limits are annoying, they are avoided by politicians because they make them unpopular, but they are effective. To downplay and avoid facing these challenges that constitute an inconvenient truth there are those who believe that there can be no insurmountable limits to economic growth because there are no limits to humanity's imagination and willpower. This view is wrong and leads mankind to expand beyond the capacity of the Planet. Proposals that have no concrete use to counter the ecological disaster are numerous. Among them, there is the idea that economic growth can be sustained by reducing environmental damage per unit of good or service. Energy efficiency can generate benefits, but reducing environmental damage per unit of good or service has not generated any environmental benefits at all. Despite these improvements, in efficiency, consumption of non-renewable resources increases.

Economic rules have already pushed beyond the operational safety space for the climate, for the loss of biodiversity, for the reduction of soil fertility, and for other factors indispensable to the well-being of humanity (lack of water and food) and of the Planet. Continuous (infinite) economic growth in a finite ecological system is impossible: this concept should be taught during the first year in university Faculties of Economics all over the Planet and in all schools for politicians. The educational and training system is increasingly shown to be obsolete, outdated, inadequate, and based on losing models: capitalism, consumerism, the market without rules, competition, individualism, injustice, inequality, environmental illiteracy. The prevailing cultural model considers natural capital to be privatizable and devaluable. It overlooks the fact that the biggest and most frightening public debt is the one contracted with nature and not by cultural inventions such as banks and finance. Unfortunately, however, we do not have time to wait for a likely better generation of economists and politicians. At the same time, we must learn to avoid being misled by both economists and politicians. There is no time, perhaps the most effective cure might be the closure of many Economics classes in Universities.

Some realistic but frightening predictions indicate that in the future we might hope to be able to live sustainably if the World's population is reduced to less than 2 billion. By dedicating at least 5,000 square metres to food production through intensive agriculture, and at least 10,000 square metres to renewable energy production, for every inhabitant, one could hope to be able to lead a sustainable and dignified life. [97] It is not an impossible goal, we could try to reach it, even if the numerical reduction could require more than a century and many sacrifices.

If the population continues to increase and consumption with it, and faster, the probability that the system will not suddenly collapse is almost nil, because the ecosystem and economy will become very vulnerable. A collapse of the balances that regulate ecosystems could generate a sudden and uncontrollable decline, both of the industrial system typical of the richest countries and of the number of inhabitants of the Planet. The conditions that regulate the economy and the relationship we have with the environment must be modified as soon as possible. Studies, surveys and unquestionably reliable estimates, even if they use the arid language of numbers, all lead to the same conclusion: the future will certainly be worse for the majority of the population but, probably, collapse is still avoidable. So there is still hope and it is worth trying to change the future more wisely.

UNITS OF MEASUREMENT

Attention is drawn to the meaning of some units of measurement and other concepts that will be used to better understand the subsequent in-depth studies.

The prefix "k" is read kilo and means 1,000 times, while the prefix "M" is read mega and means 1,000,000 times larger than the preceding unit of measurement (e.g. 3 kcal or kilocalories correspond to 3,000 cal or calories).

The prefix "G" is read giga and means 1,000,000,000, or one billion times the unit of measurement that follows it. To give an example, 1 Gkg corresponds to one billion kilograms or one million tons (Mt).

One calorie (cal) is the amount of energy required to raise the temperature of one gram of distilled water from 14.5°C to 15.5°C, at a pressure of one atmosphere (i.e. at sea level). One kcal (corresponding to 1,000 calories) is the amount of energy required to raise the temperature of one kilogram of water by one degree.

From the combustion of a kilo of methane it is possible to obtain 14,000 kcal, from a kilo of diesel 11,000 kcal, from a kilo of coal 7,000 kcal and from a kilo of firewood less than 4,000 kcal. [187]

The amount of daily energy needed to sustain the life of a human being is about 2,000 kcal, and this is the same amount of energy that can bring 23.5 litres of distilled water from 15°C to 100°C. To make 1 kg of distilled water pass completely from the liquid state to the vapour state requires about 540 kcal, a quarter of the daily energy sufficient for a human being (this is called *latent heat of vaporisation*).

Two thirds of the World's population sustain a predominantly vegetarian diet, consuming about 200 kg of cereal products each year. One third of the World's population, including Italians, consume about 360 kg of meat and meat products per year, which require at least 655 kg of cereals (and other animal feed) to be produced. If we assume that every inhabitant of the USA consumes 920 kg of food per year, it means to need about 3,800 kcal per person per day. In the

USA, to produce 1 kcal of food we can consider having to invest, on average, at least 13 kcal, that is 13 times more than the energy obtained. [425]

One "toe" (ton of oil equivalent) corresponds to the energy developed by the combustion of one ton of oil. This energy is conventionally considered to be 10,000,000 kcal (equal to 41.868 GJ, corresponding to 11,627.907 kWh).

The combustion of 1 kg of oil can produce 10,000 kcal (1.16 kWh) and 1 kg of natural gas 8,000 kcal (0.93 kWh). As an example, 10,000 toe corresponds to about 12 million m^3 of natural gas.

Overall, the Planet consumes at least 8 billion toe per year. It is estimated that each European inhabitant consumes on average at least 3 toe per year. Italy is one of the European countries with the highest index of motorized mobility (civil and commercial), allowed by the import of the majority of fossil energy used.

1 barrel of oil is equivalent to 158.99 L (about 135 kg).

A human being can, for a short time, generate a power of about 800 W [equivalent to 336 cal/second (800 x 0.24 cal/second) needed, for example, to run up the stairs], but to perform a lasting physical activity cannot develop a power higher than 50 W (12 cal/second). A radio-CD device with a power of 50 W can use energy equivalent to that produced by the work of a human being. A medium-sized car with a power output of 80 kW, travelling at cruising speed, does the same amount of work as 1,600 people producing a power output of 50 W. An airplane taking off performs work equal to that which can be done by the muscles of 1.6 million people (between 2013 and 2018, fossil fuel emissions generated by air travel grew by 6% per year, a rate equivalent to building fifty new coal power plants per year). [6, 1276]

It has been possible to exploit the energy contained in the chemical bonds of the fuels, in substitution of the muscular force, very efficiently. To produce cereals without the help of machines and chemistry a human being can manage one hectare per year, considering a commitment of about 1,200 hours/ha per year (for example in Mexico to grow corn). While the intensive, highly mechanized, fossil energy-guzzling agricultural system, such as the corn farming system that can be found in the USA, may require only 10 hours of human labour per hectare per year. [425] In the intensive farming system a single worker can easily manage 50-100 ha, but more energy is used than is contained in the food obtained.

One horse-hour (HPh) corresponds to 642 kcal (0.746 kWh). 1 HP corresponds to the maximum work capacity that a horse can sustain, working for 10 hours a day. Conventionally it is considered that the work capacity of a human being is one tenth of that of a horse, so a man working 10 hours a day can produce an energy equivalent to 1 HPh. To understand better, we may also consider that a horse can do in one hour the work that could be done, in the same time, by 10 men. Remember that probably the first horses were domesticated in Asia around 3000 b.C. [425]

One gallon (3.79 L) of gasoline can produce the energy equivalent to that produced by 3 weeks of work by a man working 40 hours a week (at 0.1 HP or 0.075 kW).

ENERGY

Energy, according to some fundamental laws of physics, cannot be created and cannot be destroyed. Furthermore, the transformation of one form into another always implies a loss (e.g. in heat). The first light bulbs (invented by Thomas Edison in 1879) were very inefficient, as they transformed only 0.2% of energy into light.

There is a possible direction of spontaneous movement of energy: from a hot body to a colder one; spontaneously the opposite never happens.

Internal combustion engines produce electrical energy with an efficiency that varies from 30% to 40%. It means that only 30-40% of the energy contained in the fuel used is converted into electricity, the rest is lost mainly as heat (in reality energy is also consumed to build the engine, to extract and transport the fuel, to handle the waste, to build the roads and many other energy intensive activities, so the real energy yield is less than 30%).

To produce energy, it must be transformed from one form into another: for example, the combustion of petroleum derivatives or vegetables exploits the chemical energy stored in the chemical bonds (between carbon atoms and between carbon and hydrogen atoms). A trigger (temperature) and an oxidiser (oxygen) are required to release this energy. With combustion, the initial mass is converted mainly into water vapour and carbon dioxide, and ends up in the atmosphere increasing the initial volume, in the case of petrol, by up to 2,000 times.

Whenever we transform one form of energy into another, e.g. electrical energy into mechanical energy (e.g. a blender), only a fraction of the initial energy can be transformed into useful energy (e.g. blade rotation). Most of the initial energy is lost as heat, which is returned to the environment (it constitutes an energy loss). Energy is lost at each step. Within the food chain, at most 10% of the chemical energy contained in food passes from one level to another. This explains why it is difficult for a food chain to sustain more than 4 or 5 transitions and why carnivores, at the top of the food chain, require very large areas for their survival. More than 100 potential prey items are also required for the survival of each carnivore.

The energy ingested with food (chemical energy) is consumed with physical and mental activity, or is accumulated in adipose tissue (a part is dispersed in the form of heat and in the organic material expelled with the manure).

All forms of energy can be transformed into heat, while the opposite may not happen. Energy can be exchanged, but it must be subject to some fundamental principles. The energy of an isolated system, that is one that exchanges neither matter nor energy with its surroundings, is always the same. It can pass from one form to another, but the total amount does not change.

Another fundamental principle teaches that natural systems tend toward disorder, i.e. it is easy to achieve disorder, while order requires work and time.

Transporting energy also costs energy. Extracting oil from wells like those in the Middle East costs 5% of the energy obtainable, and transporting it along ocean routes may cost another 1%. [6] Extracting coal can cost 20% of the energy contained. One problem experts have noted is that new deposits of non-renewable sources, such as oil or coal, require more energy to extract. So, over time, energy consumption to obtain non-renewable energy is likely to increase.

The lack of awareness of these basic principles leads some journalists and politicians to claim they can "disintegrate" waste in an incinerator, and economists to propose social models that imply infinite GDP growth. We build goods that consume huge amounts of energy, and this trend cannot be sustained for long. For example, to build a computer, it is necessary to spend an amount of energy equivalent to 250 kilos of oil. This means that a computer, before it is even used, will have consumed three times as much energy as it is likely to consume during its lifetime. Not recycling goods such as computers means throwing away the energy used to produce it, as well as wasting rare and precious molecules (called rare earths), and polluting the environment.

The current model of life in industrialized countries such as Italy is based on consumerism that offers, at least to a part of the population, a non-sustainable well-being, since it is based on the destruction of limited resources and on the exploitation of people. Probably there has never existed on Earth a species with such a high hunger for energy and, at the same time, with such an efficient capacity to accumulate wealth, far above the possibilities of consumption. We are currently witnessing the phenomenon whereby the richest classes can accumulate enormous resources, certainly not effective in improving the quality of life, as they are disproportionate. In 2017, the 8 richest people on the Planet possessed a wealth equal to that of half of the

World's poorest population. [980] Even in Italy, the three wealthiest people own more richness than one third of the population. The speed with which the gap and inequality are growing is alarming.
The current economic model is based on infinite growth and, therefore, has many negative implications also because it is impossible to have an infinite increase, for example of 2-3% of GDP per year, as many economists hope; this is because the Earth ecosystem is based on finite resources and, in many cases, easily measurable, so the very hope of obtaining infinite economic growth is lacking in credibility.

The primary energy source is that which is present in nature and, therefore, does not derive from the transformation of any other form of energy. Primary energy refers to the availability offered by primary energy sources such as the sun, wind, tides or oil. Primary energy is the energy content of the fuels used. More than 80% of primary energy comes from fossil sources, which are limited and non-renewable. In Italy the average annual *per capita* consumption of primary energy was (already more than 10 years ago) about 2.8 toe, 7.4 toe in the USA, and 9.4 toe in Canada; the World average is 1.8 toe *per capita* per year. [6]
Dividing the global supply of primary energy (about 11 Gtoe) by 7 billion people, we obtain the availability of energy that approximately one inhabitant of Western Europe consumed in the 1960s. It follows that, at least theoretically, the current supply of energy could allow all the inhabitants of the Planet a more than decent lifestyle. We know, however, that in the World some countries consume much greater quantities: a European, for example, can consume more than three times the available World average.
The primary energy source is not immediately available as it must be transformed before it can be used: oil is transformed into gasoline or solar energy is transformed into electricity by photovoltaic panels. When the transformation has taken place, we talk about secondary energy. Even biogas (methane produced by the fermentation of corn) must be cleaned of moisture, sulphur and then be enriched with methane, before being sent for combustion.
If the energy made available by the primary source, as well as being transformed, is transported to the user, we speak of final energy. The process of production and use, being not ideal, involves losses so that the energy made available to the system of interest is less than the final energy. This last energy is called useful energy and may be less than 10% of the energy produced. In order to obtain usable energy (e.g. electrical energy), a large part of the primary energy (even 2/3) can be lost, mainly in the form of heat.

On average, a European citizen consumes 50% of energy to keep the temperature of a house constant, and another third to get around by car. 65% of heat and 50% of electricity are produced with natural gas, while 97% of transport uses oil derivatives. Therefore, to reduce waste, it would take just a few simple interventions, such as those on the transport of goods and people.
A very famous formula is the one proposed by Einstein: $E = mc^2$. Mass (m) can be transformed into energy and, theoretically, the quantity obtainable is gigantic, because the constant "c" is equal to the speed of light (300,000 km per second). So, tiny masses would be enough to obtain enormous amounts of energy, if it would be simple to free them from atoms in a controlled way.
The nuclear fission of uranium (the atom is split into more than 100 lighter isotopes, some of which are radioactive such as caesium, iodine, and strontium), which takes place in nuclear power plants, releases enormous amounts of energy. The fission of 1 gram of uranium (^{235}U) can release the amount of energy that is obtained by burning 20 quintals of oil.
It would be good to keep in mind that even the use of nuclear energy produces climate-altering gases, contrary to what is often advertised: to build and dismantle power plants, to extract uranium (in Italy it should be imported), and to manage the waste that will still be deadly after thousands of years.

THE HIDDEN EXTERNALITIES OF GREEN CAPITALISM

An alternative to fossil fuels are energies that are classified as renewable, such as solar and wind power. These two forms of energy production are growing and certainly help the dialogue about planetary limits and the ecological conversion needed. The energy transition, along with the digital transition (internet, computers, phones, screens, batteries), has underestimated costs and impacts; some of them are summarized below:

- Renewable energies need fossil energies to be produced, so the former are closely dependent on the latter. The relationship between the energy invested and the energy obtained is a crucial aspect. Assessments of the fossil energy needed to obtain energy classified as "green" and therefore sustainable underestimate some costs such as those of extraction and processing of all the components needed for construction. Also underestimated are the energy costs of transporting, disposing of, and building all the technological and digital networking needed to operate, such as batteries and electronic components. Factories and digitization also have significant energy costs. It is a dark side of the energy transition, though it should all depend on these measures that a forward-thinking society should make as its first assessment.

- Technologies such as those for solar panels require the use of elements that are present in the Earth's crust at very low concentrations and chemically bound to substances from which it is expensive and polluting to separate them. They are called rare earths even if they are not necessarily unobtainable, the problem is that they are present in very low concentrations: less than 1% of the total weight of the rocks excavated. For example, almost 9 tons of rock must be extracted from the subsoil to obtain one kilogram of vanadium, 50 tons to obtain one kilogram of gallium, 140 tons to obtain one kilogram of platinum and two hundred tons to hope to purify one kilogram of lutetium. [1092] There are basically less than 40 major elements and compounds found underground that have special physical and chemical properties, as unique as magnetic and optical properties. For example, a cell phone needs at least 50 different minerals and metals to be built. [1100] In order to be purified, they must be separated chemically and physically from other substances that are more abundant and, in some cases, toxic to the environment (e.g. radioactive metals). Therefore, a lot of energy has to be spent to extract, separate, and process them in order to obtain the desired alloys. The energy costs and environmental impacts of these operations are considerable. Moreover, like all the resources of the Planet, these substances are not present in infinite quantities. Precisely because of these factors rare earths are extracted in small quantities (in some cases it is at most hundreds of tons, compared to millions of tons of copper or billions of tons of iron extracted each year in the Planet). Another consequence is that they are expensive (thousands of times more than iron).

- The production of renewable energies generates little-known environmental impacts that may obscure the sustainability of some choices. The extraction and processing of minerals is one of the most polluting industries in the World, second only to the recycling of used lead batteries. [1093] The problems generated by the entire life cycle of the extraction and processing of rare earths to support green technology are different.
The water footprint, i.e. the amount of water required for extraction and processing, can be enormous: even more than 300 cubic metres of water for every cubic metre of rock. The water used is returned contaminated with toxic substances and, in some cases, radioactive

waste is produced. The degradation of water (groundwater, rivers) can be considered irreversible; there is a remarkable damage to health that is not considered in sustainability assessments.
In order to carry out the extraction and purification of the desired chemicals, it is necessary to carry out chemical treatments and reactions using hazardous substances (e.g. sulphuric and hydrochloric acids) that are released into the environment. In addition, dangerous and toxic substances and by-products are produced, such as some radioactive compounds (e.g. radioactive thorium and uranium derived from underground mining and processing to purify rare earths). So the mines and places where these toxic wastes are managed will remain poisonous and potentially deadly for generations.
The extraction and processing of these compounds pollute water, air and soil. The resident population and workers are the most affected. [1092] The pollution generated by this industry is kept hidden or not publicized, for obvious reasons.

- The carbon footprint, i.e. the production of climate-altering gases (in terms of carbon dioxide equivalents) of the entire life cycle of products and the structures needed to operate them, is not adequately assessed. Blast furnaces are used to process the extracted rocks, which generate considerable emissions and produce very hazardous solid and liquid waste. Probably at least 7% of the energy consumed in the World is used to extract rare earths. [1103]
The need for fossil energy could increase considerably in the future for several reasons: the mines from which rare earths are most easily extracted are exhausted and the demand for minerals is constantly growing (3% - 5% each year).
We should think about mining activities, that is, places where substances are extracted from the ground, which use enormous amounts of energy, dangerous substances (e.g. acids) and water, and which generate a huge amount of waste and poisonous substances. It is probably impossible to think of sustainable mining, and it is even more impossible if we do not neglect the health and social security of the workers, of the resident population and of the future generations.
The new model proposed by green capitalism, based on digitization, on the production of electricity from sources classified as renewable, on the encouragement of private electric transport, tends to move pollution away from the eyes of the upper classes and towards the poorest areas of the Planet. This distancing from reality facilitates the illusion of having improved the situation. New impacts are generated and added to existing ones, which are shifted to the places where rare earths are mined, processed, and the technologies necessary for the planned transition are produced.

- One promise let down is the ability to recycle these components that cost money and cause environmental degradation. Unfortunately, the level of reuse, recovery and recycling of solar panels, batteries and electronic components is very low. Moreover, often the insane rules of the market push even faster towards self-destruction: the planned obsolescence of mobile phones, household appliances, cars, etc., is the only way to achieve the goal.
Regarding e-waste, the greatest producers are China, USA, and India, producing 38% of the global total, but Europe has the highest production *per capita*. An estimated 53.6 million tons of e-waste was produced in 2019, registering a 21% increase compared to 2018: only 17.4% was recycled. [1095] Northern Europeans produced the highest amount of e-waste in 2019, around 22.4 kg *per capita* (only a small fraction is rare earths, but these are indispensable crumbs as they are irreplaceable).
Less than one-sixth of electronic waste is properly recycled, resulting in huge waste: items containing precious metals such as iron, copper and gold end up in landfills; 300 tons of gold, equivalent to 11% of the World's production of this metal, ended up in the trash. [1096]

Millions of tons of hazardous materials, including lead, mercury, cadmium, and chromium, are dumped in landfills. If these materials had been recycled, they would have generated billions in wealth. Our computers and mobile phones probably contain a quantity of rare metals that would ensure self-sufficiency for many years if they could be reused. Recycling costs a lot of energy, requires expensive and polluting processing and from the business point of view is not interesting. It is much cheaper to extract new metals. Moreover, recycling is not always technically possible, as it involves separating some metals from complex alloys. It is like hoping to recycle some ingredients used in food production: theoretically it is possible to imagine chemical extraction processes but they are realistically unsustainable as they cost too much and pollute a lot. For this reason, the recycling rate of most rare metals is less than 10% or 3%. [1097] However, even if the recycling would work, at current rates of growth in consumption would not be enough: despite the current rate of recycling of lead in North America and Europe is almost 99%, the extraction activity is increasing. [1098]
Waste management generates environmental problems and illicit trade exacerbates them. A principle in favour of environmental responsibility, espoused by most countries in the World, prescribes that waste must be disposed of in the place (State) where it was produced (this is the theory). The management of technological waste is also unsustainable. The energy transition requires technologies that need the extraction of rare earths from the subsoil that are destined to return to the soil. Within a few years of their lives, computers, phones, and other products with electronic components that originated in one hole are destined to end up in another. During this move from a mine to a landfill - which may take a few years (planned obsolescence) - pollution is generated, limited resources are consumed, sites are established that will remain poisonous for a long time, and a lot of people are exploited and made ill. Pollution and exploitation of workers are relocated, so they become temporarily less noticeable, for example in big urban centres.

▪ The extraction from the underground of some chemical compounds necessary for the transition to renewable energies implies social impacts that are considered unacceptable in a civilized society. Workers in the mines, often located in countries where social guarantees are almost absent, are underpaid and exploited (e.g. cobalt extracted in the Democratic Republic of Congo; tantalum in Mozambique and Rwanda; gadolinium and niobium in Brazil; gallium, indium, germanium, and antimony in China; platinum, iridium, ruthenium, and rhodium in South Africa; chromium in Kazakhstan). [1092] Damage to the health of workers and the resident population is not considered in estimates of the sustainability of these processes.

▪ Like many other sectors, the renewable energy sector is also at risk of speculation and excessive incentives. Electricity produced through solar panels or wind turbines can be paid at a price much higher than the market price, distorting and obscuring the real costs. Unsustainable choices may affect several aspects such as the installation of photovoltaic panels on the ground. Land take increases impacts on the environment and negative externalities such as reduced food availability, increased agricultural land prices, reduced biodiversity and fertility.

▪ The distribution of the places on the Planet where some of these substances necessary for the construction of batteries and solar panels are extracted suggests geopolitical problems. Some substances are extracted from very few places so there is a sort of oligopoly or even monopoly. This is very worrying because it is another limitation. To give an example, China is probably the World's leading producer of 28 rare earths (in over ten thousand mines). [1092, 1099] China has produced the most powerful supercomputers in the World, has become the main producer of photovoltaic panels, makes the greatest investments in wind power, produces most of the batteries needed for electric vehicles and is promoting over 100 sustainable city projects. [1101, 1102] So it is leading the race towards the green transition but at the same time it is also the greatest producer of pollutants in the Planet, followed by the USA.

Actually, rare earths are not concentrated in the most active mining countries. Probably this delocalization towards poorer countries has been intentional in order to move the most polluting and most dangerous works for human health out of sight; an example is the French mining activity that in the 80’s purified at least 50% of some of the rare earths of the World to be then almost dismantled and delocalized in Asia: in any case, very dangerous wastes remain in France, such as radioactive ones. [1092] Moreover, relocating in countries where the guarantees on social and environmental safety are lower than those that a civil and far-sighted society could tolerate, means for entrepreneurs to be able to obtain the same things with much lower costs. History repeats itself also in the mining sector: rich countries transfer the most polluting and most dangerous activities to poor countries that were previously less polluted.

Every three years the European Commission reviews the list of critical raw materials for the European Union (EU). Economic importance and supply risk are the two main parameters used to determine criticality for the EU. Economic importance analyses in detail the allocation of raw materials to end uses based on industrial applications. Supply risk considers different aspects such as the EU's dependence on imports and trade restrictions in third countries. [1099] The list compiled by the EU Commission in 2020 contains 30 raw materials, 14 of which were present in the 2011 report. Some of these materials are the following: antimony, cobalt, indium, phosphorus, gallium, germanium, lithium, magnesium, niobium, tantalum, tungsten, and vanadium. The supply of many critical raw materials is highly concentrated. For example, China supplies 98% of rare earths to the EU, Turkey supplies 98% of borate to the EU, and South Africa meets 71% of platinum needs. [1099] For most metals, the EU is between 75% and 100% dependent on imports. So it is completely dependent on other countries, a very precarious and frightening condition, especially in times of crisis. The countries from which it imports may set limits to exports and may decide prices as they have a monopoly, in fact there are hundreds of restrictions on exports recorded in the World, i.e. prohibitions, taxation, maximum quotas to some countries instead of others. [1100] On the other hand, it is understandable that poor and emerging countries exercise their sovereignty over the resources they extract and that they do not want to sell them off (hoping that only a few entrepreneurs will not take advantage of them). Many countries refuse to manage their resources transparently. As a result, the rare earths’ market could be much more unstable, unpredictable, and ultimately more in crisis than one might expect.

Another food for thought is the need for many rare minerals to build armaments (planes, missiles, ships) and related equipment such as radars, sonars, night vision equipment, lasers, drones, robots, satellites, and communication systems. The industry for war needs great amounts of rare and valuable resources; this sector sees the United States in the lead.

Finally, attention is drawn to the fact that some estimates report that one third of the rare earth trade is handled by the black market. [1092]

Adding to this dependence is another problem: the forecasts for the increase in consumption are astonishing. To support the green energy transition and the digital energy transition, the forecasts for 2050, published in 2020, are a challenge to planetary limits: for electric vehicle batteries and energy storage, the European Union would need 60 times more lithium and 15 times more cobalt than the current supply of its entire economy. Demand for

rare earths used in permanent magnets, e.g. for electric vehicles, digital technologies or wind turbines, could increase tenfold by 2050.
Globally, the World Bank predicts that demand for metals and minerals will increase rapidly. The most significant example is represented by the electric accumulators, for which the demand for relevant metals such as aluminium, cobalt, iron, lead, lithium, manganese, and nickel would increase by more than 10 times by 2050 (in a scenario of temperature increase of 2°C). It is likely that, globally, at least one new mine per year will need to be opened to support the projected growth in rare earth consumption in the coming years. The environmental impacts, planetary limits and costs generated by these consumption-increase targets will surely stop this race much earlier than expected. It is incredible that pollution, resource depletion, and other limits are not covered in these official reports from accredited agencies. The myth of infinite growth becomes a curse for the health and well-being of all, especially of the future generations. There is a way out: reduce the use of resources and apply only processes that may ensure complete recovery and recycling; a utopia? Even if the resources are used in circular processes, the process is not sustainable at all, but it is more durable. It should be remembered that in Europe the recycling rate of rare and precious resources such as niobium, indium, lithium, tantalum, gallium, phosphorus (and others) is zero, and for some others such as germanium and vanadium it is less than 2%. [1099] The prospect of digging several new mines in Europe (and in the World) in order to hope to become self-sufficient and secure unrealistic growth rates is a potentially lethal and self-destructive display of omnipotence, supported by environmental illiteracy. Habituation to fairy tales and legends will encourage the waste of precious public resources and time.
Maybe the construction of new mines in the industrialized countries in Europe will contribute to reduce the ignorance about the impacts generated by this industry and to apply measures of protection for the workers and the environment superior to those adopted in the poor countries. Living close to mines, or worse, having to work in them, will certainly contribute to activate consciences and to overcome indifference. Perhaps living near sites that generate many environmental and health problems will help to accept some limits to unbridled consumerism.

The condition of conflict of interest (and selfishness) together with the lack of knowledge about the different aspects that should be evaluated in order to make objective comparisons and sustainable choices does not allow rational and conscious decisions to be made. Before investing in transitions that are considered more ecological, we should have more certainty. The lack of transparency of the whole chain that should support energy conversion fosters the illusion that it is democratic and universal, the ultimate solution to many problems. Real costs and negative externalities should be assessed more carefully to avoid market artifices that shift the disadvantages onto someone else, invisibly to the majority of potential beneficiaries. The production of solar panels, batteries, digitalization and green technologies (internet, computers, telephones, electric cars and bikes) implies impacts and costs that are underestimated or deliberately kept secret.

Green capitalism promises us a better World with energy-saving technologies and lower environmental impacts (decarbonisation). However, green capitalism espouses the insane theory of infinite growth; therefore the extraction of rare earths at the current rate of increase of renewable energy, digitalization, and technologies considered sustainable (e.g. electric cars) should double at least every fifteen years. To hope to maintain the rate of growth of the energy, technology, and digital transition in less than 30 years we probably need to extract more substances from underground than we have taken in our entire history. Reality is likely to disillusion this dream. As much as a quarter of the weight of an electric car could be made of batteries, and the amount of energy needed to build an electric car is far greater than that needed to build a traditional one (by as much as 4 times). [1092] Batteries are made up at least of lithium, nickel, cobalt, aluminium, manganese, copper, steel, graphite, and plastic. We are adding to a type of scarcity (of oil) that of valuable and scarce natural resources (rare earths). The energy balance of the entire life cycle of electric cars, when revealed, is very likely to be bad news: the benefits in terms of energy savings and climate-altering gas emissions are insignificant or even non-existent. [1092] Moreover, we must not forget that currently in countries like Italy, China, USA, India most of the electric energy is produced by fossil fuels: methane, coal, and oil derivatives. So even the electric car burns fossil fuels in large quantities. The lack of transparency on information that should be the starting point for any project and investment leads to the suspicion that too many people want us to believe that things will get better, when it is much more likely that they will get worse. Even the production of everything needed for digital technology such as telephones, computers, and internet consume great amounts of energy. The production of computers and mobile phones alone absorbs at least a fifth of the rare earths extracted annually. The shipment of a *gmail*, before reaching the recipient, may pass at the speed of light from the United States (where it is processed and stored by special computer centres that require a lot of energy). It travels thousands of kilometres and all this has costs both in terms of structures and networks and energy: an *email* with an attachment requires the energy needed to keep a light bulb on for an hour. [1094] Across the Planet, more than 10 billion *emails* are sent every hour, requiring an enormous amount of energy: more than 50 GW/hour, the energy that can be generated by dozens of coal-fired power plants; a transit and data processing centre for operation and cooling can require the electricity needed to meet the needs of more than 25,000 inhabitants. The centres for data processing are called *data centres* (in Milan there are at least 18 of them, about fifty throughout Italy). Probably all the data centres in the World to operate require at least 1% of all the energy consumed in the Planet: between 2010 and 2018 the energy consumed by data centres has increased by at least 6%. [1104, 1107] Electronic equipment, in the World, consumes at least 5% of the energy. [1105] A data centre can absorb 50 Mega Watts, the amount needed to run 140,000 televisions. A data centre operating at 4 megawatts can occupy an area of 5,000 square metres and consume 8 tons of oil per day (the equivalent of about 57 barrels of 140 kg each every day; 30% of the energy may be needed for cooling). [1110] In the coming years, this consumption is planned to grow significantly: by 2030, the electricity use in data centres is likely to increase tenfold, reaching 8% of the global demand. [1106, 1108, 1109] It is possible that by 2025, globally, the number of internet users will have increased by 1.1 billion, the energy consumption resulting from the use of network services will be multiplied by 2.9, electricity consumption by 2.7, and greenhouse gas emissions by 3.1. [1111] These figures are underestimates because they do not include many other energy costs such as those generated by the construction of structures, equipment, and their disposal (think of the cables that cross the Planet). Therefore, even the digital transition requires significant investments and the idea of dematerialization of information and communication is a great deception. We must reflect on the following question: is a connected and digital World preferable to a clean one? We can no longer ignore or continue to be deluded by the prospect of infinite growth or by digital technologies presented as sustainable.

In conclusion, green capitalism, with the energy and digital transition it supports, exacerbates the human pressure on ecosystems by adding other problems, such as those generated by the extraction and processing of rare earths, and by the increased demand for electricity consumption that is not as sustainable or even renewable as people want to believe. We need to doubt and question the validity and sustainability of the energy and digital transition, because there are many warning signs. We need more transparency and information.

CLIMATE CHANGE

AIR POLLUTION AND ROAD TRANSPORT

The failure of our society to prevent pollution such as atmospheric pollution highlights the failure of the system of rules and controls that, in countries like Italy, is managed by hundreds of public and private bodies. The environmental crisis manifests itself in the waste of important resources for infrastructures such as roads and highways; while state budgets in the environmental and cultural fields are reduced. [875]

Air pollution is the cause of enormous health costs: the air is increasingly polluted and people are dying because of it. [736] In Italy deaths due to air pollution are about 1,500 per million inhabitants, while they are 1,100 per million inhabitants in Germany, 800 in France and 600 in Spain. [196] Among the main causes are road traffic, heating, industry, and agriculture. Many studies have now confirmed that there is a positive relationship between total concentrations and frequency of effects: there is no threshold value, below which there are no negative effects.

Dust emitted into the atmosphere from combustion is harmful to health and the health risk depends on its concentration, size, and chemical nature, as well as other factors such as exposure time and individual susceptibility. Smaller particles pose a greater danger to human health, as they can penetrate deep into the respiratory system. Dust causes coughing, shortness of breath, asthma, irritation of the airways and, in the long term, promotes heart attacks, strokes, and cancers of the respiratory system. Epidemiological studies have shown a correlation between dust concentrations and the occurrence of chronic respiratory diseases such as asthma, bronchitis, and emphysema. Particulate matter contains highly toxic substances such as polycyclic aromatic hydrocarbons.

Fine dust generated by road transport has long been regarded as one of the main causes of cancer in urban areas. [572, 881] The dust emitted by diesel engines is classified as carcinogenic. Diesel combustion produces hundreds of different substances including acetaldehyde, formaldehyde, acetone, alkanes, dioxins, polycyclic aromatic hydrocarbons, sulphur, nitrogen (e.g. ammonium), zinc, cadmium, lead, and iron. [878] Some of the most dangerous components present in the fine dust generated by diesel engines are polycyclic aromatic hydrocarbons (in the urban context, up to 40% of diesel fuel is used for heating). A chronic, lifelong (70 years) exposure to diesel engine particulate matter increases the probability of recording 300 cases of cancer, per million people exposed, for every additional microgram in a cubic metre. [883] According to this study, in order to reduce the risks, theoretically, it would be necessary to live at least 500 m from highly trafficked roads, so that the dilution effect reduces the concentration to less dangerous levels.

Emissions from diesel engines cause irritation to the eyes and mucous membranes, coughing, headaches, dizziness, disturbance of the sense of smell, asthma, allergies, cardiovascular diseases and are certainly mutagenic and genotoxic; they therefore promote the development of tumours such as lung cancer. [878] Urban living is associated with twice the frequency of death from lung cancer for the same exposure to tobacco smoke.

In general, dust in the atmosphere shortens the life expectancy of a European citizen, on average, by eight months; but for Italians it is estimated to be nine months, and for those who live in the Po Valley: two years less life expectancy. [876]

The International Agency for Research on Cancer has listed some polycyclic aromatic hydrocarbons (PAHs with 4-6 rings) as possible and probable human carcinogens. Most PAHs are capable of causing cancer in experimental animals (e.g. by inhalation). Each 1 ng/m^3

increase in benzo(a)pyrene (a carcinogenic PAH) in the atmosphere may result in a risk of nine new cancer cases per 100,000 people. About 4% of occupational cancers in Italy are attributed to PAHs: the first occupational cancer described is epithelioma of the scrotum in English chimney sweeps, due to contact with soot and its PAH content. In addition to inhalation, an important route of exposure for humans is ingestion of agricultural and livestock products that have bioconcentrated molecules. [886] In animal studies, a number of toxic effects other than cancer have also been highlighted, affecting the skin, respiratory system, immune system, and reproductive system.

A study assessed the impact of air pollution on the health of some 39 million people living in 25 major European cities. [1258] An important result is that the decrease, up to 10 $\mu g/m^3$ of the concentration of $PM_{2,5}$ (particulate matter; annual level established by the air quality guidelines of the WHO, World Health Organization), may lead to an increase of up to 22 months of life expectancy, in people over 35 years of age, and residents in the 25 cities studied. If in Rome the annual average concentration of $PM_{2,5}$ dropped to 10 $\mu g/m^3$, it would be possible to avoid 1,278 deaths (997 from cardiovascular causes), and the population of 30 years of age would gain one year of life.

In conclusion, deciding what level of health or environmental risk to accept is more a political choice than a scientific one. It is not possible to set a concentration threshold below which we can be sure of no adverse effects on human health or on the environment. To date, we have not even been able to comply with the concentrations for safeguarding air quality which, among other things, we have self-regulated (for example, the concentrations not to be exceeded for fine dust). It has therefore been impossible to comply even with the arbitrary thresholds set by politicians.

Indirectly encouraging or promoting (e.g. by restricting circulation only to cars of lower Euro classes, or by introducing different tolls, depending on the emissions produced by combustion, per km) the scrapping and replacement of old cars, before they become irreversibly unusable due to old age, is very unlikely to produce a decrease in emissions and other polluting factors:

- Cars are replaced when they could still run many miles before they get unusable.
- Scrapping a used car and building a new car together may produce more emissions than will likely be gained from a better technology applied to the new car.
- Older cars are often replaced with bigger, heavier cars, so the reduction in emissions is cancelled out by the increase in power, greater use of materials, and increased performance.

By scrapping, emissions increase instead of decreasing. Emissions may be shifted from urban centres to construction and demolition sites. New cars have more and more technology and therefore new materials, which become rare and valuable, so it also negatively affects the depletion of limited resources. It would be much smarter to promote the use of other modes of transport, such as trains, in place of four-wheelers. The reduction of private transport in ever larger areas is necessary. No technological improvement will be sufficient to compensate for the uncontrolled increase in traffic volume. Promoting the scrapping of old cars with public funds, hoping to reduce emissions, is madness. It is surely more advantageous to use public resources to improve the accessibility to alternative, less polluting means of transport.

In Europe, food products, considering only the delivery phase from supplier to point of sale, travel on average 1,640 km. But if we consider the entire process, from production to distribution, it is more than 6,000 km. [27] The environmental costs, the so-called "externalities" associated with air pollution, are usually not considered, although they are very important, and are:

- water pollution;
- soil pollution;
- food pollution;

- climate change;
- ocean acidification;
- reduction in biodiversity;
- reduction in the availability of natural resources.

Air pollution generates an increase in temperature that will also affect productivity and costs in livestock farming: the availability of feed will decrease, problems caused by parasites (e.g. ticks and mites) will increase. In India, the increase in temperatures generates a decrease in milk production of at least 2% per year (equal to about 1.8 million tons). [879] Climate change, due to increased air pollution, will force farmers in many areas of the Planet to choose smaller species that are more resistant to heat and disease and require less care.

CLIMATE CHANGE: TOO LITTLE, TOO LATE

We can call fossil fuels "*buried sun*" as they are derived from solar energy received over millions of years. For example, coal comes from fossilized remains of plants that lived in swamps probably millions of years ago. [642] Most of the anthracite could come from plants that were alive between 360 and 290 million years ago, the period called Carboniferous. There were no mammals or birds, but metre-long spiders and other animals whose fossil remains amaze us today. During the Carboniferous period, it would have been possible to find two-metre-long millipedes and dragonflies with a wingspan of 70 centimetres. [14]

Oil could be derived from life forms that populated the oceans similar to phytoplankton. In order to be able to generate oil, a long and difficult process is necessary, which requires several steps such as the cooking of organic material for millions of years (waxes and fats from which it derives must be cooked between 100°C and 135°C for millions of years, before being transformed into oil). [642] Probably the transformation of more than one hundred thousand tons of organic material, after millions of years, can originate 4 kilos of oil. So we can say that every year we burn the amount of "*buried sun*" that has been accumulated by plants in hundreds of years and transformed in millions of years. From the combustion of a kilo of methane it is possible to obtain 14,000 kcal, from a kilo of diesel 11,000 kcal, from a kilo of coal 8,000 kcal and from a kilo of firewood less than 4,000 kcal. [36] Fossil energies, therefore, constitute a concentrate which, if it is easily extractable, provides a good prospect of gain. However, these different energy sources are not entirely interchangeable. Oil is today the most important source for its multiple uses, both in the energy field and for the production of manufactured goods and synthetic substances. For example, the chemical industry relies substantially on oil (e.g. plastics) and the transport sector relies almost entirely on it: 97% of transport depends on oil products. [15] Natural gas is mainly used in the energy sector (both thermal and electric), coal is very important in the electric generation sector and in metallurgical production (coal needs oil to be transported).

In the history of our Planet, geologists have difficulty in finding a period in the past when the characteristics of the atmosphere have changed as rapidly as they have today. Probably to find an atmosphere with a composition similar to the present one we must go back at least 55 million years: our species has existed for less than 2 million years but we conquered the Planet starting from 12000 years ago. [774] In 12000 years (this is the Holocene, the cradle era for humanity) the climate has been benevolent and predictable and has allowed us to invent writing, agriculture and breeding. The average temperature of the Planet has fluctuated at most ±2°C, so the climate has been constant, predictable and benevolent to mankind.

We are turning the climate clock back millions of years, but under conditions that probably never occurred. The Holocene was our paradise: the human species went from a few million to a few billion because our home was perfect. To reverse the dangerous experiment underway we

must act now, re-naturalizing at least 50% of the earth's surface and most of the oceans. We entered the Holocene probably in less than 5 million but we are about to leave it in almost 10 billion: we have entered the Anthropocene, the era dominated and poisoned by man. The human species will remain mainly accompanied by the few living beings domesticated and artificially selected in the laboratory, i.e. completely dependent on a fragile and unsustainable system: we can define this era as Eremocene; we will remain alone, therefore it will be a short era; no species can survive long without the others.
We can say that we are living in new conditions, because we have never breathed such an atmosphere: we are the guinea pig of an experiment run by ourselves. In fact, there is no longer any doubt that we are the cause of this sudden change.
Probably few other times in the history of science has there been such a widespread consensus as on the causes of climate change. [456, 458] Yet it is very easy to find information or listen to interviews of (influential) politicians who deny the evidence and the scientific consensus. [457] The universe of pseudoscience and the industry of doubt are very active in enlisting mercenaries and mass media. Skepticism is fueled by considerable investment and diabolical strategies funded by big businesses. [260] Here the attack on the credibility of science is systematic and inelegant. The short-term economic interests of a few large corporations come before everything else: collective health and safety.
An interesting aspect of the science that studies climate change is that it continuously and rapidly updates and modifies its predictions. The scientific literature is considerable and it is therefore difficult to keep up to date. However, it can also be said that in the last 20 years forecasts have almost always been updated by worse ones: for example, on the predictions of melting Arctic glaciers or the release of methane from melting permafrost in Siberia. In fact, in the course of a very short time, the speed of negative changes has increased and the risk one runs is that of being too optimistic. Being optimistic is an advantage: it increases longevity and reduces the risk of heart attacks, but we should not risk espousing optimism simply to support the denial of the evidence. [975] Conversely, climate change is too often perceived as abstract, distant, invisible, and doubtful.

Global carbon dioxide emissions from fossil fuels, in the decade 2008-2017, increased by an average of 1.5% per year and overall carbon dioxide emissions increased by around 3%: 40% from coal, 35% from oil, and 20% from methane (China +3% per year; India +5.2% per year). [680, 776] In 2018, the annual energy-derived emissions reached an all-time high of 33.1 Gt, more than a third of which was from coal: the energy demand is increasing.
In Europe, mobility in urban areas contributes to 40% of total carbon dioxide (CO_2) emissions, and 70% of other pollutants. [877] The OECD (Organisation for Economic Co-operation and Development) has estimated that in Italy transport is responsible for 31% of all carbon dioxide emissions, with road transport contributing the most (82%). Many of the problems arising from private mobility, such as pollution, accidents, noise, and reduced quality of life, are attributable to the fact that energy is very cheap. Although fossil fuel prices tend to increase, the organisation of a new society based on energy saving and collective mobility is not taking off. As a result, oil consumption in Italy is high: between 600 and 700 kilos with peaks, in some areas, of over 1,000 kilos of oil equivalent *per capita* per year.

Some emerging countries are reaching the consumption levels of Europeans. To give an example, China in thirty years has experienced the growth that in Europe was recorded in two centuries, with a population at least three times higher (between 2011 and 2013 China produced more cement than the United States of America in the entire twentieth century). [977] Three-quarters of the increase in planetary climate-altering gas emissions over the 2010-2020 decade was generated in China. The extraction and production of raw materials such as cement, steel, and aluminium are responsible for the emission of at least 20% of the Planet's climate-altering gases. [980] Our model of development is not sustainable and it is unthinkable to hope to perpetuate it indefinitely and for all the inhabitants of the Planet.

There is no longer any doubt: human activities are the major cause of climate change. We have managed to increase the average temperature of the Planet by at least 1°C compared to the pre-industrial era (1850). Cumulative carbon dioxide emissions, between 1870 and 2016, amounted to about 2,080 Gt (giga tons or billion tons); in 1870, the hydroelectric power in the United States of America provided three quarters of the industrial energy used. [977] According to some authoritative research centres (IPPC), if we want to have any chance of not generating undesirable and irreversible climate change, we should not exceed the emission of 2,350 billion tons of carbon dioxide. [762] The result is that we have at our disposal about 300 Gt of emissions, equal to those we emit into the atmosphere in less than 7-8 years. Underground fossil energy reserves constitute a carbon dioxide potential estimated between 31,300 Gt and 50,000 Gt, i.e. an amount 15 times or 25 times higher than the cumulative emissions between 1870 and 2016, which amount to about 2,080 Gt. From this point of view, we can say that underground we have a fossil arsenal capable of destroying several times the climate suitable for our survival. Fossil fuels will have to stay in the ground to contain climate change and the resulting disasters. *Per capita* emissions will have to be reduced to less than 2 tons of carbon dioxide per year, instead of over 7 tons *per capita* in Italy or over 20 tons *per capita* in the USA. If an attempt is made to burn all available fossil fuels, the Planet will surely become inhospitable and unlivable before they are exhausted.

It is possible to state that the emission of less than 600 Gt in carbon dioxide-equivalent greenhouse gases, in the next few years, will be sufficient to reach an energy level of the Earth's climate system that is very dangerous for the survival of the biosphere and, therefore, of humanity. [775] Considering that current emissions amount to about 50 Gt (53.5 Gt recorded in 2017) annually, it means that concentrations capable of causing an irreversible and devastating increase in temperature will be reached in a few years.

The World's nearly 4 billion hectares of forests, which cover about 30% of the Earth's surface, hold about 50% of the carbon (283 Gt) contained in the atmosphere. Between 1990 and 2005, as a result of forest destruction, the amount of gas trapped in vegetation decreased by about 1.1 Gt per year. [703] Instead of reforesting the Planet, which would also produce many other benefits essential to our survival, we are moving precipitously in the opposite direction.

At present it seems impossible to achieve the proposed objectives for the reduction of climate change because it would mean having to go from about 50 Gt of carbon dioxide emitted each year to almost zero, before 2050. Today, each inhabitant of the Planet on average produces about 4 tons per year of carbon dioxide, but industrialized countries produce much more: more than 20 t/*per capita/*year in the USA (Italy imports virtually all the fossil fuels it uses, such as methane from Russia and Libya). It is necessary to drastically reduce these emissions in less than 30 years: will it be possible?

Probably in order not to exceed a dangerous increase in temperature (+2°C compared to the pre-industrial era, remembering that 1°C, since 1850, has already been attained) it would be necessary to leave most of the known fossil fuels underground. [761, 774, 844] At present, even small reductions of the current emission levels, of 10% or 20% by 2050, are a mission impossible, a utopia. Yet such small reductions would not substantially delay the announced disasters.

It is unrealistic to think that the deadlines set by the (albeit voluntary) climate agreements can be met. The phenomenon is hitting us much faster than is commonly known, even if when you listen to those who deal with climate issues (experts) you breathe, in many conversations, an atmosphere of suppressed panic. Unfortunately the most realistic scenario is that average temperatures will rise due to increased emissions.

Due to climate-altering gas emissions, the temperature is currently set to rise by at least 0.2°C every ten years. [775] The promises made at important conferences, such as the Paris Agreement,

which are entirely voluntary, are unrealistic chimeras, declarations without the means to make them a reality. [776]
The global climate and environmental summits underline the subservience of politics to markets and finance. In the alarming context where environmental collapse is at the doorstep, it is increasingly evident that neither politicians nor businessmen can be considered as allies to counter catastrophic changes such as climate change. It is about protecting non-negotiable values, indispensable for humanity and non-humans, whose dependence and relationship we underestimate.
In just a few years, concentrations of some greenhouse gases such as carbon dioxide, methane, and nitrous oxide have become the highest in 800,000 years and we are ignoring the catastrophic predictions. The concentration of carbon dioxide in the atmosphere has increased, from 277 parts per million in 1750, to over 417.6 in March 2021: a 37% increase. [777] Most of these emissions came from fossil fuels and land use change: deforestation, agriculture, animal husbandry, urbanization, fires, desertification, drought, erosion, salinization, etc. The total emissions released into the atmosphere have been redistributed through natural cycles among the different areas of the biosphere: in the atmosphere about 44%, in the soil about 29% and in the oceans for 22% (decade 2008-2017). [776]

GAMBLING WITH THE FUTURE

At this point it is important to reflect on this important aspect: who should determine the acceptable amount of climate-altering gas emissions that we can afford to produce in the next few years? The scientists, the oil companies, the politicians, the environmental associations, the military disaster specialists, us? Behind this question a great weakness of the whole system is hidden, a great unknown: are we sure we know the limits of our capacity for scientific prediction? These are enormous and thorny problems that have margins of uncertainty and unpredictability that cannot be estimated in the long term. The reductionist approach, i.e. with compartments designed to solve single, partial and circumscribed forecasts, is not reliable in the short term. The real system is complex and much more vulnerable than we can probably foresee. Uncertainty, unpredictability, complexity together with fragility and lack of knowledge are elements that will not allow accurate forecasts. We therefore run the obvious and predictable risk of inaction, waiting for certainties that will not come: we will let the worst face of the Anthropocene inexorably manifest itself. Given what is at stake, it would be worthwhile to act before understanding and knowing. It is no longer a matter of waiting for the results of a scientific exercise that has yet to be elaborated, but of making necessary choices for the well-being of all. We are in danger of being presumptuous and at the same time risking too much if we continue to hope to understand environmental problems before they cause upheaval. Waiting for scientific certainty is not possible, changes are very rapid and irreversible. It is necessary to organize resilience, to build another form of society attentive to ecological balances.

We live in a finite system where all resources are limited such as land and non-renewable energies. Even renewable energies such as biomass (e.g. firewood) or solar energy have to be adapted to a system that has huge limits. Biomass takes land and resources away from food production, and solar panels require materials that are available in finite quantities and occupy agricultural land too. Therefore, important choices are on the horizon, which can only be made reasonably if aspects that are currently underestimated, such as ethical and environmental ones, are also considered. The climate change of the industrial era tells the story of a failure, of postponing important choices. The truth has been concealed by cynical and lying operations. We can say that hundreds of treaties and international agreements, which governments have made to protect the environment, have been a failure. [680] The changes needed to reduce this apocalyptic climate transformation are unpopular because they are frightening. The wealthiest nations should start by reducing emissions by at least 15% each year. It means not using cars, not travelling by plane, overturning agricultural production which today consumes at least 10 times more energy (in fossil fuels) than it produces (in terms of calories on the plate), not producing plastic, consuming locally produced food, reducing the consumption of animal by-products, not wasting land to produce agro-fuels and agro-plastics, fighting planned obsolescence. [741] We have to accept frightening limits, especially for the wealthy fraction of the Planet. Perhaps if the majority of people knew what is known about the changes taking place, we would be able to organize a more sustainable and less energy-intensive society, without falling into a very dangerous social and economic collapse. To achieve these changes, we would have to leave fossil fuels in the ground, but concrete policies in this direction are not evident: even if at this moment our house is in danger, it is on fire, and we remain indifferent. Some people believe that we should leave it to the administrators but the political will, in this field, is apparently absent. If we continue to ignore this problem and use fossil fuels, before the end of the century, the average temperature of the Planet could increase by more than 4°C, thus far above the threshold that we have arbitrarily established as a safety margin, gambling with the future. [774]

THE CARBON CYCLE

The greatest carbon reserves are found in fossil sediments, which contain about 40,000 Gt (1 Gt = 10^9 t) of carbon, of which at least 4,000 can be used as fossil fuels. The oceans contain about 38,000 Gt, which is at least 51 times the content of the atmosphere. On dry land the biggest carbon reservoir is the soil, while in the atmosphere there is the smallest carbon reservoir, about 750 Gt. [624]

The most interesting aspect of the carbon cycle are the fluxes between the various compartments. Between land and oceans there is transport of dissolved organic carbon in river waters. An equivalent flux occurs in terms of inorganic carbon. However, the most important carbon fluxes are those that occur between oceans and the atmosphere, and between landmasses and the atmosphere. The exchange of carbon between oceans and the atmosphere is about 92 Gt/year in the atmosphere to ocean direction, and about 90 Gt/year in the opposite direction. The oceans act, therefore, as an element capable of absorbing atmospheric carbon, thanks above all to the ability of carbon dioxide to diffuse in surface waters and to deposit itself in the form of carbonates in the ocean depths. [624] The oceans absorb about 30% of the carbon dioxide generated by man and more than 90% of the heat. [977]

The planetary carbon budget has other factors that depend largely on human activities: the use of fossil fuels and deforestation. The use of fossil fuels and deforestation introduce billions of tons of carbon into the atmosphere every year, with a progressively increasing trend. Reduced soil fertility, thawing of frozen soils (permafrost), melting of glaciers, and alteration of the oceans (acidification, temperature rise, pollution, and collapse of food chains) are also increasing net carbon fluxes to the atmosphere. Overall, due to human activities, there is a net emission of carbon to the atmosphere. As it is well known, in the face of these factors unknown to our atmosphere prior to the industrial revolution, the concentration of carbon dioxide in the atmosphere has increased from 280 ppm in the pre-industrial era (this concentration had little fluctuation in the 600,000 years prior to 1850) to 417 ppm today and continues to increase. In the 12,000 years prior to the industrial era, the temperature fluctuated by one degree. The safety threshold that we have arbitrarily established according to anthropocentric criteria is 350 ppm, while the point of no return is around 450 ppm but today, we have reached over 417 ppm. We have brought carbon dioxide to a concentration 20% higher than the safety threshold and 7% lower than the maximum level that we have established as a safeguard objective: it is very likely that by exceeding the concentration of 450 ppm, the average temperature of the Planet will increase of more than +2°C. Less than 6-7°C of average temperature makes the difference between an ice-free Planet and an ice-ball Planet. The difference between an inhospitable Planet and one that allows agriculture and the development of human society is a matter of a few degrees, probably even less than 5°C. We are turning the climate clock back of more than 5 million years, according to many researchers even 10 million years. We are breathing air with a composition we have never recorded in the 2 million years of evolution that separate us from our ancestors who lived on African trees.

THE AWAKENING OF THE GIANTS

Predictions have many weaknesses in the sense that some changes may occur much more quickly and earlier than we believe. In fact, some changes follow relations between cause and effect that are not linear but in steps, making sudden jumps. This is what could happen due to the sudden thawing of permafrost, i.e. soil frozen all year round: permafrost occupies about 15 million square kilometres in the northern hemisphere, the so-called tundra (Russia, Canada). As this area thaws, huge quantities of greenhouse gases (e.g. methane) could be released, several times greater than current total annual emissions. Hundreds of billions of tons of climate-altering gases could be released into the atmosphere rapidly: between 3,620 and 7,100 Gt. In this regard, we must remember that cumulative emissions between 1870 and 2016 amount to about 2,080 Gt, so there is a frightening potential in the permafrost: over 70 times the current annual emissions (about 50 Gt) and at least twice the cumulative emissions over 150 years (2,080 Gt). We could call the foreseeable thawing of permafrost "the dangerous awakening of the giant". Being able to reduce the increase of the Earth's average temperature by 0.5°C, i.e. from 2°C to 1.5°C, could mean that a permafrost area between 5 and 8 times the size of Italy will not thaw. So small increases could trigger sudden and huge releases of climate-altering gases, with effects that we cannot predict in detail. But we are sure that social security and health will be compromised.

There is no certain prediction of the effects of, for example, a doubling of the concentration of climate-altering gases in the atmosphere, which at current rates will occur before the end of this century. The uncertainty and the inability to predict mean that the climate roulette will certainly have some sad surprises in store.

There are two other giants that should be of concern and alarm: glaciers, such as the polar and Greenland glaciers, and the oceans. The seas, like the glaciers of the entire Planet, are under attack with unpredictable, though largely evident, long-term effects. Ocean temperatures are rising, acidity has increased, pollution has reached unacceptable levels (e.g. plastic), the amount of oxygen in the seas is decreasing. Biodiversity is also being drastically reduced due to many factors including overfishing, pollution, and acidification. The oceans have absorbed most of the Planet's heat (90%) and at least 30% of the carbon dioxide, produce about 50% of the oxygen, and constitute an enormous reservoir of carbon dioxide, many times greater than the amount recorded in the atmosphere. The collapse of chemical, physical, and biological balances in the oceans, and of exchanges with the atmosphere, could lead to sudden and huge releases of carbon dioxide into the atmosphere, rising sea levels, reduced oxygen production, and an increase in extreme events such as hurricanes. [680, 686] It is difficult to predict exactly how these three giants (oceans, glaciers and permafrost) will react over the next 10 to 30 years, but we have very good information on the trend. The uncertainty is focused on the speed of change and the magnitude but not the direction: [686]

- by 2100 the average temperature of the Planet could be higher in a range of +1°C to over +6°C than today;
- the oceans could be just over a metre higher and up to several metres higher than they are today (at least 4% of the World's population and production are below 10 metres above sea level);
- before the end of the century, extinction could affect between 20% and more than 50% of living species; in some ecosystems, the destruction of biodiversity could affect more than 90% of species, such as coral reefs and primary forests.

Responses to different increments are nonlinear, so small changes generate effects that vary greatly in intensity and extent.
It is also morally right to be self-critical: we are so presumptuous as to believe that we can predict all the effects of climate change, but there may be synergistic phenomena that amplify the magnitude of climate change in a way that is completely unforeseen.

CLIMATE CHANGE DAMAGES BIODIVERSITY AND FOOD SAFETY

Living beings work together in synergy to maintain important balances for the well-being of the human species. Plants produce oxygen, absorb carbon dioxide and regulate the climate. At least 50% of our breath is given by photosynthesis that takes place in the oceans that have absorbed at least one third of the carbon dioxide we have emitted. The destruction of forests and the degradation of marine ecosystems generates the alteration of the conditions that, during the Holocene and that is for 12000 years, have allowed humanity to prosper.
Small increases in the average temperature of the Planet, for example by 2°C instead of 1.5°C, may generate great differences. Changes grow exponentially, so small increases in the average temperature generate big changes that are devastating for flora and fauna. One of the important causes of the reduction in pollinator biodiversity is climate change. [1245]
The projected impacts of a range of warming scenarios on different species groups in "*Priority Areas*" for conservation are alarming (including the Mediterranean area). [621] The 35 regions analysed contain much of the richest and most extraordinary biodiversity on the Planet, including many iconic endangered and endemic species. International climate agreements, such as the Paris Agreement, propose to limit the increase in average global temperature to below +2°C, although current national climate commitments, assuming they are met, would lead to a warming of at least 3.2°C. The worst-case scenario, the so-called "*business as usual*", i.e. that nothing is changed as unfortunately is happening, will lead to an increase of more than +4.5°C: experts consider this eventuality very catastrophic. As the temperature rises, the percentage of species in danger of extinction increases. With +4.5°C warming, almost 50% of the species currently present in the Priority Areas would be at risk of extinction, but if the temperature increase were limited to +2°C this risk would be halved. [621] This prediction highlights the urgent need to reduce greenhouse gas emissions.
Plants will be particularly affected too, because they are often unable to adapt quickly enough to a climate that changes so rapidly. To give an example, in the Amazon it is estimated that there are more than 80,000 plant species, many of which are endemic. Unfortunately, if the best targets for reducing the increase in the Planet's temperature are met, an increase of less than 2°C (compared to the pre-industrial era) could lead to the extinction of 40% of plant species. If the commitments made are respected, with the consequent increase in the Planet's temperature of at least 3°C, before the end of this century the rate of plant extinction in the Amazonia could increase to 6 species out of 10. [621] These estimates do not consider other effects that could prove to be multiplicative, such as deforestation, habitat fragmentation, fires, colonization by alien

species, and other factors related to human activities. Similar catastrophic effects as a result of climate change are easily predictable in other areas of naturalistic interest. In Madagascar, the optimistic scenario of a temperature rise below +2°C suggests that this large island will become inhospitable to at least a quarter of the animal and plant species present. In the Mediterranean area, a rise of just +2°C could lead to the extinction of 30% of the plant and animal species present today, before 2080. [621] In Central Africa, to sustain a population of tens of thousands of elephants, it is necessary to pump water from underground. In Australia, meeting the most ambitious climate change mitigation targets could result in the extinction of 60% of certain animal groups such as amphibians. Due to inertia in the Earth's climate system, the Planet will warm to some extent even if we manage to reduce climate-altering gas emissions to zero; as a result, summers in the Arctic will be ice-free within the next 20 years.

The number of insect species likely to face extinction as a result of an increase in average temperature of +2°C, rather than +1.5°C, increases threefold: from 6% to 18%. The +0.5°C change in increase could cause the number of plant and vertebrate species to become twice as extinct: 8% to 16% for plants and 4% to 8% for vertebrates. These predictions were obtained by studying only 105,000 species, less than 1% of the existing species, so they could easily prove to be optimistic. [775]

The forecasts for marine ecosystems are also catastrophic: coral reefs could be reduced by between 70% and 90% if the temperature increases by +1.5°C, while they could become extinct (>99%) if the increase is +2°C. If global warming stabilizes at +1.5°C (average temperature) above pre-industrial levels (i.e. +0.5°C above today), the Arctic Ocean will be ice-free once every hundred years in September (the month with the least ice). If global warming of +2°C occurs this could happen one year in three. [776] In the Arctic, perennial sea ice occupies barely half the area it covered thirty years ago, and in thirty years it may be gone altogether. [699]

Cryosphere depletion in the Arctic and high mountains also undermines food security and water availability. Studies reveal that increased carbon dioxide in the atmosphere increases the rate of growth of some plants, but decreases their nutritional value. Atmospheric concentrations of about 550 parts per million of carbon dioxide, about 37% higher than current concentrations, in wheat reduce the amount of zinc (9.3%), iron (5.1%), and proteins (6.3%). [774] Similar reductions have been described for rice, peas and soybeans. Globally, climate change is more likely to lead to a reduction in food production, even if the increase in carbon dioxide favours the growth of some plants.

Among the effects of climate change, there is that of the spread of certain insect species. The example is given of locusts in Africa, which are close relatives of grasshoppers. At the beginning of 2020, swarms of locusts the size of cities invaded more than 15 countries in East Africa, the Middle East, and Asia, devastating crops. [778, 1276] It has been possible to record flying swarms of 70 billion insects that are four times the size of Milan's urban area. These huge swarms swoop down on crops and devour everything in a matter of hours, posing a serious threat to the food security of millions of people. Aided by the wind, they fly at a speed of about 16-19 kilometres per hour, covering 130 kilometres or more in a day. [779] In the past, in 1954, there were long and spectacular migrations of swarms moving from Northwest Africa to the British Isles and, in 1988, from West Africa to the Caribbean, a distance of five thousand kilometers in about ten days. Factors likely to have encouraged the proliferation of these insects included cyclones that, in 2018 and 2019, brought heavy rains to the desert. So there is a link between climate change and the crisis generated by the locust invasion plaguing areas such as Ethiopia and East Africa (in some of these areas there are ongoing conflicts and antipersonnel mines are used). Unfortunately, the proliferation of certain insects will lead to increased food insecurity and famine for millions of people.

In order to hope to halt the likely rise in temperature, we should achieve zero emissions by 2050: this means that anthropogenic emissions will all have to be balanced by nature's ability to absorb them. Climate experts have been warning us for several years now; if we allow even a quarter of the known fossil energy stocks to be burnt, the effects will be catastrophic: the agricultural system will be destabilised, resulting in a significant reduction in harvests at planetary level. It is quite realistic to assume that the yields of major cereals could be reduced by 10% for every one degree increase in global average temperature.

THE FREQUENCY OF EXTREME WEATHER EVENTS IS INCREASING

Today the concentrations of some greenhouse gases (carbon dioxide, methane, and nitrogen oxides) are the highest recorded in the last 800,000 years. To find carbon dioxide concentrations higher than today's, we probably need to go back 15 million years: our species has been hosted by Planet Earth for about 2 million years, so we are operating in an atmosphere with a composition we have never breathed before. Probably by the end of this century carbon dioxide concentrations will reach the levels recorded 50 million years ago, when Antarctica was full of palm trees; it is important to remember that in our days the lowest temperatures recorded on the Planet have been measured in Antarctica: -98°C. [699]

Climate change alone could be responsible for a 2% reduction in food production every ten years, while economists and demographers predict a 14% increase in demand, again every ten years.

Changing rainfall patterns and droughts could force huge agricultural areas to switch from irrigation to the exclusive use of rainwater; before the end of this century, the reduction in the availability of water for irrigation could affect millions of hectares and cause an unmanageable reduction in the calories provided by maize, wheat and rice. [736] In some European countries a reduction in maize production between 20 and 30 kg/ha per year is expected before 2050. [871] The drought in Italy, in 2017, reduced cereal productions from 30% to 50%, generating damage to the Italian agriculture of over two thousand million euros (Coldiretti). The apple production fell by 23%, with peaks in Trentino of 60%. [196] Even the production of grapes and legumes, in 2017, due to the drought, fell by 70%. Every year all over the Planet and also in Italy extreme weather events are recorded, i.e. records never measured in recent history. Italy is subject to the effects of climate change in a much more significant way than the rest of Europe. The average temperature is increasing more and extreme weather events are doing much more damage than in the rest of Europe. Temperatures are increasingly out of the norm (winter 2019-2020 was the warmest ever with an average temperature +3.4°C higher than the 30-year reference period 1981-2010, and April 2020 was the warmest in Europe since measurements were taken until then) and rainfall is reduced: in Italy, winter precipitation (2019-2020) was reduced by 43%. [1000] Hundreds of extreme weather phenomena have occurred in Italy over the last decade. These are mainly flooding from heavy rainfall with associated landslides, tornadoes, rainstorms, droughts, and heat waves. In 2019 alone, 1,668 events classified as extreme were recorded in Italy, more than 4 per day (there were 142 recorded in 2008, there were 20 in 1999 and 18 in 1990). [1180] Between 1990 and 2020, 10,274 events were recorded; these are whirlwinds of sand or steam, big hailstones falling over several areas, intense rain, tornadoes, strong wind gusts, intense snowfall or snowstorms, frost, avalanches, and lightnings recorded both on the land and water surfaces: this collection of data is very alarming. [1001] In Germany, during the same period (1990-2020) 39,861 events were recorded, in France 23,910, in the UK 4,649, and in Spain 4,081. Over time, there has been an increase in the frequency and intensity of extreme weather events across Europe: in 30 years, the frequency of extreme weather events has increased more than 80-fold in Italy, more than 64-fold in Germany (5,315 events recorded in 2020 and 82 recorded in 1990), by about 127 times in France (1,017 recorded in 2020 and 8 recorded in

1990), by more than 169 times in Spain (679 recorded in 2020 and 4 recorded in 1990) and by more than 1,000 times in the United Kingdom (1,156 recorded in 2020 and one recorded in 1990). [1001] Overall in the European area, the number of extreme weather events recorded between 1990 and 2020 was at least 204,757: from 152 extreme events recorded in 1990 to 23,876 in 2020; an increase of about 157 times. These statistics are impressive, indicating an alarming and accelerating growth. Among the main causes, there are climate-altering gas emissions, which in Europe amount to an average of at least 8.7 tons of carbon dioxide equivalent, *per capita* each year (in Italy the average is about 7.3 tons; 114 million tons of oil equivalent were consumed in 2018, including at least 36 million tons of oil equivalent from the transport sector, 32 million tons of oil equivalent due to energy consumption by the residential sector, 18 million tons of oil equivalent from waste management, and 17 million tons of oil equivalent from the tertiary sector).

CLIMATE CHANGE, POLLINATORS AND FOOD SAFETY

Climate change is characterized by drought, excessive heat, desertification, and extreme weather events. All these factors also affect floral and entomological biodiversity. The speed of climate change exceeds the ability of pollinators to adapt to new conditions, leading to their decline or extinction. In Europe, climate change could cause species numbers to decline by between 27% and 42% before 2080. [424] Most wild species are likely to see their geographical range reduced by more than 50% before 2055.

It is difficult to estimate how much the climate change will affect pollinators: according to some research, between 17% and 50% of species will experience problems due to reduced flower availability caused by the expected climate change. [588] Some plants, such as cereals and legumes, may reduce their ability to provide nutritionally important minerals and proteins with climate change: zinc in cereals, iron and zinc in legumes. As a result, some nutritional deficiencies are likely to increase.

It's a long time that evidence exists, at least for some plants, that increased carbon dioxide concentration will lead to a reduction in the protein value of pollen, the essential food of many pollinators. [239] In some flowers, drought contributes to a reduction in nectar production (e.g. in lavender flowers), while rainfall causes a dilution of nectar (e.g. in acacia flowers) making them less attractive to bees.

Both the increase in the amount of carbon dioxide in the atmosphere and the increase in ultraviolet radiation may be expected to alter flowering and nectar quantity significantly enough to affect negatively the insects' chance of survival. [361]

Pollination may be carried out by wind, insects and/or other animals. Entomophilous pollination is a crucial aspect of the reproduction of flowering plants (angiosperms). Unfortunately, because of human activities, both plants and pollinators are getting extinct. Climate change generates several negative effects such as the time lag between flowering and the presence of pollinators, reducing the possibility of pollination for flowers and the possibility of feeding for insects. Due to the increase in temperatures of each degree centigrade, in some areas of the Planet such as temperate zones, plants begin flowering at least 4 days (between 2 and 6) earlier. [588]

The increase, possibly doubling, in carbon dioxide concentrations that will occur before the end of this century may harm the interaction between flowers and pollinators. In the USA (Illinois), climate change will alter known interactions between 429 plants and 1,419 species of pollinating insects (14,983 types of flower-insect interactions were examined). [588] If carbon dioxide concentrations double, by the end of the century in this area of the USA average temperatures are likely to increase by up to +5°C. As a result of this increase, flowering stages could be brought forward by one to three weeks, harming between 17% and 50% of pollinators. Thus, the change in flowering times induced by climate change may generate very negative effects. Insects will look for flowers when there are none or too few to ensure their survival, and plants will have difficulty in reproducing. In this case we can talk of co-extinction. This simulation predicts that the change in the phenological phases of plants, generated by the increase in carbon dioxide, will lead to a reduction in both insect and plant species, in a significant and alarming way. [588] Many other factors that could generate additional negative pressure such as drought, extreme weather events or the spread of alien species were not considered in the forecast. Although only the lag or reduction in overlap time between the presence of pollinating insects and flowers were considered, the results are alarming. This prediction raises a very important question: altered species interactions may be a major driver of mass extinctions, which is of great concern and largely underestimated because these phenomena are poorly understood. The interactions in ecosystems between plants, soil organisms and animals are mostly unknown, but they constitute a network of very delicate and irreplaceable balances, unable to adapt to the speed of change imposed by humanity.

Among the easily foreseeable effects of climate change is the displacement of suitable habitats for pollinating insects to the north. The range of some bumblebees (*Bombus terricola*) is predicted to shift 230 km northward by 2030 and 550 km by 2050. [361] Climatic conditions suitable for the survival of many species are moving much faster than the ability to adapt and the ability to move (e.g. for insects).

The use of pesticides is responsible for the reduction of biodiversity: of at least 42% of invertebrates in Europe; the use of these molecules is increasing on a global scale, also due to global warming. [277]

Climate change will generate extreme events, droughts, floods and other phenomena that will affect food security and pollinators. Sea levels 22,000 years ago were probably 120 m lower than today and 8,000 years ago they reached current levels. Coastal areas at risk on the Planet due to rising ocean levels occupy 2% of the surface area, but are inhabited by 13% of the World's population who will be future climate refugees (several hundred million people). [1294] Between 2008 and 2016, it was estimated that 25 million people were displaced by environmental disasters per year. [773] The top 5 countries for the number of people displaced by environmental disasters include China (first place), India (third place), and the USA (fifth place). [773] Forecasts on the possible increase in ocean levels are very variable, but they indicate that small increases in the average temperature of the Planet are followed by big variations in the growth of the height of the seas. When the Earth was completely free of ice, ocean levels were several tens of metres higher than they are today (perhaps 67 metres). If the average temperature of the Planet were to rise by more than +4°C, as it could occur before the end of this century if we continue not to take this problem seriously, according to some scholars there is no reason to doubt that the levels of the oceans could rise by many metres. [774] Salty water will cause less fertile arable land to be available and a reduction in the amount of irrigation and drinking water. Therefore, food security near the coast will be severely compromised.

Agriculture is likely to contribute 24% of total climate-altering gas emissions, with agriculture and livestock being the most important sources of some climate-altering gases, such as methane. In Europe, agriculture is responsible for at least 10% of total emissions, but livestock is responsible for 38% of methane emissions and 32% of di-nitrogen oxide (N_2O) emissions. [871] Paradoxically, agriculture is the main source of some climate-altering gases and is one of the sectors most affected by these changes.

CLIMATE CHANGE AND ZOONOSES

Broadly speaking we can consider that about 60% of pathogenic organisms, which damage human health, are zoonoses, i.e. organisms that can also affect animals (probably, for the remaining 40% there is no adequate scientific knowledge). [114] Excluding purely food-borne zoonotic diseases, there are several zoonotic agents that can be transmitted by farm animals, either directly (direct contact) or indirectly (contamination of drinking water or through insects). Climate change will also favour the spread of zoonoses and their vectors, such as malaria-carrying mosquitoes: in the next few years, 200 million more people could contract malaria, as the degradation of natural ecosystems and climate change favour vector mosquitoes. [736]

Some insects, such as those pathogenic to plants or humans, are favoured by less severe winters and longer periods of warmth: for example, the platyhelminth that generates schistosomiasis in China, the *Anopheles* mosquito that transmits malaria in Africa and kills at least 400,000 people every year, or ticks that can transmit other diseases. Climate change favours insect vectors of diseases in new territories as it happened with the outbreaks of *chikungunya*, in France and Italy, in the years 2007, 2010, 2014, 2015, and 2017. [791] Malaria is a very dangerous disease that was defeated in Europe in 1975, but the mosquito that transmits it (*Anopheles*) is still present. In fact, the genus *Anopheles* includes at least 460 species of mosquitoes of which more than 30 are possible malaria vectors. [1073] Climate change could favour the spread of mosquitoes in Europe (e.g. the tiger mosquito in Northern Europe) and with them the diseases they vector. Within this century it is likely that climate change will generate an exposure for several hundred million people to diseases that were once relegated to tropical areas, affecting over a billion people worldwide. In the worst-case climate scenario, which might occur around 2080, Europe could be among the most affected regions. [792] We can state that in the World today the climate present before the industrial era would have the possibility of preventing the onset of tropical diseases transmitted by a few mosquitoes to more than a billion people. Measures to prevent climate change could prevent the spread of potentially dangerous diseases to a large part of the industrialised World.

STRATOSPHERIC OZONE DEPLETION AND TROPOSPHERIC OZONE INCREASE

Ozone (O_3) is a highly reactive gas, it has a considerable oxidising power, it has a pungent odour and, at high concentrations, it is blue in colour. Ozone is found both in the upper atmosphere and in breathing air, i.e. air in contact with the ground. While stratospheric ozone, i.e. that in the upper atmosphere, is useful, the tropospheric ozone we can breathe is harmful. Tropospheric ozone reduces plant growth, is harmful to humans, and has been shown to be harmful to animals in the laboratory. [978] In the stratosphere (between 30 and 50 km above the ground) the presence of ozone protects the Earth's surface from ultraviolet radiation emitted by the Sun, which is harmful to life, including humans. The absence of this compound in the stratosphere is generally called the "ozone hole". Ozone that defends us from solar radiation due to human activities tends to decrease, while harmful (tropospheric) ozone is increasing. Ozone is a powerful greenhouse gas: it is estimated that its contribution to climate change, on a planetary scale, is among the most important together with that of carbon dioxide and methane.

Ozone near the Earth's surface is measured by the air monitoring network and is a component of "photochemical smog". It occurs mainly in the summer months, when there is intense solar radiation and high temperatures. It is formed, for example, by the photolysis of nitrogen dioxide. Nitrogen oxides and volatile organic compounds emitted into the atmosphere by many processes undergo a complex system of phytochemical reactions, induced by ultraviolet light,

leading to the formation of ozone and hundreds of other very rare substances. These secondary pollutants are collectively referred to as photochemical smog, because they are generated by light-catalyzed chemical reactions. The concentration of ozone measured on the ground results from a dynamic equilibrium determined by multiple factors, which lead to its production and removal; as mentioned, the concentration increases in the summer months and there is a chemical relationship between the concentration of ozone and that of nitrogen oxides (also other molecules, such as non-methane volatile organic compounds, can promote the formation of tropospheric ozone).
The increased intensity of ultraviolet radiation, which reaches the Earth's surface due to the hole in the ozone layer (the stratospheric one), increases the health risks especially of those populations living at medium and high latitudes, mainly due to external exposure (unshielded solar radiation can damage skin and eyes). Without the stratospheric ozone filter, life on land would probably not exist. [889]
Tropospheric ozone reduces the respiratory function, increases the bronchial reactivity, causes eye burning and induces inflammatory events in the upper airway (coughing and breathing difficulties). The increase in the concentration of ozone, in the part of the atmosphere near the ground, increases the incidence of cardio-respiratory diseases, especially in urban areas.
Ozone present in the lower layers of the atmosphere (troposphere) favours the increase of carbon dioxide because it reduces the normal absorption mechanisms of plants, inhibits chlorophyll photosynthesis, inhibits the transport of nutrients from roots to leaves, and accelerates ageing. Ozone concentrations above 40 ppb cause problems for plants. This tropospheric ozone derives from photochemical reactions between oxygen and precursors that are of anthropogenic origin: methane, carbon monoxide, volatile organic compounds, nitrogen oxides. It is estimated that the increase in tropospheric ozone (expected by 2100) will reduce the amount of carbon dioxide absorbed by plants by up to 140 Gt (1 Gt or "Giga" ton corresponds to one billion tons).
Ground-level ozone is toxic to plants and humans, killing at least 150,000 people a year. Pesticides affect air quality and can damage some of the natural protective mechanisms of the atmosphere. Methyl bromide is a pesticide used in the cultivation of tomatoes in greenhouses (a fumigant), to disinfect grains and silos and has been banned by the Montreal Protocol because of its negative effects on the ozone layer. [280]

DISTRACTING ATTENTION WITH SCIENTIFICALLY AND MORALLY INCORRECT DISCLOSURES

Among the proposed but unlikely solutions to counter climate change, a few stand out:

- Increasing the use of nuclear energy (IPPC report 2018 p. 19). [775] It must be remembered that in the energy and economic budget of the life of a nuclear power plant, which usually does not exceed 60 years, many items are underestimated such as the waste that will remain deadly for tens of thousands of years and the non-existence of a safe method of their disposal. The costs and risks involved in producing lethal weapons are also underestimated. Finally, we must not forget that the isotopes used to produce nuclear energy are finite resources.

- Increasing the cultivated area to generate agro-fuels and replace fossil energies; but without fossil energies you do not get agro-fuels. This strategy is madness because more energy is consumed, in terms of fossil fuels used, than the energy obtained (in biomass that can be used for combustion or fermentation). [741] In order to implement this strategy, millions of people must be reduced to hunger. Assuming to invest 150 million hectares of cultivated areas in the Planet to produce agro-fuels and to be able to feed with the cultivation of this area 5 people per hectare means losing the amount of plants able to feed approximately 750 million people (this is 15 million square kilometres, an area equal to one and a half times that of Europe; these are ethically unacceptable scenarios described in the IPPC 2018 report page 22). [775]

- An incorrect but increasingly exploited message is the following: geo-engineering will save us. Among the fanciful works of geo-engineering proposed as a way to save the Planet, there is the introduction of cooling elements into the atmosphere with artificial volcanic eruptions. Another hypothesis is to introduce enormous quantities of nutrients (e.g. iron-based minerals) into the oceans to encourage the growth of plankton, which will accumulate more carbon dioxide. The idea behind these proposals is to distribute pollutants to hopefully reduce negative effects of other pollutants. These are technologies that do not exist to date, so they are fanciful hypotheses and will certainly have unacceptable undesirable side effects, such as distracting attention from the impending environmental collapse, and wasting time and resources. The example of sulphur, which would be distributed in the upper atmosphere and could damage the ozone layer, is one of the proven side effects. [686, 774] These proposals serve to fuel doubt and the belief that technology will save us. [846, 980] These projects are not real and are the expression of a delusion of omnipotence or madness in a terminally ill person. Unfortunately, in many climate reports and at some conferences, these technologies, yet to be invented (this is currently science fiction), are lumped into climate change mitigation estimates as if they were existing applications. [840] Another science fiction hypothesis, widely disseminated as if it were a ready and sustainable technology, is that of taking climate-altering gases from the atmosphere and sucking them underground where they should be stored forever. These sound like ideas from the dreams of mad megalomaniac billionaires. Surely these fanciful ideas will not save us as they are only imaginary projects. One concern, however, does exist and it is based on the huge inequality, in terms of available means. The richest billionaires on the Planet have such impressive resources at their disposal that they might decide to try to carry out crazy projects without needing the help of others.

- Reducing climate-altering gas emissions from animal husbandry by modifying the microbial flora of the digestive tract or by selecting, with appropriate crosses, new ones (as they do not exist today) that could perhaps generate less methane.

- Reducing the impact generated by hundreds of millions of cars, trucks, and planes on the road by reducing their weight (e.g. by 10%). [980]

It is shocking that non-existent solutions generating devastating delays are being disseminated in an attempt to reassure the ignorant. This is a disruptive exercise that is founded on a mixture of conflict of interest, fantasy, and ignorance, and proposes unacceptable assumptions.
In the fight against climate change winning slowly is like losing, so the perception of urgency must be spread, contrary to what often happens. A great danger is the financial condition in which the richest men on the Planet have such great economic wealth that they can afford experiments on a global scale without any limitation other than economic.
Realistic proposals are to reduce consumption (it is the easiest sustainable way) and not use fossil fuels. Choices such as:

- reduce consumption of foods of animal origin;

- grow vegetables that do not require irrigation;
- decrease the production of waste (e.g. food waste);
- increase recycling (e.g. metals, electronic waste);
- countering planned obsolescence (e.g. electronic products and household appliances);
- replace annual crops with multi-annual crops (e.g. orchards and arboretums instead of cereals for food);
- design and disseminate sustainable agriculture: without pesticides, with less use of fossil fuels, with greater biodiversity, without the application of harmful practices such as soil movement and burning of plant residues;
- reduce private transport and transport of goods;
- decrease the production of plastics from all trades not necessary for survival;
- safeguard drinking water and prohibit its privatisation;
- do not use public funds to encourage activities that are opaque or damaging to the environment, such as the production of methane from corn;
- reforesting the Planet;
- oblige entrepreneurs to disclose essential information on the level of environmental and social protection they provide;
- inform consumers about production methods and ingredients in a much more credible way (the resources spent on advertising absent ingredients are a measure of the lack of transparency and fairness);
- raise taxes on the rich;
- limit the maximum wealth an individual can accumulate;
- dismantle tax havens;

and other choices that can be applied immediately and easily but are unpopular. This list could go on for dozens of pages but the most important necessary change is to recognize the existence of limits and, consequently, to accept prohibitions, for the good of all and of the next generations.
Unfortunately, visionary options are being promoted, such as the application of currently non-existent technologies that could perhaps one day allow greenhouse gases to be extracted from the atmosphere (IPPC 2018 report p. 15 and 17). [775] Using science fiction to address such close and apocalyptic problems is (to say the least) naive.
Remaining with more concrete hypotheses, easily achievable without compromising health, we can start from the diet: reduce the consumption of meat and derivatives by the wealthy of the Planet. The change in eating habits, towards a greater vegetarianism, could make available an area between 4 million and 25 million square kilometres: an area about 2.5 times the size of the whole of Europe. [36, 776] Another good idea could be to prohibit the production of agro-fuels (methane and ethanol) and agro-plastics, allowing the food use of large areas: even 7% of the current agricultural area used in Europe and much more in other countries of the World. [741]
Unfortunately, the set of proposals put forward is very disappointing as they are insufficient to generate the necessary change of course. It may seem exaggerated, but scientific information indicates that it is very likely that the time available to us to save ourselves from apocalyptic climate events is only a few years, even less than 20. We are compromising the future of our children, while updates on climate change forecasts over the last few decades consistently indicate an increase in the devastating effects. Countering indifference to climate change probably requires a war-like mobilization, as happened with the Coronavirus emergency. Global warming, the destruction of biodiversity, the degradation of soils and oceans and other factors consequent to the human invasion of every corner of the Earth will certainly do much more damage and will also favour other epidemics.

FIGHTING IGNORANCE AND INEQUALITIES TO SAFEGUARD ENVIRONMENT AND HEALTH

These predictions should frighten us and set in motion all necessary actions. The time window in which this dangerous uncertainty can be positively addressed is very short and shrinking faster and faster. The worst predictions of a +2°C rise could occur in less than one human generation. Common sense should lead us to leave fossil fuels in the ground, reforest the Planet and scale back consumerism. Turning back the lifestyle of industrialized countries by at least 60 to 70 years is the only way out; that is precisely why it scares us. To peacefully obtain an immediate reduction of freedom in exchange for future benefits, even if it is a near future, probably remains and will remain a utopia. And yet in Italy the situation is worse because the growth of the average temperature is double of the global average. Italy is an area that is affected by climate change with an intensity much higher than the global average and, consequently, Italians should be even more worried: minimum temperatures have increased more and winter temperatures as well (we have recorded the reduction of freezing days and the increase of tropical nights); total precipitations have decreased as well as the total number of rainy days has decreased. [1294] In a century the glaciers in the Alps have halved and the speed of change is increasing. In the Mediterranean area, some crops, such as non-irrigated crops, could see a reduction in yields of up to 50% before 2050 (e.g. corn and wheat). [776] The climate is changing at a rate that is equivalent to a shift southward of European cities of about 20 km per year (London may soon have the climate of Barcelona) and it is 20 years that grapes have also been grown in Finland and Sweden. [871] An interesting index that tries to estimate the risk from climate change has been developed by *GermanWatch*. [203]

The effects of climate change are very evident in Italy too: [1290]

- the melting of Alpine glaciers (average temperatures in the Alps have risen by more than 2°C since 1864) and the collapse of rocky slopes famous for the achievements of climbers (e.g. mount Cervino, monte Rosa);

- cloudbursts (in some cities of Liguria in a few hours the rainfall equalled that falling in a year: in less than 24 hours 948 mm in Bolzaneto, in the Province of Genoa, on the 8th of October 2021 and 740 mm in less than 12 hours in Rossiglione, in the Province of Genoa, on the 4th of October 2021);

- on the 13th of November 2019 high water in Venice exceeded 1.87 m (tides above 1.1 m have gone from a frequency of one or two per year, until the middle of the last century, to twenty-six in 2019; on the 13th of November 2019 an event occurred that is emblematic of our inability to deal with huge problems whose solutions make those who propose them unpopular, as the seat of the Veneto Regional Council was flooded, two minutes after the political majority had rejected the measures proposed to control climate change; the representatives of the people, among other things, decided to continue the session despite the alert and the sounds of sirens, and were forced to flee in a risky and daring way);

- extreme weather events such as 14 cm hailstones fell in Pescara in July 2019;

- between 1950 and 2015, at least 348 tornadoes were recorded, causing 69 deaths and 753 injured, and generating costs of hundreds of millions of euros (on the 8th of July 2015 in the Province of Venice, a tornado moving at a speed of about 20 km/h recorded winds of up to 250 km/h; in November 2012 in Puglia, one of the largest tornadoes recorded in Europe was recorded);

- agriculture suffers serious damage (climate fluctuations could make many Italian wine-growing areas unsuitable for this crop in a few decades);

- the Mediterranean Sea is invaded by alien species from e.g. the Red Sea, and due to changes (e.g. overheating, overfishing, pollution) a lot of native species could get extinct (e.g.

Salpa salpa together with at least 21% of the 256 endemic fish species, by the second half of this century).

We are already experiencing a climate emergency even in Italy and the forecasts are catastrophic. [1289] We are pushing climate chaos in a direction that becomes out of control and we risk a lot if we rely on our forecasting ability: things could be much worse. For example, there is insufficient information on oxygen consumption and its decrease in the short and medium term. Combustion, the destruction of forests, the degradation of life in the oceans, the loss of soil fertility, all contribute to reducing the concentration of oxygen in the atmosphere. The speed of the collapse of ecosystem services could increase rapidly as critical thresholds are crossed, such as the reduction of biodiversity by more than 50%. These and other environmental issues should be investigated more thoroughly. Moreover, science, despite countless cries of alarm, has not been able to change political and social choices in any significant way. [1289] A great challenge is to ensure a good quality of life to the greatest number of people while respecting planetary limits, even in Italy. Probably some far-sighted choices such as the reduction of military spending and the fight against tax evasion would allow the recovery of hundreds of billion euros that could be allocated to ecological transition and controlled degrowth. Climate change undermines the foundations of social life and despite the fact that science no longer has any doubts, deniers continue to waste resources and hold back unrepeatable opportunities; the statement released in 2019 should be highlighted, that bears the signatures of 500 prominent people who deny the existence of a climate crisis (including 165 Italian signatories). [1291, 1292, 1293] This is one of many campaigns orchestrated by hundreds of climate-skeptic exponents (professors, lobbyists, politicians) aimed at blocking any international commitment to build a zero-carbon economy by 2050 (in 2016 with the election of Donald Trump, in 4 years, at least 176 acts and steps were taken to reduce measures aimed at reducing climate warming, including the US withdrawal from the Paris Climate Agreement signed in 2015). It is important to prevent those who have understood from remaining passive or, worse, from rowing in the opposite direction for selfish reasons. A self-destructive behaviour should be vigorously opposed.

The environmental issue is effectively represented by the climate issue, which is a striking example of the distorted World order that prevails: less than ten of the richest people on the Planet possess economic resources greater than those possessed by half of the World's poorest people; 1% of the inhabitants of the Planet possess a wealth greater than that held by the rest of the World's population. It follows that the richest 10% of people generate more than half of the planetary climate-altering gas emissions. [762] The time has come to build a social order that creates benefits for all and not only for a privileged few. The prevailing economic models are based on the expression of very negative human characteristics such as selfishness and leave no room for civic sense and empathy.

Our ignorance about the environmental crisis has become a major economic asset for some. Ignorance of ecology and of the basic principles of physics, chemistry, and biology is a prerequisite for the myth of infinite economic growth and the exploitation of nature without limits, and is therefore a real currency: the more you cultivate it, the greater the chances of profit. However, this policy clashes with the biophysical limits that actually do not allow the Earth to withstand the increasing human pressure.

Fossil fuels are finite resources and before we have even consumed the last drop of oil, the biosphere will have been severely and irreversibly altered. We have probably already consumed more than half of the known oil reserves. Indicators that signal that oil is bound to run out are:

- the ratio of energy used to obtain other energy (EROEI: *Energy Returned on Energy Invested*);
- the amount of new oil discovered compared to the amount of oil extracted.

Between 1940 and 1984, the ratio of energy invested to energy obtained rose from 1/100 to 1/8. In 2011, new fields were discovered amounting to one third of the quantities extracted that year. These two indicators are enough to predict that cheap oil will run out.

Climate change will cause an increase in deaths and illnesses due to various factors such as malnutrition, malaria, diarrhoea, mental disorders, heat waves, floods, droughts, hurricanes, fires, and wars. The effects of climate change are very uncertain: for example, the number of climate refugees in 2050 could be between 25 million and even 1 billion. Such a wide range underlines both the uncertainty and the danger. The real costs of doing nothing could be much higher than we can predict. If the voluntary international commitments promoted so far are respected, warming will exceed +3°C (90% probability of exceeding 2°C). It should be noted that climate models assume the possibility that the temperature could rise by more than 5°C before the end of this century. There is no unequivocal scientific certainty that if the 450 ppm of carbon dioxide in the atmosphere is not exceeded, an increase of 2°C will not be exceeded and climate change will not be dangerous (in December 2021 the concentration was 416.7 ppm while in 1750 it was about 277 ppm). Nevertheless, during international climate conferences an increase of less than +2°C is often indicated as a safety threshold, when in reality it is the result of a political compromise rather than a scientific certainty. It would have been much more practical to set targets in terms of the amount of climate-altering gas emissions not to be exceeded. The political compromise accepts the possibility that coral reefs will get extinct, that hundreds of millions of people will become climate refugees or displaced by environmental disasters, and accepts other events that are likely to be catastrophic.

IRREVERSIBLE SOIL DEGRADATION

SOIL IS A PRECIOUS AND NON-RENEWABLE RESOURCE

Soil is the result of a long and difficult process of formation through rock disintegration and other processes (pedogenesis) that takes from 1,000 to 100,000 years. [147] Unfortunately, the rate of erosion and degradation is far greater than the natural capacity for regeneration, so soil cannot be considered a renewable resource. In general, it is estimated that the loss of the arable layer is proceeding at a rate 17 times higher than natural regeneration in Europe, 10 times higher in the USA and 47 times higher in China. [8] At least one third of the Planet's arable land area has been lost over the last 40 years due to soil degradation. Of the remaining land 40% is at risk (at least 50% in Europe). [985] Soil degradation is proceeding at an unsustainable rate, faster than the natural capacity for regeneration: we have passed the limit of sustainability. Ecosystem services cannot keep up with the speed of change we have imposed.

The time it takes to form these few fertile centimetres on which our survival depends should make us reflect on their preciousness and on the fact that, in relation to the time span that affects the human species, soil is not a renewable resource. We can no longer ignore this alarm.

A fertile soil contains about 100 t of organic matter per hectare. [425] The amount of organic carbon compounds, in the first metre of depth, varies between 30 t/ha, in a soil with an arid climate, up to 800 t/ha. Most soils have between 50 and 150 t/ha. [49] Soil with less than 70 t/ha of organic carbon is considered infertile. [84] Intensive agriculture depletes soils of organic substances such as carbon compounds. [49]

The carbon cycle has a delicate balance that is increasingly altered by human intervention, in favour of the transfer of soil and forests into the atmosphere. It is estimated that in some areas of the Planet (e.g. USA) at least 50% of the carbon dioxide present in soils has been transferred into the atmosphere in less than 100 years due to deforestation (e.g. for grazing), erosion, and cultivation. Ploughing is one of those agricultural practices that favours the emission of carbon dioxide into the atmosphere: ploughs pulled by oxen are depicted in Egyptian hieroglyphics dating back to 3000 b.C. [985]

Industrial agriculture reduces soil fertility; in some Italian regions more than 70% of agricultural soils have a fertility level lower than the minimum values able to ensure a good agricultural production: at least 2% of organic substance from living beings.

An example of avoidable soil degradation is the cultivation of plants to generate agrofuels such as methane and ethanol. The problems generated by cultivation for the production of agrofuels are manifold: [1246]

- competition with the food chain;
- the increase in negative social impacts such as oil palm cultivation in New Guinea where workers are often underpaid, exploited, kept in unenviable conditions, and local cultures get extinct;
- waste is generated polluting the environment and damaging health.

Alternatively, the area could be used for reforestation, the renaturation of ecosystems with significant benefits for the climate and soil. Especially in the most industrialized areas of the Planet we take most of the available biomass, so hoping to meet energy needs by taking more biomass means further damage to natural areas: problems such as soil degradation, climate change, and reduction of biodiversity will increase.

On a planetary level, an area at least 15 times the size of Italy is degraded every year due to deforestation, fires, increased salinity, and urbanization, and at least a quarter of the

Earth's surface is threatened by desertification. In Italy, at least 21% of the surface is at risk of desertification; this percentage may be much higher in the southern regions. [985] Unfortunately, the degradation of soils and the consequent decrease in productivity are irreversible, as they occur faster than the capacity to regenerate themselves in natural conditions. Probably a half of the soils is already affected by fertility reduction phenomena and more than 30% of the cultivated soils lose their arable status before they can reconstitute themselves. [15] Therefore soil management must be renewed towards sustainability and regeneration criteria. It must be considered that the demand for food could double before 2050 and to support the increase in food production, soil fertility must be increased. Unfortunately, there is an increase in soil degradation, the opposite of what is needed to support the global food demand. How can one hope - as the World Bank would like - to increase food production of 70% by 2050 under these conditions? One probably needs to be an expert in economics and finance to believe in infinite growth, for example, of the GDP by 2%: this would mean having to double wealth and production every 30 years or so.

In general, agricultural soils are less fertile than those of natural ecosystems, in fact they contain between 25% and 75% less carbon. The lack of fertility is compensated, in an unsustainable way, with the use of chemicals (pesticides and fertilizers) and energy: at least 10 kcal of fossil energy for every kcal of vegetable.

Approximately between two and twelve million hectares of soil are irreversibly degraded each year and become unusable for agricultural purposes due to various factors such as erosion, salinization, desertification, drought, floods, reduced fertility, urbanization, and pollution. In the face of the demand for increased food production, even doubling within the next 30 years, in reality the capacity of the Planet to sustain the food supply is reducing at an alarming rate. At this rate, in 30 years, at least 60-90 million hectares will become unsuitable for cultivation: this is an optimistic estimate, as it could be much more. [740] To have a measure of comparison in Europe, in 2016, 173 million hectares were cultivated, or 39% of the total area. [739] In the World, the land area occupies about 15 billion hectares, while the utilized agricultural area accounts for one third of the total, or roughly 5 billion hectares: [740]

a. 3.4 billion hectares of pastureland (including alpine pastures);
b. 1.4 billion hectares of arable land;
c. 140 million hectares of permanent crops (orchards, palm groves, vineyards, tea or coffee plantations).

The surface area of arable land, since the 1970s, despite the cultivation of vast areas of forest in Brazil, Africa, and Indonesia (millions of hectares per year subtracted from nature), has remained almost constant due to losses generated by various factors such as the salinisation of irrigated areas, the impoverishment of agricultural land and the advance of urbanization.

Predictions of the food insecurity that lies ahead could be optimistic as there are many uncertain factors, such as a drastic reduction in biodiversity such as pollinators. The decrease in the service provided by pollinating insects alone could generate a 10% reduction in the volume of plant products, especially of crops with a higher income and a better nutritional value (e.g. for vitamin content). Effects such as those of climate change must also be considered, as very large areas may become unsuitable for cultivation. Other negative pressures are generated by the occupation of agricultural land to satisfy selfish economic rules, such as the production of agro-fuels. [741] The foreseeable increase in plant diseases (e.g. phytophagous insects such as locusts which are favoured by climate change and other anthropogenic factors) may also worsen the current situation. [742]

Another land use that should be restricted is the cultivation of at least between 3 and 4 million hectares to produce the tobacco needed to support over 1.1 billion smokers. It should be reflected that we cannot sustain a wasteful land use, as the arable land available to each human being today is between 1,000 and 3,000 square metres and is bound to decline. The people who live in a dangerous situation of food insecurity are many even in the richest countries: for one American out of seven food security is not granted, so it is not a right guaranteed to all but a privilege of the few. [837]

Biotic stresses (e.g. the decrease of microorganisms and invertebrates in the soil, pests) and abiotic stresses (e.g. drought, salinity, accumulation of pollutants, heat) contribute to reduce soil fertility all over the Planet. [1242] The loss of biodiversity within soils is one of the important problems as it causes a decrease in the ability to produce food and sustain plants. Many microorganisms are useful to plants as they increase fertility, facilitate nutrient uptake and improve plant health. For example, certain microorganisms in the soil help make plants more drought-resistant: in maize (*Azospirillum lipoferum* and *Bacillus* Spp.), soybean (*Pseudomonas putida*), wheat (*Bacillus amyloliquefaciens*, *Azospirillum brasilense*, *Rhizobium leguminosarum*, *Mesorhizobium cicero*, and *Rhizobium phaseoli*), and rice (*Trichoderma harzianum*). [1242] The mechanisms by which drought resistance is conferred are diverse such as the production of polysaccharides and the activation of metabolic activities, while salinity tolerance may be generated by hormone production. Thus, microorganisms involved in the formation of root symbioses (mycorrhizae) can confer resistance to both drought and salinity (e.g. in the case of lettuce and tomato). [1242] Soils in many parts of the Planet are so degraded that it is necessary to restore their fertility by increasing organic matter and inoculating microorganisms useful to plants and to healthy ecosystems. Fertilizing with the organisms needed to restore soils will be an increasingly necessary practice but will have little effectiveness if we continue to implement unsustainable agronomic management practices such as plowing, pesticides, chemical fertilization, soil not covered by year-round vegetation, and irrigation.

LIVESTOCK, FERTILIZERS, AND SOIL

The distribution of livestock manure to feed plants is an excellent way to recycle this material and, at the same time, reduce the use of chemical fertilizers. Manures contain nitrogen in both organic and inorganic forms. The organic form is usually not available for uptake by plants until it is converted into more easily assimilated forms, such as ammonia (NH_4^+). A fraction of ammonia is transformed by microorganisms into nitrates that are useful to plants, but at the same time can easily contaminate water: between 15% and 55% of the nitrogen distributed in the field is lost in water. The distribution of manure in depth (45 cm), compared to spreading with injection at 15 cm, increases the amount of nitrogen that is retained by the soil but costs more energy. [907]

Different plants behave differently: some species are more dependent on inorganic nitrogen (such as maize), others are able to take up organic forms, such as amino acids and proteins. Plants such as rice, sorghum, and carrot, although they prefer to take up inorganic nitrogen, are able to take up organic forms such as proteins, which can be an advantage. [906] Nitrate is generally considered to be the form of nitrogen most readily assimilated by plants. Plants living at low temperatures and in acidic soils are more dependent on ammonia than on nitrate.

Not paying attention to good agronomic rules means distributing pollutants to the environment instead for the plants. To give an example, alternating cultivation with legumes (e.g. cereals in summer and legumes in winter) may generate an increase in production equivalent to that which can be produced with the distribution of 15-200 kg of nitrogen per hectare per year, depending on the plants used and the cultivation conditions. [52]

The considerable availability of organic material to be used as fertilizer is a very positive aspect if the farm is located near soils poor in organic matter. It is possible to consider that every day, a cow, a pig or a chicken produce a quantity of dejection equal to 10% of their body weight, with a percentage of water of 95% for pigs, 92% for cattle and 85% for poultry. The dejection may contain greater amounts of urine than manure. Pigs produce 7,300 litres of slurry per animal per year, cattle can produce 18,000 litres per year, laying hens produce 55 kg and turkeys 183 kg of manure. The amount of water that is drunk daily varies greatly; for example, a dairy cow can drink about 200 L of water per day, a fattening cow 50 L, a pig 20 L.

Despite the great availability of organic substances such as manure, large quantities of chemical fertilizers continue to be produced and used. In the World, it is estimated that for the production of nitrogen-based fertilizers, 1% of all energy used by man is consumed (5% of all natural gas consumed is used: between 33 and 44 GJ per ton of ammonia) and at least 41 million tons of carbon dioxide are produced per year. With the Haber-Bosch process, which converts atmospheric diatomic nitrogen into mineral fertilizer, about 100 million tons per year of nitrogen-based fertilizers are produced. [114] It is estimated that at least 40% of the World's population is dependent on the use of synthetic nitrogen fertilizers. [919]

Nitrates are one of the most important chemical contaminants of water bodies on the Planet. [884] The presence of nitrogen compounds in water, such as nitrate and ammonia, indicates possible microbiological contamination from manure or sludge or wastewater from sewage treatment plants. The degree of impairment of the water resource is assessed on the basis of the nitrate concentration. In groundwater, the threshold value has been set at 50 mg/L; in some cases, the more precautionary limit of 40 mg/L is also used. The most relevant constraint for agricultural activity, deriving from the application of this regulation, is the imposition of a maximum annual limit on the input of nitrogen of zootechnical origin for crop fertilization. This limit has been set at 170 kg of nitrogen per hectare in areas considered vulnerable to nitrates (in Italy). In areas not subject to these constraints, the limit is 340 kg of nitrogen per hectare per year.

About 14,000,000 animals weigh on the Piedmont Region (Italy) such as cattle, poultry, and pigs, mainly raised in the plain. The utilized agricultural surface is about 900,000 hectares. [971] Therefore, the probability of groundwater contamination following fertilization with livestock manure is high. Overall, at least 38% of the water monitored in the agricultural area in Piedmont has already been recognised as contaminated by substances also deriving from livestock, such as nitrogen compounds (Nitrates Directive and vulnerable areas; Directive 91/676/EEC and Legislative Decree No. 152 of 11 May 1999). If we consider only the plain, the percentage is much higher than 38%. Let us assume the following assumptions, simplifying the Piedmontese situation: [741]

- more than 55% of the usable agricultural area may be regularly fertilized (500,000 ha out of a total of 900,000 ha);
- livestock holdings are uniformly distributed over the usable agricultural area, so manure transport is not a limitation (a very optimistic assumption);

for every 10,000 square meters, there is the availability of manure from 2 cattle, 3 pigs, 22 chickens; in addition, 700 kg of compost from municipal solid waste (i.e. the organic fraction produced by 3 and up to 5 people in a year) and up to 200 kg of sludge, obtained from the purification of wastewater, are available. Chemical fertilizers and digestates from anaerobic fermentation, which also use crops such as corn to produce methane (biogas), are also potentially available in each hectare. In detail, always in Piedmont, more than 240 kg of mineral fertilizers are used per hectare, but if we consider that they are distributed only on the 50% of the utilized agricultural surface (UAS), then even more than 450 kg/ha/year will be distributed. Special wastes, i.e. organic wastes originated from productive activities and used for spreading in agriculture or for environmental recoveries, are another kind of fertilizer used in agriculture. Theoretically, assuming that this material can be uniformly distributed over 500,000 hectares of agricultural land, this is equivalent to spreading approximately 2,000 kg of special waste per hectare per year (otherwise, an average of 1,000 kg/ha of special waste distributed each year

over the entire utilized agricultural area of Piedmont would be obtained). To these pollutants, others must be added such as several kilos of pesticides per hectare each year.
If we try to apply the same reasoning just carried out on Piedmont to the entire Po Valley, we can easily understand that the exclusive zootechnical effluents exceed the capacity of agricultural soils to receive them, considering only nitrogen compounds and only those deriving from zootechnical dejection. There is an excessive and concentrated availability of organic substances in a few geographical areas, which therefore become an environmental problem. We can no longer talk of fertilization but rather of distribution of organic waste on the soil. The availability exceeds the needs of the crops. The agricultural surface usable both for the production of animal feed (e.g. corn) and, in general, for the spreading of zootechnical manure, in most of the territories of the Po Valley is not sufficient to ensure the spreading of the available organic material (manure, compost, sewage sludge, digests obtained by anaerobic fermentation) without compromising the health of the environment and of water. Agricultural soil is used to dispose of excess organic pollutants.

FERTILIZATION AND METAL INPUT

Metals contaminating agricultural soils may come from fertilizers, pesticides, polluted water, the atmosphere (from fossil fuels), corrosion of agricultural equipment, food additives, and medicines used in livestock farming, or in sludge from wastewater treatment. Forest fires release great quantities of metals, dioxins, and furans into the air and soil. Fungicides containing copper, zinc, manganese, and tin are used in viticulture, fruit growing, and horticulture (up to several kilos per hectare per year).

An important source of some contaminants to the soil and to the food chain is air pollution. For some metals such as lead, deposition from the atmosphere is a major source. Due to air pollution more than one kilogram of heavy metals may be deposited in each hectare per year: 100 mg per square metre per year. An indirect method to assess the amount of metals entering the agricultural system from the atmosphere is to measure the concentration in bio-indicator organisms such as moss, lichen or leaves, e.g. of oak, poplar, and pine.

Manure has high concentrations of metals (cadmium, mercury, chromium, and nickel), salts and micro-organisms. The spreading of manure and sludge (or their digestates) presents several problems, such as the presence of dangerous substances. Metals are also introduced into the soil through inorganic fertilization with chemical phosphorus and nitrogen fertilizers. Heavy metals are defined as having a specific weight greater than $5 g/cm^3$ and are for example: arsenic, cadmium, mercury, and lead. These metals may persist in the environment and generate negative effects, both in plants and animals. Metals may remain in soils for thousands of years, and may be found as oxides, sulphites, salts or carbonates. They are known to bioaccumulate in the food chain. Some metals, such as mercury and arsenic, are volatile. Mercury can be emitted from the leaves of certain plants that have concentrated it, so it is very mobile.
Some metals are toxic to humans, animals, and plants (e.g. cadmium, lead), others are essential in small quantities for both plants and humans (e.g. copper and zinc), others have negative effects mainly on certain plants (e.g. chromium). Some metals at low concentrations are indispensable elements for plants (boron, zinc, copper, nickel), but at higher concentrations they become phytotoxic. Cadmium, nickel, arsenic, and lead generate various negative effects in humans and are also carcinogenic. Cadmium may be absent in the human body at birth, but as age progresses it accumulates, especially in the kidneys and liver, and may exceed 30 mg by the age of 50. [909]
Feeds may contain metals such as copper, zinc, selenium, cobalt, arsenic, iron, and manganese, which are fed to livestock for health reasons and as growth promoters. Copper and zinc promote

enzymatic reactions and for this reason they are sometimes added to the diets of livestock, together with iron and manganese; they are classified as micro-nutrients, i.e. needed in small quantities. Only between 5% and 15% of the ingested metals are absorbed by the animals, the majority being excreted in the faeces.
Some of the problems that may arise from the distribution of metals in the environment are as follows:

- Accumulation in the soil of contaminants with phytotoxic effects (e.g. zinc) and harmful effects on soil organisms (e.g. lead, mercury, and cadmium).
- Ingestion by herbivores.
- Bioaccumulation of pollutants in plant and animal organisms, with risks to the food chain. Metals such as copper, zinc, and lead may be bioconcentrated in plants such as wheat. [910, 911] Wheat seeds can concentrate cadmium and copper. Spinach, leafy vegetables, and forage crops accumulate cadmium. Corn tends easily to bioconcentrate several metals such as manganese, zinc, and cadmium. [911] Maize absorbs 5% of the cadmium concentration in the soil, accumulating up to 79 ppm, 14 years after the last use of the compost. [912, 913] In vegetables intended for animal feed, 0.5 mg/kg of cadmium is considered a safe concentration. Once ingested by humans, cadmium may halve its concentration in 10-30 years. It therefore remains in the body for a long time, accumulating preferentially in certain tissues: liver, kidneys, and reproductive system.
- Water contamination.

Cattle manure may have higher concentrations of metals than those required for composts derived from the organic fraction of municipal solid waste. For example, zinc may be found in concentrations up to three times that allowed for fertilizers in Italy.
The distribution of only livestock manure, in the quantity that supplies 250 kg of nitrogen per hectare per year, generates an increase in the concentration of some metals in the soil. [914] In particular, distributing every year a quantity of dairy cattle manure corresponding to 250 kg of nitrogen will also distribute (every 10,000 square meters): 1,816 g of zinc, 431 g of chromium, 305 g of copper, 109 g of lead, 3 g of cadmium, 0.4 g of arsenic, and 0.3 g of mercury. Thus, pollution of soil, water, and bioaccumulation in the food chain is promoted.
Highly contaminated matrices, used as fertilizers, are sludge derived from wastewater treatment and compost derived from aerobic fermentation of municipal solid waste. Long-term studies have shown that following the spreading of composts there is a halving in the soil of the amount of organic matter from the compost, in at least ten years, but the concentrations of metals increase compared to the same before the spreading.
In the Netherlands, 30 years of compost use have resulted in increased concentrations of metals in the soil, such as cadmium (by a factor of 3), arsenic, chromium, nickel (by a factor of 4), mercury and lead (by a factor of 4). In cultivated vegetables these metals have been found at higher concentrations: from 15% up to 50% (bioaccumulation from soil). Animals eating these vegetables can bioconcentrate them further. For example, cadmium and lead are found in higher concentrations in pig lights (bioaccumulation from plants).

In sewage sludge the concentrations of microorganisms and potentially dangerous undesirable substances are much higher than in livestock manure. For example, concentrations of bacteria such as *Escherichia coli* may be 100 - 10,000 times higher, and maximum metal concentrations 30 times higher. Due to the high concentrations of pathogens and potentially dangerous substances (e.g. heavy metals) it would be advisable to reduce the use of sludge and its derivatives in agriculture, both those used as such and those derived from aerobic and anaerobic fermentation.
Sewage sludge also contains organic pollutants such as endocrine disruptors, medicines (e.g. antibiotics, the tranquilizer diazepam which is reduced by up to 50%) and other products (e.g.

cleaning agents). There are also other materials used to make fertilizers, such as agro-industrial residues, industrial sludge, waste from the canning industry, olive pomace, and vegetable water from oil mills, slaughterhouse residues, leather industry residues: all are potential sources of different types of pollutants.

Different fertilizers have, for the same concentration of nitrogen, different amounts of metals, varying even by a factor of 10 or more. Metals play an important role in upsetting the balances that regulate soil fertility, as they can reduce the activity and biomass of microorganisms. Several scientific works have demonstrated the inhibition of the growth of microorganisms if high-dose, metal-rich fertilizers are used, such as sludge from the sewage treatment process. Therefore, fertilization plans should also take these inputs into account. Unfortunately, up to now, the amount of nitrogen is used almost exclusively for making fertilization balances. This approach greatly limits the possibility of preventing environmental and health risks. In general, the constant distribution over time of fertilizers such as compost, sludge or digestates (biomasses obtained from anaerobic fermentation aimed at obtaining methane) brings higher quantities of metals to the soil than the natural ones, generating some risks: cytotoxicity, inhibition of microorganisms, decrease in fertility, bioaccumulation, and contamination of drinking water. Fertilization plans should evaluate the risks arising from the addition of hazardous chemicals other than nitrogen compounds, as they are present in high concentrations. Neglecting this aspect leads to dangerous changes in the chemical characteristics of soils, favouring a reduction in fertility.

COMPOST: FERTILIZATION OR DISPOSAL OF HAZARDOUS SUBSTANCES?

The borderline between fertilization and pollutant distribution in many cases is not easy to delineate, and it depends also on the quantities compared to the needs. Each crop and each context (soil, rainfall, climate) requires an optimal requirement of nutrients in different ratios. Usually, however, estimates are made on the needs of the crop only for the compounds needed in greater quantities such as nitrogen, with the consequent risk of exceeding the "micronutrients", i.e. those needed in low quantities (e.g. some metals).

Compost can derive from the aerobic fermentation of different matrices including livestock manure and municipal solid waste: the duration of the process should not be less than 90 days and the temperature during the bio-oxidation phase must be maintained for at least five days above 55°C (better for two weeks). Regardless of the regulatory classification considered, compost is capable of providing useful substances to plants and is a good material recovery system. Compost classified as *high quality compost* is obtained from the composting of separately collected organic waste that meets the requirements and characteristics laid down in the regulations. *Compost of high quality* can fall into the category of soil improvers, i.e. materials to be added to the soil mainly to preserve or improve its physical and chemical characteristics and biological activity. [924] *Compost of high quality*, in addition to being used as a soil conditioner, can be used for mulching, i.e. covering the soil with a layer of material 3-10 cm thick, in order to conserve moisture, protect the soil from erosion, prevent the formation of the so-called surface crust, reduce compaction, maintain its structure, and raise soil temperature. The use of compost derived from municipal solid waste is encouraged. [741]

Sludge can be used to produce the mixed composted soil improver. The use of sludge as a soil improver increases the concentrations of substances that are hazardous to plants (phytotoxic) and soil fertility. [926, 931, 932] In order to reduce chemical and biological risks, several restrictions have been issued in Italy over time. Sludge from wastewater purification (e.g. from the sewerage system) should not exceed 35% of the initial mixture, expressed as a percentage by weight, and may be applied to soils in doses not exceeding 15 t/ha of dry matter, over a three-year period.

In the case of compost production, some dangers become more probable as a result of the use of raw materials that are not well sorted and contain contaminants such as glass, packaging, metals, plastics, batteries, etc. Among the substances found in composts from the organic fraction of municipal solid waste, classified as carcinogens or potential carcinogens, we highlight: arsenic, asbestos, hexavalent chromium, nickel, and polychlorinated biphenyls (PCB). Some undesirable substances may originate from transformations during composting (e.g. microorganisms and their spores and toxins; odorigenic substances and volatile compounds). Potential hazards that may be assessed in compost production include:

- Organic compounds such as solvents, fats, halogenated hydrocarbons, dioxins, PCBs, polycyclic aromatic compounds, and esters such as phthalates. Dioxins (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans) which are over 200 molecules in number and are formed during combustion from chlorinated organic precursors (e.g. PVC).
- Inorganic substances such as: [912, 928, 929, 930]
    - arsenic (found in soil treated with compost up to 90 ppm);
    - nickel (found in compost at concentrations between 0.8 and 1,220 ppm);
    - mercury (found in compost at concentrations between 0.9 and 23 ppm);
    - lead (found in compost at concentrations between 11 and 1,312 ppm, with a maximum bioavailability of 75%);
    - cadmium (found in compost at concentrations between 0.3 and 12 ppm, with a maximum bioavailability of 52%, depending on the soil pH);
    - asbestos, copper, hexavalent chromium, and zinc.

These substances may derive from contaminants in municipal solid waste, such as batteries, plastics, glass, ferrous material, paper, etc.

The concentrations of metals permitted in fertilizers such as composts are much higher than the natural concentrations in soils, even over 10 times higher, for example for mercury and cadmium, or at least 5 times higher for lead. [925] As a result, the composition of the soil changes dangerously over time. In some cases, the high phytotoxicity of some substances reduces bioaccumulation. For example, nickel is usually phytotoxic at concentrations below those dangerous to human health, so the route of bioaccumulation in the plant food chain does not seem to be important. Metals such as copper and zinc may also have some phytotoxic effects.

Risks such as those posed by the systematic distribution of contaminants into the environment and the food chain at concentrations higher than those naturally present can easily be foreseen. The quantities of some contaminants released in composts are far higher than those that could result from, for example, contaminated air alone. Therefore, the hazardous substances present in composts can lead to the risk of bioconcentration in the food chain, as well as environmental problems (e.g. phytotoxic effects, water contamination). Some of them, such as zinc, may have negative effects on the soil's microbiological activity and fertility. [926]

The concentrations of some contaminants in the compost (e.g. heavy metals or particulate matter) will be higher than in the waste from which they are derived, due to the decrease in mass generated by water loss and, in some cases, due to the formation of new substances.

All types of compost, and especially those derived from municipal solid waste or sludge, contain dangerous substances such as metals (e.g. cadmium, chromium, mercury, nickel, lead, copper, and zinc) at concentrations higher than those naturally present in the soil and, therefore, the composition of the soil will be modified. The compost obtained from sludge from wastewater treatment always has a higher metal content than a compost obtained from mechanical treatment of undifferentiated waste, and a compost obtained from separate refuse collection.

Polycyclic aromatic hydrocarbons (PAHs) may be found in composts at concentrations between 1,500 µg/kg (organic household waste) and 12,000 µg/kg dry weight (green raw material from rural areas; analyses carried out in Germany). Some scientific papers find PAHs in 100% of compost samples. Composts derived from municipal wastewater or organic material from heavily urbanized areas contains higher concentrations of PAHs. By examining the profile of polycyclic aromatic compounds, it is possible to know whether the organic material has been polluted by the combustion of petroleum derivates, fossil carbon or oils, and to trace the place of contamination (e.g. industrial or urban area).

In general, PAHs are found at higher concentrations in leaves and green waste: up to 6 times more concentrated (maximum concentrations) than in organic kitchen or household waste. The higher concentrations found in green waste and leaves can be partly explained by the phenomenon of bioaccumulation from the air.

Arable soils may have average PAH concentrations of 200 µg/kg dry matter, and urban soils of 1,100 µg/kg dry matter. Composts derived from raw materials collected in urban areas has an average PAH concentration of 2,700 µg/kg of dry matter.

Compost is used in large quantities, between 8 and 300 tons of dry weight/ha/year, resulting in more organic pollutants being delivered to the soil than from aerial deposition or other fertilizers, such as livestock manure. Animal manure also has quantities of contaminants, such as metals, in concentrations that may be higher than those regulated for composts, and than natural soil concentrations.

In summary, it can be stated that the concentrations of PAHs detectable in the various types of compost (at a concentration of 1,800 µg/kg of dry matter), and in particular in that derived from the organic fraction of municipal solid waste, are higher than those naturally present in the soil, by a factor of even 10. Polychlorinated biphenyls (PCBs) during the composting phase double their concentration compared to the initial one, due to the reduction in mass (a very small fraction degrades or volatilizes). The concentration of PCBs may also be higher by a factor of 15 than the natural concentration in arable soil. The same applies to dioxins (polychlorinated dibenzodioxins and polychlorinated dibenzofurans).

In composts, some pesticides of the organochlorine category are found up to concentrations of:

- 15 µg/kg dry matter, for aldrin;
- 850 µg/kg dry matter for DDT, Dichloro-Diphenyl-Trichloro-ethane molecules (in green waste compost);
- 32 µg/kg dry matter for thiabendazole;
- 76 µg/kg dry matter for cyfluthrin.

Thus, even for these molecules, composts may contribute higher concentrations of pesticides than those present in the soil.

In conclusion, unwanted molecules are often brought by the compost to the soil with concentrations several times higher than those present as a result of other anthropogenic contaminations. Therefore, it would be appropriate to manage and regulate fertilization plans based not only on a few parameters such as the amount of nitrogen or a few other nutrients such as phosphorus and potassium. The risk of negative and, in some cases, irreversible changes to soil characteristics should be prevented.

In the organic fraction of municipal solid waste some contaminants such as metals, glass or plastic are an unavoidable contamination: the lowest contamination that can be achieved with the best separate collection (estimated in Italy) is about 3% of metals, plastic, and glass.

The negative change in soil characteristics will increase over the years due to accumulation. Estimates for the situation in Piedmont suggest that after 10 years of using composts derived from sorted waste, a quantity of 200 g of plastic, iron, and glass per square metre is likely to be distributed, or 1,500 g if the compost is derived from undifferentiated waste. Even composts that meet the most restrictive criteria will contain high amounts of potentially negative materials.

Other risks associated with composting activities are as follows:

- spread of leachate generated by stormwater;
- attraction of animals such as birds, rodents, and insects;
- spread of weeds;
- spread of pathogenic organisms in agriculture (e.g. the phylloxera insect, *Daktulospharia vitifolia*, can be spread in viticulture as a result of the use of contaminated composts, if used in sites close to vineyards; some plant viruses, such as the tobacco mosaic virus, can also be spread by composts).

The logical conclusion of what has been said up to this point is that compost from undifferentiated waste or organic material that contains hazardous contaminants, such as sewage sludge, should not be used for agricultural purposes. Moreover, in many areas of Italy, such as the Po Valley, the availability of potential fertilizers is higher than the needs, so it is possible to choose. National and regional regulations provide for chemical, physical, and microbiological criteria of acceptability of a waste to be used to produce composts. The concentrations allowed in the raw materials that are used to produce composts from poor quality waste are too high, such as those established for sludge originating from the purification of sewage water. The acceptability criteria for producing a compost from sludge are much higher than those allowed for a compost from separately collected organic waste. Therefore, sludge should not be used in agriculture either directly or after a composting process. For sludge and unsorted waste, landfill is the least harmful disposal for the environment and the food chain compared to fertilization.

In order to be able to distribute the quantities of organic material coming from livestock farms, from the purification of sewage water (civil and industrial), from the management of urban and industrial waste, respecting only the criterion dictated by the quantities of nitrogen (a maximum of 340 kg of nitrogen per hectare per year), it would be necessary to have, in Northern Italy, at least another Po Valley (not considering other contributions such as that of synthetic chemical fertilizers). In some provinces with a high density of livestock farms it would be necessary to have three times the surface area currently available for agricultural use. Transport over long distances (more than 15 km for manure or compost) is not and will be less and less convenient, as costs will increase (e.g. fuels). Until now, externalities, i.e. environmental costs such as air, water and soil pollution, have not been properly taken into account. Therefore, in a context like the current one in Piedmont (and the Po Valley), i.e. an excess of organic material compared to the capacity of the agricultural system to recycle it in an eco-sustainable way, it could be convenient:

- Reduce the amount of organic waste by decreasing landless breeding farms.
- Increase energy recovery from manure, through anaerobic fermentation, especially on landless farms. This reduces the input of excess organic matter into the environment.
- Increase energy recovery by installing biogas plants in wastewater treatment plants, and in plants treating municipal and industrial waste.
- Decrease the quantities of low-quality organic matrices for agricultural use, such as sewage sludge or compost from undifferentiated waste collection, which could be destined for landfills.

The distribution in agriculture of an excess of organic and inorganic substances is one of the most efficient systems imaginable for polluting the environment and contaminating the food chain.

THE ECOLOGICAL FUNCTIONS OF THE SOIL ARE UNDERESTIMATED

Soil is abused, degraded and, at the same time, we still do not know most of the life forms that inhabit it: these contribute to the balances essential for plant growth and our survival. Soil biomass is largely made up of living beings; the countless organisms in the soil are the source of its fertility. One hectare of fertile agricultural soil may contain 100 t of organic matter, a biomass of invertebrates and microorganisms of 10,000 kg, and contain at least 4,000 different species of microorganisms such as bacteria. [425] A very fertile soil may probably contain more than 20,000 species of bacteria with a biomass of more than 3,000 kg/ha. In one cubic metre of soil, 50,000 insects and mites, and 12 million annelids can be found. A single gram of soil may contain billions of bacteria, hundreds of thousands of fungi, tens of thousands of protozoa and algae. This biodiversity useful to agriculture is seriously threatened by modern methods that use strategies that are not very conservative and sustainable. Monoculture of genetically identical individuals, soil tillage and fertilization, and the elimination of wild species lead to an irreversible reduction of biodiversity.

Plant roots are part of a very complex soil ecosystem in which microorganisms and plants exchange substances and favours. The roots can secrete large quantities of carbohydrates and proteins to attract micro-organisms. In turn, the microorganisms produce compounds that are useful to the plants (e.g. substances with a hormonal action). The organic matter in the soil is therefore made up of living beings, decomposing plant and animal residues, molecules synthesized mainly by micro-organisms and produced in the soil by chemical reactions. The greater the amount of organic matter in the soil, the better its fertility.

The interactions between different living things in and out of the soil are largely unknown. Among nature's extraordinary curiosities, there are some species of cicadas found in the USA. Their juvenile stages spend years underground, feeding on tree roots. The adult forms emerge for mating every 13 to 17 years. It seems that some species of cicada synchronize their life cycles to prime numbers, those numbers divisible only by 1 and themselves. [848] Another peculiarity of some species of these insects is that the males can produce very intense sounds (up to 120 decibels).

The soil performs several functions, some of which are obvious and others are underestimated: besides being the place where an enormous biodiversity lives, necessary for fertility and not only (e.g. it hosts some pollinators and some parasites of plant enemies), it guarantees food production, provides oxygen, stores carbon dioxide, holds and cleans water, can protect us from extreme hydrogeological phenomena. The cultural, aesthetic, recreational, and educational functions, which are fundamental for our well-being, are neither negligible. The wealth of vital functions provided by the soil, however, is not adequately realized and evaluated, especially if we look at the choices of the government of the territory or if we pay attention and observe the state of widespread degradation. Soil is one of the main carbon dioxide reservoirs in the biosphere, much higher than the atmosphere: probably the first centimetres of soil contain a quantity of carbon at least 3.3 times higher than that in the atmosphere and at least twice that present in plants and in the atmosphere together. [8] In this respect it is useful to remember that a forested soil can store between 70 and 100 t/ha of carbon dioxide, an agricultural soil half that amount and a sealed soil zero (covered by concrete). Probably 60% of the carbon that was present in soils since 1800 has been lost due to human activities. [8] These are ecological values to which we are blind, they are difficult to monetize, and we easily forget that they are the indispensable services to sustain future generations.

Industrial agriculture treats the soil as if it were an inert substrate and can afford to do so because it invests much more energy than it can obtain. By exploiting fossil energies, we can temporarily delude ourselves that we do not have to take care (it would be more intelligent to say worry) about soil fertility. When so much energy is available, it is possible to cultivate even the desert. This approach generates irreversible and therefore unsustainable changes. We should not allow it, the soil is necessary for the survival of everyone and of the next generations, no one should have the freedom or the right to destroy it at will, as happens today with impunity. We should be frightened of the current degradation and we should rush to curb these individual freedoms.

Few are horrified by the existence, in Italy, of thousands of different urban plans, managed autonomously by local administrators and which in fact allow the soil to be managed in complete freedom. In Northern Italy about 50% of the soils of the plains have a very low content of organic substance, less than 2%. With such a low concentration of organic substance a soil is considered at risk of desertification and has compromised its ability to retain and degrade pesticides, which will therefore be more likely to end up contaminating water.

We have let slip the notion that the destruction or taking away of an environmental resource from future generations does not come at a price. Putting a value on this legacy is difficult, but it is certainly much higher than that of the market for soils in agriculture or the construction industry.

The different types of land use, even in the agricultural sector, are not equivalent and should be chosen with foresight and care. To produce one kilogram of beef requires at least 18 square metres of soil per year (and releases at least 0.5 kilos of methane), but to produce one kilogram of wheat requires less than 2 square metres of soil per year.

In order to determine what can be produced from the soil, account should be taken of the quantity of calories and nutrients obtainable per unit area, the ratio between energy invested and energy obtained, water requirements (per caloric unit), the quantity of carbon dioxide emitted (per caloric unit), the impact on ocean acidification, water quality, and the health of farmers and consumers.

Considering only the rules of the market or those established by a few economically very strong and politically influential subjects is a blindness that we can no longer tolerate. The needs of local communities and the health of the Planet must become the decisive elements on which to plan the agricultural system. Agriculture in Europe occupies 40% of the total surface area and directly employs at least 22 million people: an equivalent number of workers are employed in the industrial processing sector and in related services. [871] Of this 40%, 18% is occupied by grassland and 46% is not irrigated. In 2016, less than 7% of the European agricultural area was occupied by organic farming: overall an area approximately as large as the agricultural area used in Italy: 11.9 million hectares. In Europe there has been a reduction in the agricultural area for over 20 years: between 2000 and 2017 by 5% (about 80,000 ha per year). Between 2010 and 2015 the reduction in the agricultural area was about 0.3% per year. [871]

Leaving control to the freedom of action of a few entrepreneurs and to stupid economic rules has led to counterproductive concentrations: in Europe, 3% of farms control more than 50% of the surface area. These farms have an average size of more than 100 hectares, while 65% of farms have an area of less than 5 hectares; farms with more than 50 hectares account for 68% of the total European agricultural area. [871] Theoretically it should be easier to spread a more ecological model of agriculture if there are few stakeholders, but in fact the opposite happens.

The value of land should no longer be calculated solely on the basis of what can be cultivated or built on it. It should be assigned an ecological value (protection of biodiversity, storage of carbon dioxide, clean water), a value for the community (recreational, reduction of temperature, reduction of noise and air pollution) and another for future generations. These categories of values are ignored today, but if we were farsighted, more intelligent and less selfish we would discover that they are at least orders of magnitude higher than the price assigned by markets managed to satisfy the fleeting and material interests of private individuals. Soil is one of the most precious goods for humanity, it is formed in a very long time, as a result of complex bio-geochemical processes, but to devastate it a few minutes of agricultural work or the time necessary for a sale-purchase aimed at urbanization are enough.

FERTILITY AND FOOD SAFETY ARE CLOSELY LINKED: ZERO LAND CONSUMPTION IS NEEDED

Most of the Planet's biomass is found within the oceans but over 99.7% of human food comes from the terrestrial environment and less than 0.3% from aquatic ecosystems. [14, 263] Humans together with farmed animals make up at least a quarter of all the terrestrial animal biomass. [114]

Soil damaging practices (but not limited to it) include ploughing, fertilization (e.g. distribution of sludge, poor quality compost, and chemical fertilizers), distribution of poisons such as pesticides, pollution (e.g. metals deposited from atmosphere and waste), monoculture, irrigation, leaving soil uncultivated and unvegetated, not returning the organic matter taken up, employing techniques using plastic (mulching, irrigation). These practices, in addition to degrading the soil, also promote erosion and lead to a loss of fertility and eventually to desertification. The rate of erosion may be much faster than the rate of regeneration, so it becomes an irreversible degradation process. [97] It is estimated that at least 10 million ha of agricultural land are abandoned each year due to the loss of productivity caused by erosion. [425]

Erosion driven by water and wind moves huge amounts of material. If erosion occurs at a rate of 2 t/ha per year on 20 ha, during 50 years, enough sediment will be transported to form an area 10,000 m^2 wide and 15 cm deep.

In nature, a forested soil usually experiences erosion of less than 0.05 t/ha/year. It may be considered that erosion of more than 1 t/ha/year is not sustainable as it is greater than the natural regeneration capacity. In general, the erosion of agricultural soils is 500 to 1,000 times faster than that of forested soils. [91] Over the years, the soil loses its capacity to support plants, unless artificial inputs are made by man: fertilizers, pesticides, irrigation, etc. In Europe the agricultural surface amounts to 47% of the total surface and it is estimated that the average erosion rate is about 3.2 t/ha per year; in Italy at least one third of the surface is subject to intense erosion and at least 27% of the territory is affected by drought and desertification. Crops with high levels of erosion include vineyards, olive groves, and orchards.

The water used for irrigation contains salts. If you distribute 10 million litres per hectare per year, for example in the case of maize, this means that up to 5 t/ha of salts may be released into the soil. [425] Every year at least 10 million hectares of farmland are rendered unusable for agriculture due to salinization and approximately 50% of all irrigated soils suffer from increased salt concentrations. [425] If the predicted sea level rise occurs, the concentration of sea salt in water and soils will increase, creating problems for agriculture and drinking water supply.

The rate of soil loss is very rapid. The fertile layer is normally only a few centimetres thick (between 30 and 80-90 cm) and plowing may generate the loss of one millimetre per year. [761] In the past, the loss of soil (thousands of years ago) was a hundred times less: 1 millimetre every 100 years. Chemical fertilization and cereal cultivation reduce the amount of carbon (a fertility indicator) in the soil between 0.5% and 1% each year. So the rate of loss of fertile soil is much greater than it would be in the absence of human intervention.

Fertile soil retains water more efficiently, a precious commodity that is bound to become increasingly scarce and polluted. Increasing soil organic matter by 1% to 3-4% not only improves fertility but also doubles the water retention capacity.

A very simple basic principle is as follows: in nature, soil is never ploughed or tilled and is almost always covered by a bed of decaying plant matter. Soil movement and the absence of

plant cover cause serious damage to the fertility necessary for the survival of the Earth's biosphere. Tillage also harms pollinating insects. It should be remembered that probably 70% of bee species build their nests in the soil. [362] Therefore, ploughing destroys the possibility of survival of a very complex ecosystem, largely unknown but necessary to maintain fertility.
Soil fertility is very important, as a lack of plant nutrients may reduce or cancel out the benefits to pollinators. It is known that pollinators (*Apis mellifera* and *Bombus terrestris*) increase the amount of seeds produced by sunflower plants only if good fertility conditions exist. [476] Good fertility conditions and pollination are necessary to record an increase of between 18.5% and 19.8% in seed production in sunflower plants. [476] It must be remembered that sunflower plants can generate an acceptable seed production even in the absence of entomophilous pollination. The obvious conclusion of some scientific studies is that the reduction in soil fertility may cancel the benefits provided by pollinators, i.e. seed production does not increase in the presence of insects if nutrients are deficient (this result was expected but has been confirmed). Soil management must therefore ensure that fertility is maintained and increased.
Creating soil in nature means having to wait for centuries, and artificially it is only possible if great amounts of organic matter and energy are available, so regeneration of infertile soil is only feasible on small areas.
We use at least one-third of the land area free of ice to grow feed and livestock, and in Europe that is 40% of the total land area. We have already degraded at least a third of the soil used for agricultural purposes worldwide. One third is a lot. It means seriously compromising our ability to produce food, altering the capacity of the biosphere to support biodiversity and to provide services essential for our survival. Soil participates in the process of water purification, climate regulation, carbon sequestration and many other functions other than enabling food production. We are destroying this thin layer on which we are inextricably dependent. Chemical fertilization cannot restore soil fertility because it only adds a few substances.
On a planetary level (a 2015 estimate) soil degradation reduces the capacity to produce food by almost 0.5% per year. In Europe, agricultural land is reduced by 0.3% per year. [871] American soils have already lost 50% of their fertility. [761]
Currently 19% of the Earth's surface is arid and about 10% is covered by ice, so it is not arable. Less than 69% of the Earth's surface may be occupied by plants: 26% of the Earth's surface is covered by forests, and 34% is agricultural land of which 23% is occupied by grasslands. The availability of fertile soil *per capita* is constantly decreasing due to various factors (e.g. urbanization, desertification, industrial agriculture, deforestation, erosion, salinization) but population increases and degradation grows faster than the population increase: between the mid-1950s and the end of the century, the increase in urbanized areas was at least 2.5 times higher than the population increase (78% vs. 33%). [773] In 2050 the World population could be between 9 and 10 billion and, according to some forecasts, food production will have to increase between 60% and 110% in order to sustain nutritional needs. [851] How can we hope to double World food production before 2050? Alarming indicators point to the opposite phenomenon:

- Reduced fertility.
- Decrease in the area occupied by agriculture.
- Increased soil sealing and urbanization.
- Reduction in productivity due to climate change (drought, hail, torrential rains, heat waves; the latter in Russia, in 2010, led to the loss of 30% of wheat and the consequent ban on exports with negative effects in other countries). [871]
- Reduced water availability for irrigation while, at the same time, the demand for irrigation is expected to increase due to a decreased rainfall, especially when needed for agriculture.

- Reduction in services provided by nature such as those provided by biodiversity, e.g. pollination by animals and biological pest control.
- Increased pollution and salinity of soils and waters.
- Increased desertification and fires.
- Increased losses due to pests caused by, for example, milder winters. Some pests are favoured by the climate change, such as those in olive trees, and an increased risk of mycotoxins in cereals is expected, which are very dangerous to human health (some are carcinogenic).
- Reduction in the nutritional value of crops such as a decreasing sugar content in grapes or protein content in grains due to changes in climate. The death of pollinators will increase deficiencies of certain vitamins.

In Europe, the climate change alone could reduce food production by more than 15% before 2050. [871] So we need to start taking action before it is too late. We need to achieve zero soil consumption and increase fertility and biodiversity as soon as possible.

CONTAMINATION OF SOIL AND WATER BY NEONICOTINOIDS

Most of the pesticides distributed with seeds, such as neonicotinoids and fipronil, are not absorbed: the percentage of imidacloprid absorbed varies between 1.6% and 4.5% for cotton, potato or rice plants and, in the case of maize, may reach 20%. [356] Some active molecules are very soluble, so they will easily contaminate water (e.g. acetamiprid and thiamethoxam). [359]
Most of the active ingredients that have been distributed with the seeds remains in the soil: as much as 98%. Plants that are sown the following year (or wild plants) will absorb these active molecules. It must be remembered that many insects (e.g. wasps and ants) can build their nests or spend a phase of their lives in the soil.
The molecules of the active ingredients and/or their derivatives (metabolites) remain in the soil and contaminate water (they are used in rice farming), as the active molecules are persistent. The half-life of neonicotinoids in the soil may be over 1,000 days. [359] This means that 10 grams are transformed into 5 grams in more than 1,000 days and, to find less than 2.5 grams (a quarter of the initial dose), it is necessary to wait at least 5 years. In the meantime, however, equally dangerous derivatives (metabolites) may be formed.
Once the soil is contaminated, wild plants growing in and around the treated areas, or subsequent to cultivation, will contain neonicotinoids or other pesticides. In fields where maize had been cultivated, with seeds impregnated with clothianidin, researchers found this molecule in wild plants (dandelions or *Taraxacum*) at a concentration of 9 ng/g after maize cultivation; clothianidin takes up to 7,000 days, i.e. more than 19 years, to halve its concentration in the soil, and it must be remembered that it is also a metabolite of thiamethoxam. [359]
In general, pesticides can remain in the soil for a very long time. Imidacloprid persists and may accumulate in the soil making the plants that will grow there in subsequent years toxic and harming non-target species such as soil-dwelling organisms that decompose plants. Clothianidin (a neonicotinoid insecticide) may have a variable half-life in the soil, ranging from 148 to 1,155 days. [368] The search for neonicotinoids in 74 French soil samples, 7 of which came from organic farming where the use of pesticides is prohibited, shows that imidacloprid is not found in these 7 samples. [363] Unfortunately, 62 soil samples were contaminated: in 65% of these samples imidacloprid was found in concentrations higher than 1 µg/kg. Only 10% of the soil samples containing imidacloprid were treated with this pesticide in the year of the survey. 91% of the samples contained the neonicotinoid but only 15% had been sown with seeds treated with this insecticide. The contamination is due to treatments that took place long before, and

confirms the persistence of this molecule. In 7 of the 11 soil samples, which had not received imidacloprid in the previous two years, it was found at a concentration between 0.1 and 1.5 µg/kg (in this case a half-life of imidacloprid of up to 1,230 days, i.e. more than 3 years, is estimated). [359, 363] In 9 of the 10 soil samples, where maize, wheat or barley had certainly been sown with seeds containing imidacloprid, concentrations between 2 and 22 µg/kg were recorded (average concentration of 12 µg/kg; one sample recorded less than 1 µg/kg). If sampling is carried out shortly after sowing, much higher concentrations can be recorded: hundreds of millionths of a gram in each kilogram.
In another survey, the presence of imidacloprid was investigated in soils that had been treated the same year or two years earlier. Of these 33 samples, 97% showed the presence of the insecticide and 78% measured a concentration between 1.2 and 22 µg/kg. In soils that had received imidacloprid the year before, an average concentration of 6 µg/kg was detected.
Neonicotinoids applied on seeds enter the plant only in a small part: between 1.6% and 20%. [434] Between 80% and 98.4% of the molecules are dispersed in the soil and the environment, they may also contaminate water.
The results of this research show that the molecule remains in the soil for years, can accumulate and move around the biosphere. Moreover, the concentrations found are not negligible as they are higher than those that cause chronic effects to bees.

Neonicotinoids also contaminate water: in the Netherlands, imidacloprid is one of the three substances most easily found above the eco-toxicological limit (13 billionths of a gram per litre) and, in surface water, it is found at concentrations 25,000 times higher than this safety concentration. [359]
In 2010 and 2011, 89% of 75 California surface water samples contained imidacloprid, and 19% of the samples exceeded EPA's proposed reference concentration (1.05 millionths of a gram in a litre). [359] Imidacloprid was also found in California groundwater at a concentration of 1 µg/L.
Waters in Canada are contaminated with imidacloprid and/or clothianidin and/or thiamethoxam and/or acetamiprid: in 2012 and 2013 between 16% and 91% of 440 samples were contaminated, depending on the area. In water, clothianidin was the most frequently found molecule and the highest concentrations were as follows: 54.4 ng/L for acetamiprid after planting treated rapeseed (average concentration of 1.1 ng/L); 256 ng/L for imidacloprid, in 2012, in wheat fields with treated seeds (average concentration of 15.9 ng/L); 1,490 ng/L for thiamethoxam in fields sown with treated rapeseed (mean concentration of 40.3 ng/L); 3,110 ng/L for clothianidin after planting treated rapeseed (mean concentration of 142 ng/L).
In Quebec, imidacloprid and three of its metabolites are found in 35% of well water samples.
In New York State, imidacloprid concentrations in water ranged from 0.2 to 7 µg/L.
In Spain, imidacloprid was found in 58% of water samples in 2010 and in 17% of water samples in 2011 at concentrations between 2.34 and 19.2 ng/L.
In Sweden, imidacloprid was found in 36% of the water sampling points, including concentrations above the limit of 13 ng/L: in 21 samples (the highest concentration recorded was 15,000 ng/L, i.e. 1,154 times higher than the regulated safety threshold). Acetamiprid was also found at maximum concentrations four times higher than the safety concentration: it was found at concentrations of up to 410 ng/L, with a limit value of 100 ng/L. [359]

Neonicotinoids widely contaminate waters such as surface waters. Imidacloprid is among the most frequently found molecules. [1173] In some cases, problems with the aquatic fauna such as crustaceans and insects have been confirmed (e.g. in Sweden and in Maryland, USA). Aquatic macro-invertebrates and insectivorous birds may be harmed by very low concentrations of neonicotinoids in surface waters (a few billionths of a gram per litre). [1173]
The risk analyses carried out by the various countries are very different and as a result tolerance threshold for eco-toxicological risks are set at different orders of magnitude. Unfortunately, the

maximum allowable concentrations set by regulations do not adequately account for chronic effects on aquatic invertebrates and subsequent levels of the food chain. In spite of the limits of the regulations, some checks record water concentrations far above the threshold values established by the regulations (e.g. in the Netherlands, Poland, Belgium, Italy, California, and the USA). [1173] For example, in California, imidacloprid concentrations over 1,050 ng/L have been recorded in surface waters contaminated by irrigation, while in Maryland (USA) concentrations up to 27,000 ng/L have been recorded and in the Netherlands up to 320,000 ng/L. These concentrations are over 10,000 times higher than those capable of harming aquatic and other wildlife. Even in groundwater throughout the Planet alarming concentrations of neonicotinoids have been recorded (e.g. Quebec, USA): concentrations of over 400,000 ng/L have been recorded in groundwater in the New York State. [1173]

A lot of studies show that pesticides in the environment alter nutrient cycling, organic matter decomposition, and respiration in the soil. Both soil invertebrates and aquatic organisms are harmed by very small doses of insecticides (e.g. neonicotinoids and fipronil). [415] Pesticides can seriously undermine the ability of ecosystems to perform functions essential to our survival, such as ensuring a safe and steady supply of food. Pesticides damage the soil biodiversity (ants, termites, bees, worms, bacteria, protozoa, fungi) even at very low concentrations. Some insecticides (e.g. imidacloprid) reduce the ability of soil microorganisms to decompose organic matter (e.g. leaves). Nitrogen-fixing bacteria (e.g. *Rhizobium* in symbiosis with pea plant roots) also reduce the ability to sequester atmospheric nitrogen due to the presence of pesticides (e.g. imidacloprid). [415] Acetamiprid decreases the respiration capacity of the soil. Therefore pesticides such as insecticides can generate serious damage to the soil ecosystem at very low concentrations.

ALTERATION OF THE BIO-GEOCHEMICAL CYCLES OF NITROGEN AND PHOSPHORUS

At the current rate of growth in demand for food, it may be expected that at least another 1 billion hectares will need to be farmed in 2050 and removed from natural ecosystems (an area comparable to the USA). [604] This increase in land consumption is likely to be accompanied by an increase in the use of nitrogen and phosphorus fertilizers: between 2.4 and 2.7 times higher than the end of the last century. The nitrogenous fertilizers produced in 2000 were about 87 million tons, the forecast for 2050 is 237 million tons. For more than 20 years, mankind has been releasing more nitrogen and phosphorous-based compounds into the environment each year than the ecosystems put into circulation naturally. This irreversible alteration of ecosystems is unprecedented in the history of mankind and will therefore be accompanied by a simplification of habitats, mass extinctions, and eutrophication.

We have reached a worrying level of impact: human activities introduce more nitrogen into ecosystems than all natural sources, due mainly to nitrogen-based fertilizer chemistry. Chemical nitrogen-based fertilizers have the characteristic of being more readily available and more easily assimilated by plant roots than organic forms (e.g. manure). We can state that at least 50% of the World's population is fed with industrial nitrogen. [846]

Phosphorus is derived from mineral deposits and is therefore a non-renewable resource. This mineral will probably be still available cheaply for less than 25 years. [837]

This knowledge points to the existence of several problems:

- We introduce large quantities of nitrogen and phosphorus compounds into the environment in very mobile forms, so they contaminate the water. The resulting negative phenomena include eutrophication of lakes, rivers and seas, and nitrate pollution of the drinking water.

- Alteration of the natural flows of the biosphere.
- Alteration of the soil ecosystem.
- Large amounts of fossil energy are consumed to synthesize nitrogen-based fertilizers and to extract phosphorus from underground. These resources are available in finite quantities: nitrogen depends on the availability of fossil fuels, while phosphorus underground has already been mostly extracted from the few mines in China, USA, and Morocco.

In natural systems, phosphorus and nitrogen are recycled in bio-geochemical cycles, so they are not wasted and do not need to be added. We consume these minerals in the form of food and feed and do not return them to the soil, but accumulate them in landfills or end them up in surface water (sewage). We need to improve the reuse of organic waste such as food waste and animal dung including human's one. A challenge will be to return food waste and manure to the soil, economically and safely. A paradox of our society is that between 30% and 50% of food ends up in the trash when it would still be edible.

Dangerous microorganisms and pollutants must be avoided by recycling organic matter such as that from municipal solid waste (compost) and sewage systems (e.g. plastics, detergents, medicines, solvents, dyes, etc.). [741]

Continuing to extract substances from the soil (and from the atmosphere such as nitrogen) without recycling them means compromising the future generations: in the USA, in 2010, less than 3% of the food waste was returned to the soil. [837] To reduce catastrophic effects, before the middle of this century, phosphorus inputs to the oceans should be halved and inputs to rivers should be reduced by 70%; nitrogen inputs, overall, should be reduced by 70%. [851] We know that we have to reduce the artificial input of nutrients used in agriculture by 50% or even 70%, but food production is expected to double by 2050. Respecting planetary limits and hoping to increase food production will be impossible. If we continue to ignore the natural cycles and waste resources, the collapse of the food supply system will be inevitable.

PREVENTION OF SOIL DEGRADATION

There are many ways to protect the fertility and health of the soil: [761]

- Do not move the soil, i.e. disturb the soil as little as possible: ploughing should be avoided. Moving the soil permanently destroys the life of insects useful to farmers, such as many pollinator bees which carry out part of their life cycle in the soil. A great many seeds are stored in the soil and can remain in a dormant state for years. Ploughing is one of those practices that encourages the destruction of this biodiversity. Soil is a carbon sink because it contains much more carbon than the atmosphere, animals, and plants combined (in the first three metres of depth). Ploughing is one of those practices that reduces the amount of carbon stored in the soil. Increasing soil fertility is equivalent to increasing the amount of carbon in the soil (climate change is countered). Reducing or eliminating tillage alone (e.g. ploughing) may generate an increase in the concentration of organic carbon and nitrogen compounds (and thus in the fertility of the top 20 cm) between 7% and 17% over 8 years. [52, 80]

- Soil must always remain covered by vegetation: the benefits are countless. Microbial biodiversity increases, the water content of the soil increases, the maximum soil temperature may be reduced by many degrees: even more than 20°C between a forested soil and one exposed to the sun. Soil temperatures above 33°C damage life forms in the rhizosphere (the part of the soil around the roots). Erosion by water and wind is slowed down considerably by the presence of a plant cover, which has another benefit too: it reduces the spread of weeds or unwanted species and, therefore, reduces the need to use herbicides.

- Plant species must be differentiated both in time (rotation) and in space (polyculture or intercropping): cultivating at least three crops at the same time (diversification). Rotation and intercropping increase biodiversity in the soil and in the biosphere (insects and birds), and make life more difficult for parasites; for the farmer it means running much fewer risks: if one crop goes wrong, the others are there. Monoculture does not exist in nature and is an unstable and vulnerable artificial system. One of the principles of the rotation of annual plants is that the same species must not be cultivated for an interval of at least two years (better if the alternation lasts longer). The use of leguminous plants in rotation and intercropping increases nitrogen content and reduces the need for fertilizers. Corn-soybean rotation decreases nitrogen requirements by more than 50 kg/ha per year and increases corn yield by as much as 10%. Alternating cultivation with legumes (e.g. cereals in summer and legumes in winter) may generate an increase in production equivalent to that produced with 15-200 kg of nitrogen per hectare per year, depending on the plants used and the cultivation conditions. [52] Growing leguminous crops in winter has the advantage of protecting the soil from erosion (the loss of up to 18 t/ha of soil per year is avoided).

- Use organic fertilizers instead of synthetic chemical fertilizers. In this regard, in the USA only 5% of the crops receive organic substances in the form of manure. Surveys conducted in nine US states, which produce 80% of corn (*Zea mays*) and soybean (*Glycine max*) in rotation, found that only 18% of corn crops and only 6% of soybean crops received manure as fertilizer instead of chemical fertilizers. [56] Spreading manure such as dung with immediate deep burial (45 cm) in addition to increasing the amount of available nitrogen in the soil, which can be measured even after 30 months, may be considered a technique to save energy and reduce water contamination. [94]
It is necessary to return the substances that are taken with the harvests, therefore the greater the extractions, the more easily the soil degrades, as it is unable to regenerate fertility. If the extracted substances (organic and inorganic) are not returned, the ecological balances of the rhizosphere will be altered and the soil will not be able to ensure the growth of healthy plants. Over the years, the soil dries out and is transformed into a substrate that is poor in life and poor of organic substances.

- Do not distribute organic substances rich in pollutants, such as sludge from wastewater treatment or poor quality compost. [741]

- Implement soil and plant chemistry analyses to better manage nutrient needs.

- Reduce the time between fertilization and when the plants need the nutrients.

- Bury non-food residues instead of using them to produce energy (e.g. straw). Organic material that is not used or is a waste should be recycled back into the soil to avoid the need for external inputs.

- Do not use pesticides.

- Avoid contamination by plastics that can alter the soil through direct and intentional contamination such as residues from mulching, irrigation systems, materials used to cover crops or to facilitate harvesting (tarpaulins, nets).

- Reduce soil compression and compaction generated by the pressure of mechanical equipment (tractors, trailers, harvesters, etc.).

- Dedicate a fraction of the land to natural vegetation. All forms of disturbance such as ploughing, mowing, and distribution of pesticides should be avoided in this part of the plots. Maintain buffer zones such as distances from watercourses (at least 20 m).

- Reduce the need for irrigation: use systems that save water and avoid erosion. In this regard it must be highlighted that the availability of irrigation water is decreasing due to several factors such as climate change, in many areas of the World like Italy.

- Lengthen the time between fertilizing and the time of irrigation or rainfall to reduce nitrogen loss to water.

Adopting at least the three basic principles of soil-conscious agriculture (no plowing, keeping the soil covered with plants, increasing the number of crop species through rotation and intercropping) could result in a significant increase in carbon dioxide sequestered in the soil. If these practices were theoretically adopted in all the cultivated areas of the Planet, it would probably be possible to sequester between 5% and 15% of the carbon dioxide emitted by the combustion of fossil fuels: in this way we could partially compensate the emissions generated by the various agricultural activities. [761]

Lastly, it is important to highlight another aspect: agricultural subsidies should only be paid out in exchange for guarantees on the implementation of practices that protect soil health. European agriculture benefits from huge subsidies, amounting to around a third of the estimated 180 billion of euros in 2018: in 2015, at least 50 billion euros were paid out, helping around 7.5 million farms. According to some estimates, subsidies to European agriculture and livestock, in the period 2014-2020, amounted to at least €480 billion. [871, 985] Public support for agriculture, which is dominated by chemical monoculture farming, is a form of direct encouragement of unsustainable practices such as pesticide use. Mono-successional monocultures cannot survive without pesticides and vice versa.

The economic support of agricultural production with public resources without asking for guarantees on the adoption of sustainable practices in return, participates, in a non-obvious way, in the dominant strategy of the entrepreneurial sectors: socializing the costs and privatizing the gains.

It is much worse when subsidies finance predatory and uneconomic practices such as public incentives for the production of agrofuels (methane, ethanol, biodiesel) that require the occupation of land with corn, wheat, sugar beet, and rape. [741] Europe had planned to dedicate 7% of the European agricultural surface to the production of agro-fuels by 2020. [741, 871] Monocultures and energy generation systems passed off as useful, because they are classified as renewable, when instead they consume more fossil energy than that obtained in agro-fuels and alter the soil and water. This waste should be vigorously opposed instead of being sponsored by substantial public resources.

Another huge waste is that of subsidies to fossil fuels (about 6% of the World GDP) and activities that damage the environment. Remember that farmers pay for diesel fuel a price below the market price. This is another incentive to waste non-renewable energy.

An examination of the principles underlying soil-healthy agriculture shows that we need to do the exact opposite of what industrial and chemical agriculture usually does. Soil should not be treated as a private, unrestricted commodity. Defending soil health and fertility should be considered a priority for the public good and, therefore, should be protected by national strategies and targets.

WATER: A PRECIOUS ASSET TO SAFEGUARD

WATER, A UNIVERSAL RIGHT, NOT A COMMODITY

Two-thirds of the Planet is covered by water and our bodies are made up for the most part of this indispensable and increasingly precious substance (the human embryo contains 94% water, at birth the content drops to 77% and decreases over the course of life until it becomes about half of the body weight). [979] On a planetary level, 70% of water is used for nutrition, 22% to produce objects and materials and the remaining part for domestic use.

In countries with an advanced economy, such as Italy or the USA, at least 45% of the entire demand for water is used in the industrial sector; in Italy, in decreasing order, for chemical products, rubber and plastics, iron and steel and base metals, paper and paper products, and textiles. In Europe at least 52% of the water is used in the industry, 32% in agriculture and 15% for domestic use; in emerging countries up to 98% of the water withdrawn is used to satisfy agriculture. [979] It is estimated that at least one billion human beings do not have access to drinking water and at least twice that number lack basic sanitation (dysentery kills more children in the World than AIDS, malaria and tuberculosis combined).

The reduction in the availability of water, the pollution of this resource and the alteration of its cycle (e.g. due to the climate change) are some of the signs that warn us that the safe operating space for humanity has been exceeded. The use of water, on a planetary level, is unprecedented: on average more than 3,350 litres are used *per capita* per day. In Italy, the overall water consumption footprint is over 6,300 litres *per capita* per day (this estimate includes the use necessary to support imports). Italy is among the World's biggest importers of virtual water and most of the water footprint is attributable to food production. These consumption levels underscore that we are not in a safe operating space, as they are unsustainable.

On average a European citizen consumes 700 cubic meters of water per year, an American 1,280. Over time there has been a reduction in the availability of water both on a planetary level and in Italy: World availability *per capita* has decreased from 9,000 cubic metres in the ’90s to 5,921 cubic metres in 2018, and could drop below 5,000 cubic metres around 2025; in Italy its availability has decreased from 3,587 cubic metres *per capita* per year in 1962, to less than 3,000 in 2018 (in Italy there is an average *per capita* consumption of 6,400 L per day, corresponding roughly to 2,334 cubic metres per year; over 50% of this virtual water comes from abroad). [979] In Italy most of the available fresh water is withdrawn and it is important to underline that it is returned to the environment polluted. Faced with a reduction in availability, forecasts predict an increase in the demand for water over the next few decades, so it is destined to become increasingly precious. The main causes of this growth in demand is population growth and increased consumption, especially in emerging countries.

Between 2012 and 2017, at least 40% of the grains grown on the Planet were used to feed animals, and at the same time, meat production nearly doubled between 1980 and 2004. The average water footprint per calorie from beef is at least 20 times greater than from grains and other vegetables. With what water (and soil) resources will it be possible to sustain the doubling of meat production that some organizations hope to achieve by 2050? Even the forecasts of cereal consumption estimate an increase of at least 30% before 2050.

Other causes of the reduction in water availability are drought (the water evaporated from the soil and transpired by the plants is more abundant than that received with the rains) and climate change. The latter manifests itself more in the coldest and most inhospitable places on the Planet. In the last forty years, the melting of the polar ice caps has accelerated (by a factor of 4 in the 10 years from 2003 to 2013). One third of the European population lives less than 50 km from the coast so there is a possibility that rising ocean levels will generate unfavourable costs and conditions for many. Sea levels could rise between 4 and 6 metres before the end of the century; cities such as London, New York, Venice, Shanghai, Bangkok, Copenhagen and Amsterdam will be seriously damaged by a rise of just one metre. Italian Alpine glaciers have also lost more than a half of their surface area in a century. Before 2100 the Alps could be completely without glaciers.

It is easy to foresee an increase in disputes over the appropriation of available water. It could be the case that, before the middle of this century, more than 60% of the World's population will find itself in water shortage. In 2018, an urban centre with over 3.7 million inhabitants (Cape Town, South Africa) became infamous for being without water. Another striking example is the Aral Sea (it was a salt lake located between Uzbekistan and Kazakhstan) that in 1960 was the fourth largest lake in the World, today it is not even counted among the top 50: in 2007 the surface had been reduced to 10% of the original one and the salinity had exceeded 100 g/L, generating the death of most organisms (to have a comparison the marine salinity is about 35 g/L). One of the causes of the reduction of the Aral Sea was cotton irrigation: in less than 50 years the surface of the lake, which had been constant for over 11,000 years, was inexorably reduced with irreversible changes. [985]

Energy production also depends on water availability, and there have already been interruptions in electricity supply due to water shortages: for example, in 2012, 620 million people (10% of the World's population) were without electricity in India. [979] Worldwide, between 4% and 13% of electricity consumption is used to manage water (withdrawal, transport, purification).

Among the unsustainable solutions there is the increase in the consumption of bottled water (by 7% per year worldwide) which costs hundreds of times more than tap water (for bottling, companies pay fees that reach a maximum of a few thousandths of a euro per litre).

These few numbers easily suggest a relationship between food, water needs and potential crisis situations: most of the water used in a country, in the World, comes from a neighboring country or is imported; for example, more than 20% of the World's population resides in China but they have approximately 6% of the global fresh water. In 2018, at least 75 armed conflicts over water were recorded in the World; at least 263 were recorded between 2010 and 2018; these include disputes in the Nile River Basin, the Cauvery River Basin in India, the conflict over the Tigris and Euphrates, the conflict between Turkey and Armenia: some of these conflicts have lasted for decades. [979] In some cases the water shortage is not the trigger of the war but the water is taken from the enemy as it becomes a military objective. Before the middle of this century, two-thirds of the World's population could experience water shortages, which may be considered a powerful promoter of conflicts and wars. Fortunately, water shortages are often dealt with peacefully: between 1946 and 1999, 1,931 conflicts over water were recorded, of which 67% were dealt with peacefully, through cooperation. [988]

There is only one way out: reduce consumption and waste and, secondly, recycle and reuse.

THE WATER CYCLE

The circulation of water through plants and ecosystems represents the greatest chemical-mediated energy transfer in the biosphere. Plants are able to assimilate water through root uptake and transport it along the stem to the leaves from where, through stomata (the tiny pores on the leaf surface), it is returned to the atmosphere in the form of water vapour. The amount of water that plants can extract from the soil is considerable. A leaf can transpire an amount of water equal to about 0.2 litres per day, a tree about 200 litres per day and a hectare of forest over 10,000 L per day. [624] Water transport and subsequent evaporation are thermodynamic processes that require great amounts of energy: 0.6 kcal are required for the evaporation of 1 g of water. The terrestrial ecosystems may be considered gigantic and silent thermodynamic machines capable of transforming, day after day, relevant quantities of energy. The energy that moves all this is always the solar energy, which is absorbed by plants and used in various processes, including transpiration and photosynthesis.

Through plant transpiration, evaporation, and precipitation (as well as through ocean currents), water transfers most of the energy received from the Sun from tropical to polar regions, almost like a pipeline that transfers heat from a boiler to the various rooms of a house. The movements of water in the atmosphere determine the distribution of precipitation on Earth and the annual availability of water resources and, consequently, the growth and development of plants and, more generally, the conditions favourable to the life of organisms. If rainfall is too abundant, erosion and the transport of solid material towards the oceans takes place.

The quantities of water involved in the global hydrological cycle are traditionally expressed in units of km³. Most of the Planet's water is contained in the oceans; less than 3% is unsalted and, of this fraction, the most is frozen (73%), 20% is found in lakes: only a small fraction of the freshwater is available for withdrawal. [979] River water (including marshes) and groundwater make up about 0.02% and 0.6% of the extant water, respectively. [425] Biological water accounts for less than 0.3% of all freshwater. The oceans, as one can easily guess, are the largest reservoirs of water on the Planet: they contain 1,400,000,000 km³, about 97% of all the water on the Earth's surface. Polar ice and continental glaciers, with 27,900,000 km³, provide the second largest contribution to our water supply. The soil contains about 122,000 km³ of water, about 48% of which is found in the root zone of the plants. Human needs are satisfied by a relatively small amount of water, contained in lakes and rivers; the water present in underground aquifers is not easily estimated: probably between 4,000,000 and 16,000,000 km³. The atmosphere contains 0.04% of the total unsalted water. [880] Very little water is available to humankind: less than 1% of all available water, about 200,000 km³.

The amount of water in the atmosphere is relatively small (about 13,000 km³), yet large amounts of water move as a result of surface energy exchange. Water is continuously renewed by rainfall: 110,000 km³ of water fall on the Earth's surface every year. This water, 59-64%, evaporates immediately or is transpired by plants, while 36% reaches the sea, passing through surface and underground streams. Evaporation removes about 500,000 km³ of water from the oceans every year, more than 90% of which returns in the form of precipitations. [979] From this we can deduce that the average residence time of water in the oceans is about 3,000 years. Only part of this water, about 400,000 km³/year, returns to the oceans as precipitation; the rest, about 40,000 km³/year, is transferred as water vapour to the Earth's surface. [624]

On the continents, the average annual rainfall is 700 mm, corresponding to 7 million L/ha/year: less than what corn needs during the months in which it grows and which, at our latitudes, are the warmest months. [425] In the period 1996-2005, the World's water footprint, i.e. the amount of water used by humans, was at least 9,087 cubic kilometres per year. [5]

Plants are able to absorb water through the root system and return it to the atmosphere in the form of vapour: this process is called transpiration. The combination of transpiration and evaporation, which occur in the presence of wet surfaces, is called evapotranspiration, a term used to describe the flow of water from plant surfaces to the atmosphere. The movement of water due to land evapotranspiration amounts to about 70,000 km³/year, which together with the 40,000 km³/year coming from the oceans determines a precipitation on land of 111,000 km³/year. This value testifies to the great importance of the terrestrial vegetation in recycling water from the atmosphere. The average residence time in the atmosphere of water coming from evapotranspiration is much shorter than that of the water in the oceans. These data show

that the terrestrial ecosystems play a crucial role in the water cycle and associated energy transformations. Consequently, if the vegetation cover is reduced, the rainfall is reduced too, with a consequent alteration of the vital conditions of many species.
Water vapour is found in varying proportions below 12 - 15 km in the atmosphere, depending on the processes of evaporation and condensation. A kilogram of air at a temperature of 0°C contains less than 4 g of water vapour when it has a relative humidity of 100%, while at +30°C it contains about 28 g. Increasing the temperature by 30 degrees increases the amount of water by seven times and consequently increases the energy that can be released. This is why the ice of the Poles is a large frozen desert. The air at +40°C contains 470 times more water than the air at -40°C, while the air at +30°C contains up to four times more moisture than the air at +10°C. In general, it can be considered that every increase in the temperature of the atmosphere by 1°C leads to an increase in the capacity of the atmosphere to hold moisture by 7%. [423] So the increase in air temperature causes changes such as an increase in the intensity of precipitations and the amount of water contained in the atmosphere: the fuel for hurricanes. [642]

WATER AND AGRICULTURE

The Planet is threatened by a water crisis because it is a limited resource and because of the increasingly widespread pollution of water resources. The World demand for water doubles approximately every 20 years and, in 1995, eighty countries, representing 40% of the World population, were already affected by water shortages. [847] *Per capita* water availability is decreasing very rapidly with population growth: from 16,800 m^3/inhabitant/year in 1950 to a probable 4,800 m^3/inhabitant/year in 2025. [15] This problem also seriously affects the Mediterranean basin and Italy. Before the end of the century, the flow rate of European rivers, on average, could be reduced by more than 40% with harmful consequences for agriculture. [985]
Overall, the Planet is experiencing a water crisis. At least 18 countries, where half of the World's population lives, are drying up their aquifers. More than 60% of the Planet's rivers have been modified by dams and, from 1900 to 2000, 70% of the wetlands have been destroyed or irreversibly altered. There are at least 900,000 dams in the World, over 40,000 of which are big ones (the first dam was probably built in Egypt in 3000 b.C.). [736, 979]
Humanity has already appropriated more than a third of the production generated by ecosystems and at least 50% of usable fresh water. Probably before 2050 one billion hectares of natural ecosystems will be converted for agricultural use and this will lead to an increase, at least 2 or 3 times, in the distribution of nitrogen and phosphorus in the environment. [604] This increase will exacerbate many existing problems such as eutrophication, reduced biodiversity, and increased health problems.
Agriculture is the greatest user of freshwater on the Planet, which is returned contaminated. It is estimated that 70% of all the non-saline water withdrawn from rivers and aquifers in the World is used for agriculture and especially for irrigation. [776, 851] In Europe, at least 25% of the water withdrawn is used for irrigation purposes; this quantity rises to 55% in southern Europe. [871] An exemplary model of water waste is agro-fuels: to produce one litre of agro-fuels between 1,400 and 20,000 litres of water (virtual water) are needed. [736, 741]
In 2050, the irrigated agricultural areas in the World might increase 1.9 times: from 280 million hectares in 2000 to over 529 million hectares in 2050. [604] The increase in irrigation not only fosters eutrophication but also increases water contamination and worsens the problem of salinization. Irrigation combined with the increased use of nitrogen and phosphorus fertilizers contaminates drinking water (e.g. nitrates and nitrites), increases the release of greenhouse gases (e.g. nitrogen oxides), acidifies soils, and increases the tropospheric ozone. Irrigation, pesticides, and fertilization will generate water pollution and a serious reduction in biodiversity.

Agriculture returns used water contaminated by fertilizers, pesticides, medicines and, in some cases, plastics or substances contained in them. Pesticide use could increase of 2.7 times (from 1.9 to 4.8 times) by 2050: from 3.8 million tons produced in 2000 to 10.1 in 2050. [604]
A diet rich in foods of animal origin requires between 10 and 20 times more water than is necessary to obtain the same calories from vegetables. If mankind instead of using the land to feed over 70 billion animals used the same agricultural land for a vegetarian diet, it would probably be possible to feed 4 billion human beings more. The diet that is based on derivatives of animal origin is very inefficient: less than 10% of the energy contained in vegetables can be transformed into calories in foods of animal origin, and this also causes a great waste of water: 15,000 L for a kilogram of beef steak.
One devastating consequence is that 25% of the World's major rivers no longer have enough water to sustain an acceptable ecological level and are unable to reach the oceans due to alarming periods of scarcity. Water consumption could increase by more than a third before 2050, worsening the current situation irreversibly. [851]

How can we hope to continue not to care about the limits of a finite Planet? Probably in the next few years there will be the last massive increase in agricultural production to satisfy economic rules: between 8.5 and 10 billion people are expected in 2050. This increase will be followed by a reduction as the scarce resources of the Planet will be governed by the financial laws in the opposite direction to that desired. Due to the insufficient ability to translate scientific knowledge into actions, such as those aimed at increasing a disaster resilience and reducing the speed of the disastrous changes we are generating, many opportunities are being lost. Dissemination and communication may probably increase the awareness necessary to activate change.

NITROGEN, PHOSPHORUS, AND EUTROPHICATION

The presence of nitrates and phosphates in surface water and groundwater is a major environmental problem, as they are responsible for eutrophication. Eutrophication is a degenerative process of the aquatic ecosystem due to the excessive enrichment in nutrients. One of the main causes is agriculture, which, through fertilization, distributes in the environment a much higher quantity of nitrogen than the plants are able to absorb. This excess of nutrients, besides polluting the environment, alters many balances, for example it favours nitrophilous plants. At least a quarter of Europe's rivers and over a third of its lakes show signs of eutrophication, i.e. oxygen deficiency. [985]
In aquatic ecosystems, if the oxygen content is excessively reduced as a result of the decomposition of dead plants, this may lead to the extinction of the various forms of life. This phenomenon is known as eutrophication. The distribution of nitrogen compounds such as ammonia promotes the fertilization of ecosystems, eutrophication, and acidification. There is also an increase in denitrification and an increase in the production of nitrous oxide (N_2O), which is a potent greenhouse gas. The nitrous oxide that reaches the stratosphere is converted into other nitrogen oxides, promoting the destruction of the ozone layer.
Surface water may be considered impaired, and therefore at risk of eutrophication, if it contains more than 40 mg/L nitrate (NO_3), more than 2 mg/L phosphate (expressed as orthophosphate), more than 1 mg/L total phosphorus, and more than 75 µg/L chlorophyll (this indicates the development of algae). The World Health Organization recommends not to exceed 45 mg/L of nitrate in drinking water, but as little as 10 mg/L may generate adverse health effects. [52, 114]
The most significant factor that may lead to nitrate enrichment in groundwater is the use of synthetic fertilizers and livestock manure in agriculture. Discharges from the sewerage system

and waste water treatment plants also increase the likelihood of detecting high concentrations of nitrates and other contaminants.
The negative health effects of nitrates include: the production of methemoglobin (hemoglobin that loses the ability to reversibly bind oxygen), miscarriages, and stomach cancer. [114, 147] A methemoglobin level of 10% is enough to cause cyanosis (blue baby syndrome). Nitrates are also implicated in the formation of carcinogenic compounds (N-nitrosamines and N-nitrosamides), which are formed inside the stomach. It is now established that nitrates are carcinogenic not only to humans but also to at least 40 other species of animals, so that it may be said that they damage the biosphere. [147]
Nitrogen compounds are partly derived from manure used as fertilizers and partly chemically produced as synthetic fertilizers. The process of synthesizing nitrogenous fertilizers is named after its inventor Haber, who won the Nobel Prize in 1918, and Bosch, who initiated its industrial production (the Haber-Bosch chemical process). Synthetic nitrogen compounds (produced from diatomic nitrogen, which is the main atmospheric gas, i.e. 78% of the air we breathe) make up at least 40% of all the nitrogen used by crops. [114] The production of chemical fertilizers, such as ammonia, increased from 4 million tons in 1940 to 150 million tons in 1990. It should be noted that to produce one ton of fertilizer requires at least three tons of fossil fuels.
Modern agriculture has altered the normal nitrogen cycle, greatly increasing the flux to the cultivated soil. Atmospheric emissions may also be a significant contributor. Anthropogenic nitrogen emissions reduce biodiversity and promote acidification and eutrophication of the ecosystems. [907]

Animals eat large amounts of phosphorus and, as with nitrogen, only a fraction is retained, while the majority is eliminated with manure. Phosphorus is involved in several important functions such as bone growth, milk secretion, energy metabolism, lipid synthesis and transport, protein metabolism, and DNA construction. Most of the phosphorus ingested with feed (which is often imported) will not be exported with meat and milk, but will remain in the territory where the farm is present, promoting problems such as water eutrophication. Livestock farms contribute to water contamination with phosphorus compounds: between 7% and 48% of all phosphorus compounds in surface waters are generated by livestock farms. [908] A large proportion of the phosphorus distributed in the environment comes from mineral fertilizers.
Phosphorus is easily washed away from the soil as a result of rainfall or irrigation, and may end in surface and deep water. It may therefore cause problems for drinking water too. The accumulation of elements such as phosphorus in surface water and the sea causes the proliferation of microscopic algae which, in turn, not being eaten by primary consumers, lead to an increased bacterial activity. This increases the consumption of oxygen and generates conditions of insufficient oxygen concentration which, in the long run, causes the death of fish. Finally, the water may be coloured and smelly.

STEROID HORMONES IN THE WATER

Animals, including humans, release steroid hormones into the environment through faeces and urine: between 0.02 and 2.3 milligrams per animal per day. In one year, a human being produces over 500 L of urine. Between 110 mg and 990 mg of hormones are excreted per year by cows, between 43 and 830 mg per year by pigs. [915]
Many countries around the World have authorized the use of certain hormones in livestock production. Among the most widely used, there are estradiol (estrogen), progesterone, and testosterone. There are also synthetic molecules such as zeranol, melengestrol acetate, and trenbolone acetate. Some antibiotics are used for their growth-promoting effect, rather than as

antimicrobials. Hormones are used because they produce several desired effects, such as accelerating the rate of daily weight gain, up to 25% more in cattle. [114]
Among ruminants, the oestrogens are secreted mainly in faeces, while in pigs in urine. These hormones, directly or indirectly, end up in the environment (e.g. manure and sewage sludge, used as fertilizers). One effect that has been measured, as a result of this distribution in the environment, is the feminization of male fish, which may be induced by concentrations of 1 ng/L (i.e. one part in a billion). It means that 2.3 mg, i.e. the amount that can be released daily with dung from animals such as pigs, corresponds to the dose potentially sufficient to generate problems for more than 2,000,000 fish per day. It has been calculated that in the year 2000 livestock farming released 49 tons of estrogen into the environment in the USA, and 33 tonnes in the European Union. [915] This chemical contamination may have devastating effects because these molecules alter several functions in animals at infinitesimal concentrations.
A research that investigated the presence of different substances, including steroid hormones in the Danube River and its tributaries, found estrone (after menopause it is the most produced estrogen, and has a lower activity than estradiol) at concentrations up to 71 ng/L. [916]
In conclusion, through the discharge of wastewater and the spreading of fertilizers, such as sludge, livestock manure or their digestates, hormones are released into the environment that may affect wildlife, especially aquatic wildlife. Hormones, if they end up in the human food chain (e.g. drinking water), may generate a lot of adverse health effects. This possibility is favoured by the presence of landless farms, which in increasingly urbanized areas are forced to discharge manure directly into watercourses and in excessive quantities compared to their natural purifying capacity.

ANTIBIOTIC CONTAMINATION

Antibiotics are molecules naturally produced by many microorganisms such as *Bacillus*, *Cephalosporium*, *Penicillium*, *Pleurotus*, and *Streptomyces*. Researchers have started from natural molecules to design and produce similar molecules that can be used as medicines. In industrialized countries, medicines used in animal husbandry account for a high proportion of the national total. For example, in the USA, over 70% of the marketed antibiotics are administered to farm animals. A substantial proportion of the medicines administered is not absorbed by the animal and leaches into the water via effluent discharge or onto the land. The contamination of water with antimicrobial agents causes the selection of antibiotic resistance in bacteria, while the presence of dissolved hormonal substances may affect crops and may cause endocrine disruption in humans and wildlife (some molecules have both an antibiotic and a hormonal activity).
Antibiotics can be almost completely eliminated in feces and urine, without being metabolized. It is estimated that 75% of antibiotics are not absorbed by animals, but are eliminated with faeces. [899] Tetracyclines are eliminated, for 25% of the oral dose, in the faeces and between 50% and 60% of the oral dose in the urine, either in their original form or as an active metabolite. [900] Information on the fate of antibiotics, in some cases, is limited and some studies have estimated half-life times of antibiotics in manure: 100 days for tetracyclines, 30 days for aminoglucosides and between 8 and 30 days for sulfonamides. [901] Antibiotics distributed with manure may be found in the upper soil layers at concentrations higher than 150 µg/kg, in the case of tetracyclines (the concentration in the slurry was 4 mg/kg). [902, 903] With manure spreading, 330 g tetracyclines, 7 g chlortetracyclines, 28 g sulfamethazine, and 57 g sulfadiazine may be distributed in one hectare per year. [902]
Several studies found antibiotics in surface and deep water in the vicinity of farms: up to 67% of water samples taken in the vicinity of pig and poultry farms were contaminated. [904]

Sulfamethazine was found in groundwater at a depth of 1.4 m. [902] Microorganisms with antibiotic resistance genes were found in soils contaminated by antibiotics in manure. [905]
The massive use of antibiotics, especially in animal husbandry, has the side effect of encouraging the emergence of resistant micro-organisms. These enhanced bacteria, which spread mainly through water, are the cause of serious, often incurable infections. Due to the increased use of these medicines, it is likely that by 2050 the number of deaths from incurable infections could rise to over 300 million. [979]

PESTICIDES CONTAMINATE WATER DANGEROUSLY

No one knows exactly how many chemicals are released from human activities into the environment. To have a baseline number to think about, it is estimated that at least 1.5 million tons of persistent, bioaccumulative, and toxic chemicals (carcinogens, endocrine disruptors, etc.) are released every year in North America: these are substances that manifest adverse biological effects in living beings at doses of millionths of a gram or less. We spill millions of tons of substances that are biologically active at very low doses and that are persistent and bioaccumulative. So we are carrying out an unprecedented experiment in global poisoning of the biosphere. Some catastrophic consequences concern the oceans which are warmer, more acidic, with a lower oxygen content, less productive, more polluted, and less covered by ice: unfortunately these negative indicators are bound to get worse.

Environmental monitoring carried out all over the Planet indicates the alarming presence of pesticides in water. Often the concentrations recorded exceed the safety levels regulated by the Authorities, and it must be stressed that there is no concentration at which no harmful effects can be measured.

Insecticides are among the most widely used pesticides in agriculture and have been associated with a number of problems, such as the emergence of resistant target organisms. Over time, new categories of molecules have appeared on the markets that have always been more toxic than their predecessors. The table below summarizes some of the information. [1265]

Category of insecticide [1265]	**Year of introduction on the markets**	**Percentage of the pesticide market (1990-2008)**	**Year of registration of the emergence of resistant target organisms**	**Quantity used in grams per hectare**	**Maximum concentrations measured in surface water*.**	**Maximum recorded concentrations in sediments****
Organochlorines	1940	-	1946	1,000-4,000	6.3 mg/L	52.4 mg/kg
Organophosphates	1950	59%	1965	50-2,000	9 mg/L	20.4 mg/kg
Carbamates	1962	24%	1972	10-200	-	-
Pyrethroids	1973	18%	1978	10-200	0.25 mg/L	10.6 mg/kg
Neonicotinoids***	1991	23%	1995	10-100	0.32 mg/L	-

* Data from 8,166 measurements at 2,500 sites in 73 countries around the World.
** Data from 3,134 measurements at 2,500 sites in 73 countries around the World.
*** Acetamiprid may be found in surface waters at a maximum concentration of 44 µg/L and imidacloprid at 225 µg/L.

These data highlight the dangerous and rapid selection of resistant organisms and the increase in toxicity over time, which means that smaller quantities can be used. An alarming fact are the maximum concentrations recorded in surface waters throughout the Planet, which exceed by even more than 100 times the doses at which negative effects are certain to occur in these fragile ecosystems.

The consequences of pesticide contamination in surface waters are underestimated. The examination of more than 800 studies conducted at more than 2,500 sites in 73 countries confirms a potential risk: more than 52% of the 11,300 surface water samples from all over the World register the presence of the 28 insecticides sought (pyrethroids, organochlorines, and organophosphates; 18 of the 28 active molecules are authorized in Europe and 24 in the USA). [1263] Some categories of molecules, such as pyrethroids, are found at concentrations that are certainly hazardous to aquatic organisms. Concentrations of pesticides in water, equivalent to the maximum limits allowed by regulations, are however able to generate the reduction of biodiversity: the richness of macro-invertebrates decreases by about 30%. [1263] Overall, more than 40% of the positive determinations (52% of 11,300) record concentrations of insecticides above the maximum concentrations permitted for that country (54% in the USA and 35% in Europe). [1263] Unfortunately, insecticides may be found in surface waters at concentrations hundreds of times higher than those regulated by the environmental protection laws: in these cases the damage to biodiversity will be much greater (a limitation is that each country designs different rules and, therefore, different thresholds of acceptability). In 82.5% of the sediments of the entire Planet, concentrations of insecticides higher than the maximum regulated concentrations may be found. [1263] This exposure is also very harmful to aquatic organisms.

Monitoring the level of insecticide contamination of surface water at 644 agricultural sites in the USA (over 5,800 measurements) reveals the presence of active molecules at concentrations above maximum stable levels, in 49.4% of samples. [1266]

The widespread discovery of pesticides in the surface waters of the Planet is an easily foreseeable result, given that from 1955 to 2000 production increased by 750% and agriculture occupies at least 40% of the surface area. The damage that agriculture, through insecticides, generates to aquatic organisms is underestimated or unknown. Another negative aspect is that most of the monitoring is unable to assess maximum concentrations, i.e. the peaks reached when the molecules are released. Concentration peaks may generate devastating ecological effects concentrated in the few months of the year when most active molecules are distributed: spring and summer. Toxic molecules used on a massive scale in agriculture reach surface waters and compromise biodiversity on a planetary scale.

40% (174 million hectares) of Europe's land area is occupied by agriculture using pesticides, irreversibly compromising aquatic ecosystems: algae, micro-organisms, invertebrates and vertebrates. Legislation and the authorization process have not been able to prevent the irreversible contamination of these delicate ecosystems. At least 66 insecticides, 42 herbicides, and 27 fungicides (in 608 samples) have been found in surface waters in Europe: 90% of the samples have at least two residues among those sought. [1273] Regulations and the prevention and control system have not been able to control the environmental chemical contamination, as was the case in Europe for insecticide residues in surface waters: 45% of water samples (at 1,566 sites) record concentrations above the maximum recommended levels. [1273] It is likely that in European freshwater ecosystems, minute concentrations of insecticides may lead to a reduction in biodiversity of at least 29%. [1273] While our knowledge does not allow us to establish concentrations at which no adverse effects are measured in aquatic ecosystems, the following maximum acceptable concentrations for some insecticides in surface waters have been established in Europe (in μg/L): [1273]

- organochlorines: endosulfan 0.01;
- organophosphates: azinphos-methyl 0.32; chlorpyrifos 0.1; malathion 1.25; parathion-ethyl 0.024; parathion-methyl 0.073;
- carbamates: carbofuran 0.02;
- pyrethroids: acrinathrin 0.008; bifenthrin 0.005; cyfluthrin 0.0068; β-cyfluthrin 0.00068; cypermethrin 0.0006; α-cypermethrin 0.015; deltamethrin 0.0032; esfenvalerate 0.01; fenvalerate 0.0022; λ-cyhalothrin 0.0021; permethrin 0.025;

- neonicotinoids: acetamiprid 0.5; imidacloprid 0.3; thiacloprid 1.57; thiamethoxam 2.8.

Unfortunately, almost 45% of the European surface waters have insecticide concentrations above these economically and politically regulated thresholds (concentrations of up to 125,000 times the recommended threshold values have been recorded). [1273] A dangerous phenomenon is that less extensive surface water bodies have higher concentrations of insecticide residues. In the case of sediments, 93% of the 214 samples exceed the recommended maximum concentrations, even by more than 31,000 times (the recommended maximum concentrations in sediments are much higher than those for surface waters). [1273] In surface waters, all cypermethrin findings (29) show concentrations above the recommended safe threshold, in the case of endosulfan 73% (60) and in the case of chlorpyrifos 19% (57 findings). Pyrethroids are the category of insecticides most frequently exceeding the maximum regulated concentrations in surface waters: in 70% of the detections, organophosphates in 38% and neonicotinoids in 24% of the cases. The situation is more serious in sediments: 100% of the organophosphates, 95% of the pyrethroids, and 90% of the organophosphates exceed the maximum regulated safety concentrations (in the case of neonicotinoids until 2015, the year of publication of this scientific article, no maximum recommended concentrations were established). These data confirm the high degree of contamination of surface waters in Europe:

1) 40% of the samples of surface water bodies record more than 5 insecticides and up to 13 simultaneously;

2) the ban on the use of certain insecticides has not, over time, led to any recorded reduction in concentration (banned substances are used);

3) the fraction of samples with insecticide residues has been increasing over the years, exactly the opposite of what should be expected from the application of the safeguard principles;

4) the less abundant fraction (about 35%) of the samples, the one that does not register concentrations of insecticides higher than the thresholds recommended by the regulations, is however contaminated by toxic molecules able to exert acute and chronic toxic effects (synergic, cumulative, and sub-lethal effects are unknown);

This has serious implications for biodiversity and the ability to provide essential ecosystem services such as sanitation and clean water supply.

The environmental protection measures that have been dictated over time by numerous European regulations have not led to a reduction in environmental contamination. Studies and observations confirm that the situation is worsening: contamination by insecticides in water is increasing and other factors such as climate change will worsen the situation (e.g. global warming could lead to an increase in the use of insecticides). Risk assessments carried out before insecticides are marketed cannot protect our health and that of the future generations. Alternatives to chemical agriculture must be designed.

Pesticides are also used in urban environments: in 2012 in the USA, 48 million kilograms of active molecules were distributed in gardens, houses (including indoors), streets, commercial and industrial areas (in the USA 34% of spending on pesticides is not related to the agricultural sector). [1266] So a large portion that is distributed for non-agricultural uses contaminates urban surface waters. The quantities of active molecules distributed per unit area, in some urban contexts, may be high (up to 1,300 kg of insecticides per km^2), so urban surface waters are contaminated. [1266]

A review of the results of 10,755 measurements of active ingredient concentrations in surface water and sediments at 609 urban sites in the USA confirms a high level of risk to aquatic life: [1266]

- Pesticides found most frequently in water were: atrazine (an herbicide), diazinon (an organophosphates insecticide), simazine (an herbicide), prometon (an herbicide of the

triazine family, like the formerly mentioned ones), metolachlor (an herbicide), fipronil (an insecticide), chlorpyrifos (an organophosphate insecticide), tebuthiuron (an organophosphate insecticide), bifenthrin (a pyrethroid insecticide), fipronil desulfinyl (a fipronil-derived molecule), 2,4-D (an herbicide), diuron (an herbicide).
- Herbicides in addition to being the most easily found molecules also recorded the highest concentrations: up to 1.58 mg/L (2,4D and diuron recorded a median concentration of over 100 ng/L).
- The examination of sediments taken from 352 urban sites (1,655 measurements) in different states of the USA reveals the presence of many molecules. Those found most frequently were: chlordane (an organochlorine insecticide), DDT (an organochlorine insecticide which recorded the highest concentration: 6.9 mg/kg), bifenthrin (a pyrethroid insecticide), DDE (derived from DDT), nonachlor (an organochlorine insecticide). Pyrethroid insecticides were the most sought molecules and, consequently, the most frequently found.
- 74% of the surface water samples (3,022) and sediment samples (2,239) record the presence of more than two active molecules with an average of 4.5 molecules per sample and a maximum of 17 substances per sample: more than 20% of the samples record 10 or more molecules simultaneously. 84% of the urban sites surveyed in several US states had at least two active substances present (in surface water and sediments). Sediments were found to be more contaminated: mixtures of pesticides are detected in 82% of samples and 85% of sites (average of active molecules per sample is 4.7). Hydrophobic molecules are more easily concentrated in sediments.
- The concentrations of some molecules are 32,000 times higher than those which are definitely dangerous for aquatic organisms (e.g. in the case of the insecticide dichlorvos).

Another monitoring of 354 surface water samples taken at 97 sites in 12 states records bifenthrin and imidacloprid (neonicotinoid insecticide) most frequently.
These data, although having many limitations (some categories, such as fungicides and the herbicide glyphosate, are searched less frequently) confirm the chronic exposure of aquatic organisms to very dangerous molecules such as insecticides, even in non-agricultural contexts. The study confirms that agricultural surface waters are more contaminated by insecticides than urban ones. The ecotoxicological effects are largely unknown but certainly negative: the reduction of biodiversity recorded in river systems, in marshy areas, in lakes is among the most evident and disastrous in the whole Planet.

Pesticides are recorded in 70% of US surface waters at concentrations above regulatory safe thresholds. Among the most frequently found molecules, there is the insecticide fipronil - which is also toxic to birds and fish - and its derivatives (sulfide, sulfone, desulfinyl, and fipronil amide, which are more persistent than fipronil). In the USA fipronil is used against ants, termites and in many crops such as corn (in this case it is also distributed in seeds) and potatoes. Due to its massive use in agriculture, fipronil may be found in surface waters at dangerous concentrations (up to 6.4 µg/L): in 22% of surface waters taken from 444 sites in 5 different regions. [1264] The sensitivity of different aquatic invertebrates to fipronil and its derivatives may vary by more than an order of magnitude and, in general, the complexity of the aquatic ecosystem is reduced as the concentration of fipronil and its derivatives increases (as is to be expected). The ability of some invertebrates (e.g. Chironomidae) to grow and develop is reduced to 50% by very low concentrations: 0.03 µg/L of sulfide, 0.06 µg/L of sulfone, 0.78 µg/L of fipronil, 0.97 µg/L of amide (these concentrations are more than 20 times lower than those that can be recorded in surface waters). [1264] The fipronil amide derivative was found to be less toxic and the sulfide and sulfone derivatives are 26 times and 13 times more toxic than the starting molecule (fipronil), respectively. This information confirms that infinitesimal concentrations of fipronil and its derivatives are sufficient to alter balances in aquatic ecosystems. Aquatic invertebrates (including insects) may be much more sensitive to fipronil and other pesticides than the organisms used to make eco-toxicological assessments in the laboratory (*Daphnia magna* and *Hyalella azteca*). Furthermore, the effects on the whole ecosystem cannot be assessed in advance in the laboratory: for example, the reduction in the abundance of aquatic herbivorous insects generates an increase in algae, with consequent significant alterations in the trophic chain and in carbon and nitrogen fluxes. The concentrations of fipronil and its derivatives recorded in surface waters are higher than those which are certain to have devastating effects on the delicate balance of these ecosystems.

Dangerous concentrations of pesticides such as neonicotinoids are found in waters in many places around the World. These molecules are found in more than 50% of the rivers monitored in the USA. [483] Also in California, 89% of 75 surface water samples contain imidacloprid and a fifth of the samples exceed the concentrations considered dangerous (1.05 µg/L for chronic exposure). [434, 435] In Australia, 93% of river water samples tested contain two or three neonicotinoids at concentrations between 0.06 and 4.5 µg/L. [439] In Australia (rivers in the Sidney city area), 93% of the samples contain at least two active molecules with an average concentration of 118 ng/L. In Canada (Ontario), clothianidin and thiamethoxam are found in 100% and 99% of water samples near corn fields, at average concentrations of 2.3 µg/L and 1.1 µg/L, respectively. [1272] A monitoring conducted in the USA, between 2012 and 2014, confirms the presence of neonicotinoids in 53% of 149 water samples taken at 38 sites: imidacloprid was the most frequently measured molecule. [1272] The presence of neonicotinoids in surface water is also confirmed by other studies in Switzerland (up to 65 ng/L), Canada (up to 2,800 ng/L), and California (up to 50 ng/L). [1272]

In southern China, in the Guangdong area, the river (Pearl River) that flows through the city of Guangzhou (over 14 million inhabitants) records high concentrations of neonicotinoids. Imidacloprid, thiamethoxam, clothianidin, thiacloprid, and acetamiprid were sampled at 14 sites: all surface water samples recorded the presence of imidacloprid, thiamethoxam, clothianidin, acetamiprid, and 93% of them recorded thiacloprid; the total concentration of the 5 active molecules ranged from 92 to 321 ng/L (average concentration of 192 ng/L). [1272] Imidacloprid is found at concentrations up to 154 ng/L. The 9 sites where sediments are sampled also register a dangerous contamination of the 5 neonicotinoids sought: between 0.4 and 2.6 ng/g dry matter. All sediment samples record the nenonicotinoids (acetamiprid and thiamethoxam are found in all samples).

The concentrations of neonicotinoid insecticides recorded in surface waters and sediments all over the World are higher than those that are certainly capable of generating acute and chronic effects in these delicate ecosystems (e.g. in sediments they damage microorganisms). As a result, biodiversity may be expected to be severely compromised.

In Romania, neonicotinoid contamination was investigated in the Danube River and some of its tributaries (these insecticides are very soluble). [438] At 16 sites 48 water samples were taken. The results obtained show a high correlation between the presence of these insecticides and agricultural practices (e.g. sowing of seeds coated with insecticides). In surface water samples, the detection rates and concentrations were as follows: thiamethoxam 68.7% (0.9-3.8 ng/L); clothianidin 64.6% (0.84-9.6 ng/L); nitenpyram 52.08% (0.39-11.1 ng/L); imidacloprid 31.2% (0.5-8.2 ng/L); acetamiprid 16.6% (0.84-12.7 ng/L). [438] The maximum total concentration of neonicotinoids reached a value of 31.6 ng/L: this quantity is certainly capable of irreversibly damaging the aquatic fauna. The highest concentration was recorded for acetamiprid: 12.7 ng/L.

In the Netherlands and in some countries in Europe, safe concentrations of neonicotinoids in surface water of 0.2 µg/L for short periods and 8.3 ng/L for chronic exposures have been established. [438] In Canada, the safety concentration established for surface water (for

short periods) is 0.23 µg/L, while in Sweden it is 13 ng/L. In the USA, the concentration at which chronic effects cannot easily be measured has been set at 0.01 µg/L.
Contaminated water distributes the toxic active molecules into the soil where they can remain for a long time. The half-life in soil is about 1,386 days for clothianidin, 228 days for imidacloprid, 100 days for dinotefuran, and 72 days for thiamethoxam. [438] This study recalls that imidacloprid represents 41% of the neonicotinoid market and is the second most used pesticide in the World. In 2018, the European Commission banned the use of imidacloprid, thiamethoxam, and clothianidin in the field (they are allowed in greenhouses), however, other similar molecules have not been banned; unfortunately, they remain authorized in many other countries around the World.
In the Netherlands, imidacloprid is recorded in surface water samples: in 2% of the monitored sites it exceeded 320 µg/L. [412] Imidacloprid concentrations of 240 µg/L were recorded in water from rice fields (immediately after field distribution). [412] This concentration decreased to 5 µg/L after one week. Surface waters near rape fields recorded clothianidin concentrations as high as 3.1 µg/L. [412] These concentrations are only 2 to 7 times lower than the doses capable of killing 50% of the exposed amphibians or fish in a few hours. Compared to neonicotinoids, the concentrations of fipronil in surface waters are closer to the doses capable of killing 50% of the fish. Another study, also carried out in the Netherlands, showed a correlation between the reduction in the abundance of several aquatic organisms (amphipods, basommatophora, diptera, Ephemeroptera, and isopods) and an increase in the concentration of imidacloprid (between 13 and 67 ng/L). [434] Sub-lethal effects recorded in aquatic invertebrates due to neonicotinoids (e.g. thiacloprid) include: damage to larval development, changes in moulting, reduced adult longevity, altered fecundity, altered sex ratio, altered mobility, orientation, feeding and egglaying behaviour.
In the Netherlands, imidacloprid was found to be one of the top three most important pesticides as environmental contaminants. [434] The highest concentration, found in Dutch waters (320 µg/L), is 25,000 times higher than the maximum tolerable limit and 56 times higher than the dose that kills 50% of the larvae of aquatic organisms (these are chironomids, a large cosmopolitan family of insects of the order Diptera that resemble mosquitoes). These results show that neonicotinoids can easily contaminate water and generate lethal effects.

78 different pesticides are found in Swedish waters: 33 herbicides, 13 insecticides, and 7 degradation derivatives. [436] Among the molecules that exceed the concentrations considered hazardous, there are: bentazone, boscalid, azoxystrobin (at a maximum concentration of 3.9 µg/L, i.e. 4 times higher than the concentration considered hazardous; the guiding value not to be exceeded in water is 0.9 µg/L), BAM, propamocarb, mecoprop, metalaxyl, imidacloprid (at a maximum concentration of 15 µg/L, i.e. more than 1,100 times the safe value not to be exceeded in water, which is 0.013 µg/L), metamitron (at a maximum concentration of 4.4 µg/L), and MCPA. The fungicides boscalid and propamocarb are found in all six study areas. Imidacloprid is the active ingredient with the highest number of samples exceeding the maximum permitted values in the guidelines: 23 samples, followed by endosulfan and its metabolite (11 samples). [436]

In France, 92% of the 2,950 water quality monitoring points contain at least one pesticide and 60% of the samples contain more than 10 different molecules. [437] In total, 400 different molecules are found out of the 670 sought. In 20.2% of the sampling points, more than 25 molecules were found at the same time, and in 37.2% points between 10 and 25. Only 8.3% of the samples did not contain any of the substances sought. The most frequently found categories are, in descending order, herbicides (glyphosate, atrazine and their metabolites; it should be stressed once again that the use of atrazine has been banned for years), insecticides (imidacloprid) and fungicides (boscalid). Bentazone, glyphosate, boscalid, and imazalil are found dangerously over the safety threshold with regard to eco-toxicity. [437]

In Canada (Quebec), 99% of the 68 St. Lawrence River water samples analyzed in 2017 contain at least one of the pesticides sought: 2 herbicides (glyphosate is found in 84% of the samples, up to 3,000 ng/L; atrazine is found in 82% of the samples), 8 systemic insecticides (acetamiprid, clothianidin found in 46% of the samples, dinotefuran, fipronil, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam found in 59% of the samples) and some metabolites (e.g. desethylatrazine found in 47% of the samples). [440] Atrazine and one of its metabolites are found up to a concentration of 860 ng/L and the sum of the 6 neonicotinoids is detected up to concentrations of 115 ng/L. [440] 31% of the samples record concentrations above the safety threshold established in Australia for neonicotinoids in rivers, which is 8.3 ng/L.

The herbicide glyphosate is one of the most widely used active molecules in the World and acts by inhibiting the synthesis of aromatic amino acids in plants and many microorganisms. Thanks also to the spread of genetically modified plants resistant to its phytotoxicity, the quantities distributed have increased 12 times, between 1995 and 2014 (826 million kilograms in 2014). [1269] The active ingredient is mixed with other substances that are secret because the manufacturing companies are allowed not to disclose them. As a result, the adverse effects on non-target species generated by different trademarks may be very different. Due to its massive use, glyphosate can be detected at dangerous concentrations in surface waters (10 mg/L) and seas (up to 1.7 µg/L in the Pacific Ocean; up to 1.4 mg/L in the Baltic Sea). [1269] The concentrations that are certainly capable of killing or harming some species of marine crustaceans are lower than those recorded in the oceans. Unfortunately, these concentrations are likely to increase if the use of glyphosate continues, and the toxic effects may be enhanced by global warming (there is a synergy between toxicity and water temperature). [1269]

For drinking water in Europe, the safety limit of the maximum concentration of 0.1 µg/L for each single pesticide and of 0.5 µg/L for the maximum concentration of all pesticides (total pesticides; Italian law decree no. 31/2001) has been regulated. [211] Due to the huge amount of pesticides used, these safe concentrations are exceeded in Europe (and not only). In 2013, it was found that about 7% of groundwater drinking water recorded concentrations of at least one of the 31 pesticides researched, with concentrations above the maximum allowable concentration. [276] Even 5% of surface water (rivers) used to supply drinking water is contaminated with pesticides at concentrations above the maximum allowable limits. [211] This case study is probably optimistic as only 31 molecules of the several hundred used are searched; moreover, waters are also contaminated by other substances (e.g. the inventory of chemicals marketed in Europe classifies 106,211 substances). [215]

The monitoring of pesticide residues in surface and groundwater in Italy confirms an alarming situation: [164]

- In 2016, pesticides were found in 67% of the surface water sampling points and 33.5% of the groundwater sampling points (national average figure). The presence of mixtures - with an average number of about 5 pesticides and a maximum of 55 molecules - in single samples is worrying. Overall, 205 different molecules are found in surface waters and 171 in deep waters. [504] Herbicides are the most frequently detected molecules (55.7%). Among the substances found at concentrations above the limit for drinking water (0.1 µg/L; Italian law decree no. 31/2001) are some fungicides (dimethomorph, tebuconazole, iprovalicarb, metalaxyl, flucopicolide) used mainly in viticulture, some herbicides (metolachlor and terbuthylazine), and the neonicotinoid insecticide imidacloprid. Banned molecules such as atrazine and its metabolites are found in surface waters. This herbicide has been banned since 2004, but is found in 66.7% of the surface water samples and 33.8% of the groundwater samples. Aldicarb, which has been banned since 2003, is also found in 20.7% of the surface water monitoring points and 8.8% of the groundwater monitoring points. [504] The active ingredient hexaflumuron (an insecticide growth regulator: it inhibits the formation of the chitin carbohydrate that forms the exoskeleton of insects), banned in Italy since 2004, is present in one fifth of the groundwater samples monitored; carbaryl (a carbamate insecticide), withdrawn since 2009, is found in 11% of surface water samples. Glyphosate is found in 40% of surface waters; imidacloprid is found in more than 50% of the surface water samples and 10% of the groundwater samples. As a result, rivers such as the Arno and the Po are highly contaminated with dozens of different pesticides.

- In some Italian Regions, the presence of pesticides is much more widespread than the national average, affecting more than 90% of surface water sampling points in Friuli Venezia Giulia, in the Province of Bolzano, in Piedmont, and in Veneto, and more than 80% of the sampling points in Emilia Romagna and Tuscany. The presence of pesticides is recorded in more than 70% of the surface water samples in Lombardy and in the Province of Trento. In groundwater, the presence of pesticides is particularly high in Friuli, with 81% of positive samples, in Piedmont with 66% and in Sicily with 60% of positive samples. In Emilia Romagna, imidacloprid was found in 40% of the samples and thiamethoxam reached a concentration of 9.8 µg/L (they are neonicotinoid insecticides). [504] Overall, the contamination is more widespread in the Po Valley.
- Herbicides and some of their metabolites are the most commonly found substances, particularly in surface waters. Insecticides capable of seriously damaging pollinators such as neonicotinoids are also found.
- Unfortunately, substances that have been banned for years (and their metabolites) such as atrazine, metolachlor, oxadixyl and DDT are detected.

In 2016, in Emilia Romagna, the herbicide glyphosate was searched for in 20 water samples and only 3 met the safety threshold of 0.1 µg/L: the maximum concentration was found to be 6.1 µg/L. [500] In some areas such as Ferrara, 65% of the drinking water samples, analysed in 2018, show residues (89 substances were searched for). [499]

This information provides an alarming picture as pesticides are widely present in water even when they have been banned for years. Increased groundwater concentrations of atrazine, simazine, alachlor, and metolachlor have been associated with a likely 3% increased risk of registering Parkinson's disease, for every 1 µg/L increase. [491] The presence of methomyl, chlorpyrifos, and propargite in drinking water was also associated with an increased likelihood of recording Parkinson's disease. [491]

Pesticide pollution of aquatic ecosystems such as lakes and rivers is a major global threat. [415] Harmful effects to freshwater ecosystems are recorded at concentrations below those considered safe, i.e. those measured through acute toxicity tests on the aquatic organism *Daphnia magna*, a small planktonic crustacean native to North America and widespread in Eurasia and parts of Africa. [413] It is an organism that has been widely used in the laboratory for eco-toxicology studies since 1934. Another crustacean, *Gammarus pulex*, living in freshwaters (it is important for waste degradation) is also damaged by concentrations of pesticides commonly found in waters (e.g. imidacloprid between 0.8 and 30 µg/L), as well as other crustaceans. [415, 416]

The situation recorded by environmental water monitoring is dramatic despite numerous limits, including:

- Only a few substances are sought because it is economically unsustainable to search systematically for all molecules.
- Different Italian regions use different monitoring methods. For example, in Emilia Romagna 89 substances are searched, in Marche 22, and in Piedmont 28. So there are many territorial differences. In 2014, in Emilia Romagna, glyphosate was not searched when in Lombardy it was the cause of many non-compliances. In 2017, in Tuscany,

pesticides were found in 95% of surface water samples and, in 10% of them, high concentrations were recorded. In Tuscany, glyphosate was found in 60% of the samples analyzed and at concentrations higher than 1 µg/L; an important note is that glyphosate, after inorganic products such as copper and sulfur products, results to be the most sold pesticide in Italy and in Tuscany.

- The summations of the amounts of pesticides in each sample are not evaluated. As an example, in Emilia Romagna, the average summation of pesticides among those detected in surface waters exceeded 2.4 µg/L.
- Metabolites and derivatives of pesticides are not researched, partly because they are largely unknown. Both the active molecules and the other molecules with which they are mixed in commercial products are transformed in the environment and in organisms into other substances that can be equally or more dangerous. Derivatives, compared to the starting active molecules, can exert toxicity in different ways and on different targets. At least 50 degradation products from 15 families of pesticides are known to contaminate water. [533] Some metabolites result from the action of micro-organisms in the soil, others are generated within organisms, both plants and animals. Others are generated by reactions that take place outside the living beings (by the action of water, oxygen or light). In Italy some metabolites, such as those of glyphosate, which is one of the most widely sold active molecules on the Planet, are found in groundwater. In the United States, fipronil was found in 78% of the water samples monitored, while its metabolites were found more frequently: fipronil sulfone in 81.7% of the samples and fipronil sulfide in 90% of them. [359] Fipronil and its metabolites in water contribute to a reduced abundance and diversity in the aquatic invertebrate community and harm the fish. [359] Fipronil and its metabolites may accumulate in fish. Neonicotinoids such as thiamethoxam, at concentrations up to 9 µg/L, and imidacloprid or clothianidin, up to 3.34 µg/L, are found in groundwater in the USA. [359] Some metabolites of pesticides can be found in water decades after the last authorized use, as it happens for those of the famous organochlorine insecticide DDT (the metabolites are DDD and DDE). [178, 181, 183]

Despite the limitations of monitoring the concentration of pesticides in water, the information available to us is sufficient to justify banning the use of these poisons. Investigations into the presence of pesticides in water, such as neonicotinoids, make it possible to state that they move easily from soil to water and vice versa, that pollution is widespread and that the concentrations recorded are capable of harming aquatic organisms. One aspect to highlight is that the safety thresholds established in the various States are very different, as they are the result of political and economic rather than health and ecological evaluations. Although the safety thresholds are established according to subjective criteria, dictated by economic assessments or convenience, they are regularly exceeded. Even the active molecules sought in official monitoring actions are very different, even within national borders, as is the case in Italy.

If this problem continues to be ignored, it is not far-fetched to predict that in some areas the water will be so contaminated with pesticides that it may be used to control plant pests without further input of active molecules: for example in Holland to control aphids and in Italy against weeds. [166] It could also happen that due to the ubiquitous spread of pesticides many organisms will become resistant, as has happened with weeds and pathogenic insects. In a lot of areas of the Planet and in Italy it should be difficult to hope for the certification of production according to the organic farming method, as pesticides are contained in irrigation water (and soils) and reducing their concentrations is impossible.

The contamination of water by pesticides also harms insects that are linked to non-salt water, as they carry out part of their life cycle, usually their juvenile life cycle, in watercourses:

e.g. trichoptera, plecoptera, and ephemeroptera[I] . Some aquatic insects are more sensitive to insecticides than bees (*Apis mellifera*). In particular, five of the six species tested (three trichoptera, two plecoptera, and one ephemeroptera) are more sensitive to the neonicotinoid insecticide imidacloprid than honeybees, and all six aquatic insect species are more sensitive to the pyrethroid insecticide lambda-cyhalothrin. [620, 687] In these aquatic insect species, the different doses capable of killing 50% of the exposed animals (LD_{50}) vary, showing non-negligible differences. In detail, the LD_{50} of imidacloprid vary between 0.12 and 15.4 µg/g and for lambda-cyhalothrin between 0.01 and 0.51 µg/g. All six species tested are more sensitive to lambda-cyhalothrin than to imidacloprid and all six species are more sensitive to both insecticides than honeybees (between 0.8 and 81-fold). [620] The larval stages of insects in the ephemeral group (genera *Baetis* and *Epeorus*) are damaged by imidacloprid concentrations below 0.1 µg/L. [697] This study highlights the fact that the negative effects of pesticides on insects that perform at least part of their life in non-saline waters is underestimated and cannot be extrapolated from results obtained in the laboratory with honey bees. Very low concentrations of imidacloprid (0.5 µg/L) are also known to harm aquatic earthworms, such as *Lumbriculus variegatus*, and terrestrial earthworms such as the common earthworm (a soil concentration of imidacloprid of 2 mg/kg is toxic to *Eisenia fetida*). [697]

Surface water contaminated by pesticides, used for irrigation, is the cause of the discovery of some dangerous substances in honey: a beehive needs even more than 100 L of water per year, some of which is taken from the plants (the guttation drops). [178]

The exposure to pesticides in water and the resulting damage is also underestimated for other insects such as wild pollinators. We must hope that we do not reach conditions that seem apocalyptic to us today, but which are beginning to occur somewhere in the World: humans pollinating apple orchards in China; one must be naive and ignorant to believe that this could be a way out. [200]

Honey bees do not store water in the colony so they must have sources continuously, especially in the warmer months as they also use it for the thermoregulation of the entire colony. Worker bees can carry 44 mg of water in one trip. [1232] Some pesticides such as neonicotinoids are very water soluble so they are easily found in water. These substances can be toxic at very low concentrations: 4 billionths of a gram of clothianidin, 3.4 of imidacloprid or 5 of thiamethoxam are enough to kill exposed bees by ingestion. Water contamination is so extensive and widespread that beekeepers are advised to provide colonies with clean water near the hive. [1232] The goal is to reduce bee mortality by decreasing their exposure through contaminated water.

[I] Ephemeropterans (Ephemeroptera) are an order of terrestrial hemimetabolous insects that in their larval stages live in surface freshwater streams. [687] Adults are poor flyers because of the very small hind wings (sometimes they may even be missing). The wings are often transparent, rich in nerves and are present only in the adult form: at rest they are always kept in an upright position. Another characteristic is the presence of two tails at the end of the body, sometimes accompanied by a third median filament. Starting from the adult stage, Ephemeroptera stop feeding because of the atrophy of the mouthparts. It is not by chance that in Greek *ephemeros* means "living one day". Once they reach the winged stage, their life span is short: most species live less than a day, while only a few live up to a week. Having such a short time at their disposal, they are immediately looking for a partner. Mating takes place in flight, always near the water. The male literally grabs the female. To facilitate the identification of females, which at the time of mating fly in large swarms near the water, males generally have bigger eyes. The female lays her eggs, dropping them on the surface of the water a few hours after mating. In some genera, the females instead lie on rocks, stems or leaves; or even, as in the case of the genus of *Baetis*, swim underwater to find a safe shelter. Most of the females of the Ephemeroptera lay from 500 to 3,000 eggs, whilst some species, like those belonging to the genus *Palingenia*, are able to lay even 12,000 of them. Their hatching may take place almost immediately as well as, in extreme cases, after 11 months. The short life span of the adults is compensated by a long process of larval development. Some larvae take up to 2 years to reach their first moult (*Ephemera danica*), although the average is one year. The larvae of some species make as many as 27 moults. Their development takes place completely in or near water. They feed on plants and algae and breathe through lateral gills.

Another potential positive effect is that the bees will have to consume less energy to replenish their water supply as they will not have to travel long flights to get it to the hive. This cannot be a solution.
The effect on the possible reduction of mortality following the provision of clean water was tested in bees reared near blueberry, soybean, and corn cultivations in Canada (Québec; 2014 and 2015). [1232] Interesting results include that bees prefer salty water (0.5% sodium; 8 L per week) or water contaminated by chicken farming, to water without additions. In this study, the provision of clean water to bee colonies strangely did not record the reduction in mortality, despite the fact that surface water was contaminated with at least 17 chemical compounds, 2 insecticides (including at least one neonicotinoid), 2 fungicides and herbicides (up to 19 ppb clothianidin and up to 5 ppb thiamethoxam). Ambient water samples recorded at least one herbicide and one neonicotinoid. Water supplied within the colony did not contain the pesticides sought but no positive effects were detected. [1232]
If bees take 44 mg of water on one trip and neonicotinoid concentrations are up to 19 ppb of clothianidin and up to 5 ppb of thiamethoxam, this means that they are exposed to 0.84 ng of clothianidin and 0.22 ng of thiamethoxam on each refueling flight. [1232] Bees at 35°C drink about 11 millionths of a litre (µL) of water per day so, in a few days, they could reach an exposure at concentrations just below those capable of registering lethal effects. However, sub-lethal effects will occur at these exposure levels.
It has come to the point where we must suggest beekeepers to supply their colonies with water other than that present in the environment: we need a different vision, we need to design a pesticide-free agriculture.

An experiment is underway that is also destructively affecting the precarious freshwater ecosystems. The effects of the residues of thousands of chemical molecules that we voluntarily distribute, such as pesticides, are largely unknown: for example, herbicides reduce the amount of algae (phytoplankton), promote increased acidity of the water, and increase the passage of light (this is an indirect effect due to the death of photosynthetic organisms); the exposure to contaminated sediments (from polycyclic aromatic hydrocarbons, polychlorinated biphenyls, hexachlorobenzene, and other substances) in fish promotes cancers, malformations, reduced fertility, and an impaired immune function. [1267, 1268, 1270] When comprehensive and conclusive information on the eco-toxicity of countless combinations of substances that do not exist in nature will be available, it will be too late: they will no longer be needed because aquatic ecosystems, from the biological point of view, will have become inhospitable deserts. The effects on biodiversity, on food chains in very fragile ecosystems such as ponds, marshes, lakes, rivers, and estuaries are certainly negative, but we do not know the details. [1267, 1268, 1270] Chemical analyses carried out to assess the quantity of a small number of substances are not able to predict eco-toxicological effects or even to explain them (in some cases the detection limits of analytical methods are higher than the concentrations that are certainly capable of generating negative effects on aquatic organisms). The analytical approach is not able to allow an adequate risk analysis. [1270] In addition, the chemical contamination interacts with many other disturbing factors such as climate change, alteration of the nitrogen and phosphorus cycles, cementification, deforestation, civil wastewater, industrial and livestock emissions, fishing, aquaculture, and the introduction of alien species. To highlight some largely unknown possible interactions, it must be stressed that recently it has been discovered that increasing ocean temperatures increase the toxicity of active molecules such as glyphosate (in some marine crustaceans). [1269] We are perpetuating a real assault that is fulminating for aquatic organisms and is self-destructive.

SOME DERIVATIVES AND METABOLITES OF PESTICIDES THAT CAN CONTAMINATE WATER (and not only)

In the following pages, we provide in-depth information on some derivatives of different active molecules of pesticides, which may be found in water at dangerous concentrations: even in the order of tens of micrograms per litre. [533] It is important to note that:

- some metabolites may be derived from different active molecules;
- some active substances are transformed into other active substances: for example, the fungicide benomyl is transformed into carbendazim;
- metabolites can be much more persistent in the environment than the starting molecules and may be found in the soil years after the application of the active molecules, therefore they have the potential to accumulate in aquatic organisms (e.g. 1-naphthol from carbaryl) or in food products (e.g. endosulfan sulphate in cow's milk);
- some derived compounds are much more toxic than the active molecules from which they are derived: in animals, chlorpyrifos-oxon is about 3,000 times more toxic to the nervous system than its parent compound (the insecticide chlorpyrifos).

In the following table some information on toxicity is given but it must be stressed that in the majority of cases there is no information on eco-toxicologicy, carcinogenicity or the possibility of bioaccumulation in the food chain. In addition, almost nothing is known about the toxic effects on insects, and soil and water organisms. A number of metabolites that are certain to be found in water are highlighted (in bold and underlined).

Family	**Active ingredient**	**Metabolites derived from the active substance**	**Some information on derivatives of the active substance** [533]
Benzimidazoles	benomil (F)	<u>**Carbendazim**</u>	Carbenzadim has a fungicidal activity like the starting active ingredient. In adult rats, carbendazim alters sperm production and testicular development. When benomyl and carbendazim, together, are administered orally to rats, an increased incidence of hepatocellular tumours is observed. In female mice, carbendazim generates a statistically significant increase in the incidence of hepatocellular neoplasms. It is teratogenic, impairs mammalian development in utero, and is very toxic to aquatic organisms.
Benzonitrile	dichlobenil (H)	<u>**2,6-dichlorobenzamide**</u>	It can be found in orchard soils, fruits, and leaves (e.g. apple orchards).
Benzotiadiazine	bentazone (H)	6-hydroxybenzone, 8-hydroxybenzone, <u>**2-amino-N-isopropyl-benzamide (AIBA)**</u>	6-hydroxybenzone is formed by soil microorganisms.
	carbaryl (I)	<u>**1-naphthol**</u>	It is formed by soil micro-organisms and is the main metabolite of carbaryl. 1-Naphthol, following chlorination of water (carried out to control potentially harmful microorganisms), may give rise to products that can form DNA adducts and be genotoxic (in human cell lines). For aquatic organisms, it is much more toxic than the starting compound and may bioaccumulate in fishes and other

			organisms (BCF or bioconcentration factor of 86).
Carbamates	aldicarb (I)	**aldicarb sulfoxide, aldicarb sulfone**	Both aldicarb sulfoxide and aldicarb sulfone are strong cholinesterase inhibitors, approximately 76 times more potent than the starting compound aldicarb. Aldicarb sulfone can originate from aldicarb sulfoxide as a result of degradation by soil microorganisms and has an acute toxicity, by oral administration, about 25 times higher than the starting compound. It was found in soil 2 years after the application of the parent compound.
	benfuracarb (I)	**carbofuran, 3-ketocarbofuran, 3-hydroxycarbofuran**, carbofuran-phenol	Carbofuran is found in groundwater 12 to 16 months after its application on potato and wheat crops, up to concentrations of 30 µg/L. It is a metabolite produced by microorganisms in the soil but also by animals and plants (such as 3-ketocarbofuran which may be derived from carbofuran). Sister chromatid exchange and mutations have been observed in human lymphocytes. 3-hydroxycarbofuran is a metabolite produced by microorganisms in the soil, plants and animals. 3-ketocarbofuran is detected in the urine of mice and rats following oral administration of the parent compound and in the urine, feces, and milk of cattle following exposure to the parent compound. Nitroso derivatives of this metabolite, which are believed to form in the human stomach, have been found to be mutagenic in tests on *Salmonella typhimurium* and have been reported to cause chromosomal aberrations in cells. Carbofuran-phenol is persistent in the soil and is found up to 90 days after the application of the starting compound. Carbofuran and/or its major metabolites may cross the placental barrier.
	maneb, mancozeb and zineb (F)	**ethylentiourea (ETU)**	It is produced by the degradation of soil microorganisms and may be detected in fruit and vegetable samples. It is found to possess mutagenic, teratogenic, and carcinogenic properties. The EPA (American Environmental Protection Agency) has classified ethylentiourea in group B2, i.e. that of probable human carcinogens.
	methiocarb (I)	**methiocarb-sulfone,** methiocarb-sulfoxide	Methiocarb-sulfone and methiocarb-sulfoxide are products of the metabolism of microorganisms in the soil, plants and animals (e.g. in rat liver). They are inhibitors of acetylcholinesterase.
	molinate (H)	**molinate-sulfone,**	In vitro studies (on cell cultures in the

		molinate-sulfoxide	laboratory) have confirmed the ability for both metabolites to act as potent esterase inhibitors in rats. In the testes, molinate-sulfoxide inhibits testosterone synthesis by Leydig cells and esterase activity; it also causes sperm alterations like the parent compound.
	thiobencarb (H)	**thiobencarb-sulphoxide**	It was positive in the mutagenesis assay on *Salmonella typhimurium*, although to a lesser extent than the parent compound.
Cyclogens	endosulfan (I)	**endosulfan-sulfate**	It is a metabolite formed in plants and mammals. In aquatic environments it tends to be adsorbed in sediments and can bioaccumulate as it is very persistent. In soil it is degraded very slowly compared to its parent compound (half-life greater than 150 days). It appears to be the metabolite most commonly found in tissues, organs, and feces of rats treated with oral or intraperitoneal doses of endosulfan (4-8 mg/kg). A large amount was found in the liver of rats 24 hours after the administration of a single dose of endosulfan. Cows fed with endosulfan (2.5-5 mg/kg) for 30 days released endosulfan sulfate into milk at concentrations of 0.2 mg/kg. A bioconcentration factor of about 130 was estimated.
Chloroacetanilides	alachlor (H)	**2,6-diethylaniline, 2-chloro-2',6'-diethylacetanilide, 2-hydroxy-2',6'-diethylacetanilide, alachlor-ethanesulfonate (ESA)**	2,6-diethylaniline is an alachlor product generated by the metabolism of mammals, soil microorganisms and aquatic insect larvae. In rat liver it can be converted to 2,6-diethylnitrosobenzene which is a potent mutagen (Ames test). A BFC (bioconcentration factor) of 120 has been reported in the mosquitofish (*Gambusia affinis*) which is a small freshwater fish. 2-chloro-2',6'-diethylacetanilide is formed as a result of biotic reactions and photodegradation. In plants it is formed by rapid degradation of the parent compound and is subsequently converted to 2,6-diethylaniline.
	metolachlor (H)	2-ethyl-6-methylaniline, **metholachlor-ethanesulfonate (ESA)**	2-ethyl-6-methylaniline is a potential mutagen.
Chlorotriazine	atrazine (H)	**desethylatrazine (DEA), deisopropylatrazine (DIA)**	Desethylatrazine (DEA) is readily found in drinking water and is phytotoxic like atrazine. In the pituitary gland and hypothalamus of rats it alters the activity of certain enzymes that

			metabolize testosterone, and reduces receptors for prostate hormones. A bioconcentration factor of 8.3 has been reported in aquatic organisms. Deisopropylatrazine (DIA) is a degradation product of the herbicides atrazine, simazine and, to a small extent, cyanazine. It appears to be more soluble than the compounds from which it is derived and has been found in groundwater at higher concentrations than its parent compound.
	simazine (H)	**deisopropylatrazine (DIA)**	*See above*
	terbuthylazine (H)	**deisopropylatrazine (DIA), desethylterbuthylazine (DET)**	Following the application of terbuthylazine to the soil, an increase in the metabolite desethyl terbuthylazine (DET) was observed from day 12 to day 45.
Phenoxycarboxylic acid derivatives	2,4-DB (H)	**2,4-D, 2,4-dichlorophenol**	2,4-D, in addition to being a metabolite of 2,4-DB, is itself an herbicide. 2,4-D is formed in the soil due to microbial metabolism and in plants. The International Agency for Research on Cancer (IARC) has classified chlorophenoxy herbicides in group 2B, which is the group of agents possibly carcinogenic to humans. 2,4-dichlorophenol is also a metabolite of 2,4-D (e.g. in plants). In several fish products it is recorded at concentrations between 0.1 and 14 mg/kg; it is also found in plants such as cereals and strawberries.
	MCPB1 (H)	**MCPA,** 4-chloro-2-methylphenol	The metabolite MCPA is itself an herbicide. It is derived from the metabolism of microorganisms in the soil, plants and animals. In rats it is rapidly absorbed and eliminated, mainly in the urine, and partly binds to plasma albumin. It is readily absorbed in the rat intestine. After exposure it was found in all organs of the rats examined (it is metabolized in the liver). MCPA, in orally exposed rats, is eliminated within 24 hours (90% in urine and 7% in faeces). In humans, 50% of the total dose was found in urine within 48 hours from the treatment. It is classified by the IARC as a group 2B substance, i.e. a substance that is possibly carcinogenic to humans. 4-chloro-2-methylphenol is also a metabolite of MCPA and was found to have a BCF of 76.3 (bioconcentration factor).
Dithiocarbamates	metam-sodium (F)	**methylisothiocyanate**	It shows fungicidal, nematicidal, and herbicidal properties.
Phenylureas	chlorotoluron	3-chloro-4-methylaniline	It causes anaemia and

	(H)		methaemoglobinaemia*, it interferes with the endocrine system and is phytotoxic.
	diuron (H)	monuron, 1-(3,4-dichlorophenyl)urea (DCPU), 4-chlorophenylurea (CPU), **3,4-Dichloroaniline (DCA)**	1-(3,4-dichlorophenyl)urea (DCPU) interacts with the endocrine system and is a potent inhibitor of photosynthesis (particularly of the photosystem II). 3,4-Dichloroaniline (DCA) is a product of the degradation of the herbicides diuron, linuron, and propanil that occurs in the soil. It is also an intermediate compound in the industrial production of many agricultural products. It interacts with the endocrine system and is nephrotoxic in rats. In mammalian studies it has been found responsible for forms of cyanosis. Sublethal effects have been observed in freshwater and marine organisms at concentrations between 0.01 and 1.0 mg/L.
	linuron (H)	1-(3,4-dichlorophenyl)urea (DCPU), **3,4-dichloroaniline (DCA)**	1-(3,4-dichlorophenyl)urea (DCPU) was found in the urine of rats treated with linuron. 3,4-dichloroaniline (DCA) was detected in carrots, 117 days after linuron treatment.
	monolinuron (H)	**4-chloroaniline,** 4-chlorophenylurea (CPU)	4-chloroaniline may also be released into the environment during the processing of intermediates for dyes, pesticides and pharmaceuticals. It may cause methaemoglobinaemia*, DNA damage, mutations, and chromosomal aberrations have been observed (*in vivo* studies). It is classified by IARC in the Group 2B: possible human carcinogen. It is highly toxic to insects, dangerous for freshwater crustaceans (*Daphnia*) and embryos of water frogs (*Xenopus*).
	metobromuron (H)	**4-bromoaniline**	It has been detected in soils of tobacco plantations treated with metobromuron. In humans it could cause methaemoglobinaemia* and probable liver and kidney damage.
Phosphonates	glyphosate (H)	**aminomethylphosphonic acid (AMPA)**	It is a degradation product in the soil but is also the main metabolite in plants and is phytotoxic. It is very persistent in the soil: it has a half-life between 240 and 958 days.
Phosphororganics	fenamiphos (I)	fenamiphos-sulfone, fenamiphos-sulfoxide	Fenamiphos-sulfone and fenamiphos-sulfoxide have similar nematicidal properties of the parent compound and are potent acetylcholinesterase inhibitors. Sulfoxide, in most soils, reaches maximum concentration in about 3 weeks, a period corresponding to the half-life time of its parent compound.
	disulfoton (I)	**disulfoton-sulfone, disulfoton-sulfoxide**	Disulfoton-sulfone and disulfoton-sulfoxide are metabolites present in the

			urine of rats exposed orally with disulfoton. In the soil, cisulfoton-sulfone has a half-life of 120 to 294 days. It is highly toxic to mammals and freshwater invertebrates. Disulfoton-sulfoxide, after a rainfall event following the field application of disulfoton, was detected in surface waters at concentrations ranging from 29.5 - 48.7 µg/L. It inhibits cholinesterase, is toxic to mammals, and is very toxic to birds and freshwater invertebrates.
Propionanilide	propanil (H)	3,3,4,4-tetrachloroazobenzene (TCAB), 3,4-dichloroaniline (DCA)	3,3,4,4-tetrachloroazobenzene (TCAB) is present as an impurity of 3,4-dichloroaniline and the herbicides propanil, linuron, and diuron. It is formed by photodegradation of 3,4-dichloroaniline. It is more persistent than its parent compound (propanil) and has been detected in soils cultivated with rice. It is more toxic than its parent compound: symptoms described are chloracne and methaemoglobinaemia*. In rats and mice it has shown dioxin-like properties.
Thionophosphates	chlorpyrifos (I)	**3,5,6-trichloro-2-pyridinol (TCP)**, chlorpyrifos-oxon	3,5,6-trichloro-2-pyridinol (TCP) is the main degradation product of the insecticides chlorpyrifos and chlorpyrifos-methyl. It is also derived from the degradation of the herbicide triclopyr. It is a metabolite of soil, plant, and animal microorganisms. In animals, chlorpyrifos-oxon is about 3,000 times more toxic to the nervous system than its parent compound (chlorpyrifos).
	diazinon (I)	diazoxon, oxy-pyrimidine	Diazoxon is a more potent enzyme inhibitor than the starting compound. Oxy-pyrimidine is formed by hydrolysis of the parent compound or the metabolite diazoxon. It is much more persistent and mobile in the soil than the parent compound.

Legend: I = insecticides; H = herbicides; F = fungicides.

* Methaemoglobin is a form of metalloprotein haemoglobin, in which the iron in the heme group is in the Fe^{3+} (ferric) state, instead of the Fe^{2+} (ferrous) of normal haemoglobin. The change in oxidation state deprives the molecule of its ability to bind oxygen reversibly and, therefore, it loses its physiological function of transporting oxygen to tissues. In human blood a trace amount of methaemoglobin is normally produced spontaneously, but when present in excess the blood becomes abnormally dark bluish brown. [445]

WE MUST DEPLASTICIZE OUR LIVES

When we talk about plastic we actually mean thousands of different chemical molecules, the main part of which is made up of synthetic polymers derived from petroleum. Among the best known substances, there are polyvinyl chloride (synthesized in 1931), nylon, and plexiglas (synthesized in 1938), polypropylene (discovered by the Italian Giulio Natta in 1954 and marketed under the name of Moplen). [982, 983] Plastics are not biodegradable and degradation times are very long: 600 years for nylon (e.g. fishing line thread), 850 years for polystyrene or at least 1,000 years for a plastic plate or bottle. Some polymers can be synthesized from plant materials, such as polyethylene and polyamide, but not all bio-based polymers are biodegradable.

More or less 4% of all the fossil fuel extracted in the World is used to produce plastic materials, at least one third of which is used to produce packagings. In 2015, more than 320 million tons of plastic resins were produced, of which 79% is dispersed into the environment and landfills: 12% of the plastic was incinerated and 9% was recycled. The amount of plastic that ends up in the sea varies between 5 and 13 million tons per year. Fishing gear accounts for 23% of the total debris found in the sea. [982] Some plastics are less dense than water so they tend to float: polyethylene (bottles), polypropylene, and polystyrene; other plastics tend to sink such as nylon, polyvinyl chloride, and polyethylene terephthalate. Floating plastic due to surface currents tends to concentrate in certain areas forming "floating plastic islands" that may have extensions greater than those of a State like Italy. The most important floating plastic islands in the seas are at least 11. [984]

In the fragile, dynamic and closely related balances between air, soil and water, pollution generates catastrophic phenomena. The introduction of pollutants into the waters alters the ability of marine organisms to survive and render services useful and necessary for the survival of the human species as well. In the oceans, photosynthesis generates about 50% of the oxygen we breathe. In one litre of water there can be 200 billion viruses and 20 billion bacteria. The species that form plankton are not limited to feeding fish and whales, but are also divided into prey and predators, parasites and hosts, organisms that live in symbiosis with each other. What discriminates the presence of plankton in the oceans, which accounts for 90% of the mass of all marine life, is the level of heat in the water. The discovery that temperature determines which species are present in an area is very important in view of the ongoing climate change. [783]

In the surface waters reached by sunlight, thousands of species of microorganisms live and produce 50% of the oxygen of the Planet through photosynthesis. Part of the oxygen generated in the oceans, probably up to 10%, is produced by a bacterium: *Prochlorococcus*. It is the most abundant photosynthetic bacterium in the oceans: a cyanobacterium first identified in 1988. [693, 780] This bacterium has only recently been discovered even though it is one of the most abundant organisms in the Planet's tropical and subtropical seas and, perhaps, we owe it one breath in 10. It lives down to 200 m of depth and can reach a density of over one hundred thousand bacteria in a millilitre: it could form between 20% and 40% of the photosynthetic marine biomass present in the open ocean (between 40° North latitude and 40° South latitude).

So far, we have been concerned about the ingestion of plastics or entrapment by big animals, but the most dangerous threat is more subtle. A variety of substances are used in the production of plastics, such as catalysts, solvents, plasticizers, antimicrobials, antioxidants, substances that protect polymers from ultraviolet rays, flame retardants, metals, dyes, etc. These substances are found in the water (e.g. in river estuaries) at high concentrations (micrograms per litre). In the sea, some of these substances, such as those contained in polyvinyl chloride (PVC) and polyethylene (HDPE or high density polyethylene, which is the most widely used material to produce plastic bags), are released slowly, even a year after spillage.

Among the effects generated by plastics on the most important photosynthetic microorganism (*Prochlorococcus*), the following are known:

- the reduction of growth;
- the reduction in the photosynthetic efficiency and a decline in oxygen production;
- the alteration of the expression of genes, such as those that enable photosynthesis to take place.

Plastic placed in the seas undermines the basic life functions of this marine microorganism and the ability of the oceans to produce oxygen, which is very evident in some areas. [782]

The extent of marine areas where oxygen deficiency, to the extent that marine life is compromised, has doubled every 10 years and this trend is increasing. [988] These areas are referred to as dead zones and pose a serious threat to the marine ecosystem.

We found that the exposure to chemicals, dispersed by plastic pollution, interferes with the growth, photosynthesis and oxygen production of *Prochlorococcus* (and not only). [781]

Plastic invades the oceans, some 8 million tons each year, and it is estimated that there are at least 165 million tons in total. By 2050, plastic will be more abundant than fish (more than 2 million tons flow into the oceans through rivers); today it is estimated that there is at least one ton of plastic for every five tons of fish. [979, 980] On the Planet, plastic bottles alone (e.g. PET) intended to carry water were produced, in 2018, at a rate of at least one million per minute: an unsustainable waste. Probably 70% of the plastic ends up in the depths, so the huge areas covered by floating material are just the tip of the iceberg. [977] Some areas of the Planet have dramatic situations: in the seas of California the (dry) weight of plastic is six times higher than that of plankton. [980] Studies reveal that, in the Planet, plastic materials are found in up to 90% of the seabirds examined. [980] The Mediterranean Sea represents 0.3% of the total volume of the oceans but receives at least 4% of the plastics released into the seas of the entire Planet. [982]

In the oceans, samples of 35,000 species of marine microorganisms that form plankton were collected between 2009 and 2013: the smallest 0.02 micrometres (thousandths of a millimetre), the largest two millimetres. This kaleidoscope of life, which is also the basis of the entire food chain in the sea, is driven by 40 million genes. Thanks to advanced genome sequencing technologies, the 35,000 plankton species analyzed have provided researchers with a sort of DNA map of the sea. Man, to make a comparison, has 20,000 genes, but within each of us we host so many bacteria that we can put together 10 million genes, 73% of which are the same as those of the plankton examined. [783] A portion of the sea, therefore, seems to live also inside us. It is useful to remember that the average number of bacteria present in our bodies is at least ten times higher than the number of cells in our bodies (the set of these micro-organisms, most of which are symbionts, useful and indispensable, is called the *microbiome*). This is possible because bacteria are much smaller than human cells. At least 500 different species of bacteria have been identified in the mouth alone, and if biological taxonomy were to be based solely on the abundance of DNA within each organism, humans could be classified as a community of bacteria hosted by eukaryotic cells. No one knows how many bacteria are present in the biosphere but they are certainly far more than we have classified. Bacteria have been found in tunnels 2.8 km deep (gold mines in South Africa) that can survive at +60°C, in the absence of light and oxygen (*Desulforidus audaxiviator*). [793] Perhaps the most surprising thing about this extremophile bacterium is that it probably does not need to interact with any other organism to survive. Its special characteristics make it capable of surviving in extreme conditions such as those under the ice caps of moons, for example Europa, Jupiter's fourth satellite (by size). [794]

Current estimates predict that abandoned plastic waste in the environment could increase 10-fold in the next 10 years and, therefore, its impact on the sea will be even greater. The spread of plastics in the biosphere is much more extensive than previously believed. Polyester, polyamide, polyethylene, and polypropylene, i.e. plastic, are found for the first time on an Italian glacier (in the order of 75 particles per kilogram): the one in the Stelvio National Park. [784] Although with due caution, it has been estimated that the tongue of the Forni Glacier (in the Stelvio National Park), one of the most important Italian glacial bodies, might contain between 131 and 162 million plastic particles. The origin of these particles could be both local, given for example by the release and wear of clothing and equipment of mountaineers and hikers who frequent the glacier, and diffuse, i.e. particles transported by air masses. [785] The most effective solution should be to ban the use of disposable plastics and to improve waste management.

It takes a lot of effort to try to know at least some of the effects generated on ecosystems and on our health by the thousands of new molecules that are released into the environment. For example, what will be the effect of the 6,000 tons of sun creams that human beings pour into the water when they dive? It is already known that some of the sea's inhabitants may be seriously harmed by them. [874]

One of the first records of fish dying from plastic probably dates back to 1931: a shark blocked in a tyre. [982] In 1966 plastic was found in the digestive tract of dead albatross chicks. Since then, plastic has been found in marine organisms such as seals, fish, and walruses. One of the first whales to die from ingesting plastic was found beached in the mid-1970s. Ingestion can be direct (turtles mistake floating plastic for their prey, jellyfish, or seabirds for molluscs) or indirect (through their prey). The number of marine species in which plastic is found is increasing: in at least 164 species of seabirds, 92 fish species, and 62 species of marine mammals. [982] Plastics cause harm even when they are not ingested as they can, for example, trap animals.

Floating plastic helps to anchor and transport hundreds of marine species, making it easier for them to move greater distances. This is a potential spread of alien species, a phenomenon that has already been recorded in 2011: due to a tsunami, the floating material allowed the transport of dozens of species from Japan to the American coast, where they do not exist.

In the oceans, due to various phenomena such as erosion, plastic is broken down into smaller pieces until it becomes invisible. Microplastics (the size of thousandths of a millimetre) are found everywhere, not only in the oceans but also in glaciers and in our bodies. Microplastics are actually not only generated by the degradation of plastics as they are used in many products such as cosmetics, toothpastes, pesticides (micro-encapsulated), and medicines. Another source of microscopic plastic pieces are household washings that detach fibres from clothing (thousands from each garment per wash). These microplastics are found in the deep seabed such as the Mariana Trench (at about 11,000 m depth, nearly 2,000 microfibres per litre are recorded).

Microplastics are able to move inside the living beings and may be found not only in the digestive tract but also in other organs such as the respiratory system. Some lipophilic materials can easily cross membranes and enter cells. Plastics contain very dangerous substances that are persistent, bioaccumulative, and toxic. Some of these substances have been shown to provoke adverse effects on reproduction, cell division (cancer), on the immune system, and on the endocrine system. Among the incriminated molecules, there are the phthalates, which are used in large quantities (up to 60% by weight) to produce polyvinyl chloride (PVC). Phthalates are produced in quantities exceeding 12 million tons per year (2013) and are suspected of being endocrine disruptors (they can manifest an estrogenic activity and, consequently, impair reproduction). PVC may release carcinogens such as vinyl chloride.

Another category of hazardous substances added voluntarily are flame retardants such as brominated organic compounds (these substances are also potential endocrine disruptors).

Among the many substances that are added to plastic polymers, there are solvents, catalysts, surfactants, stabilizers (some of these substances contain metals). For most of the substances used and released into the environment there is not enough information available to establish their hazard to humans and other living beings. Unfortunately, microplastics and various molecules contained in plastics are also found in our bodies, for example in faeces. One way of contamination is through fish, another is through the transfer of packaging materials to the food or drink they contain (e.g. some phthalates or microplastics transferred from water bottles). At least one third of the production of plastics is used to make packagings.

Cooking salt (sodium chloride) taken from sea water is rich in plastic, wherever it comes from on the Planet (up to thousands of particles per kilogram).

An alarming figure is that we may be ingesting several grams of plastics per week (of which a few milligrams may be contained in one litre of bottled water). [982] Major sources of human exposure to plastics include bottled water and fishery products. For example, phthalates are found in blood, urine, breast milk, and amniotic fluid. [982] Some substances can also easily reach the brain, as has been shown by the discovery of polystyrene in the brains of fish. In the human intestine polystyrene can alter iron absorption.

Honey has also been found to be contaminated with polymeric fibres (170 per kilogram) that could be derived from the sum of those taken from bees in the environment (think of micro-encapsulated pesticides) and those given off by packaging. Plastic enters the agricultural world in a number of ways. One of the wellknown ways of contamination is compost, which may contain up to 4% plastic, so in the form of micro-particles several kilos of plastic per hectare per year may be distributed. [741] Other sources of agricultural contamination are mulching (phthalates are found in plants and soil), irrigation (with plastic pipes in corn fields, for example) and sewage sludge (microplastics will be distributed both through irrigation and the use of sludge for agricultural and environmental purposes). In confirmation of the dangerous experiment under way, phthalates are also found in earthworms. [982]

Microplastics, i.e. those with a diameter of less than 5 mm (particles that may be as small as a few thousandths of a millimetre), are ingested by various marine organisms such as molluscs, including those used for human consumption. 84% of oysters farmed in China contain microplastics. The examination of the digestive system contents of seven species of shellfish from two important Chinese aquaculture areas confirms this widespread contamination: between 70% and 100% of samples from different shellfish species are contaminated with microplastics. [1259] The size of the synthetic fibres found inside these filter-feeding organisms varies between 10 and 5,000 µm but the most frequent size is less than 500 µm. The fibres are identified with nine different colours and at least 18 different types of polymers are confirmed (the most frequent was rayon followed by chlorinated polyethylene, polyvinyl chloride or PVC, polyethylene or PET, polyvinylidene fluoride or PVDF, polytetrafluoroethylene or PTFE, and others such as polyacrylonitrile or PAN). [1259] Other studies also confirm the contamination by microplastics in shellfish from all over the World: India, Vietnam, Italy, France, United Kingdom, Canada and USA. [1259, 1260, 1261]

Microplastics invade the oceans and therefore can be found in many fishery and aquaculture products important for human nutrition such as squids (cephalopods) and crabs (crustaceans) caught in India, which may contain between 3 and 20 particles (between 100 µm and 5 mm) per kilogram of edible part (5 colours were identified and the polymers found most frequently were: polypropylene followed by polyethylene, and polystyrene). [1260]

The worldwide annual consumption of fishery and aquaculture products (e.g. mussels and oysters) exceeds 125 million tons per year, so even a few tens of microplastic fragments per kilogram is a potentially dangerous exposure. [1261] European consumers of aquaculture products such as mussels and oysters may ingest, through the consumption of two farmed species (*Mytilus edulis* and *Crassostrea gigas*), between 1,800 and 11,000 microplastic fragments per year. [1261]

These data confirm that the consumption of shellfish and other fishery products constitutes a potential hazard to human health due to the ingestion of synthetic polymers and other substances (polybrominated diphenyl ethers or PBDEs which are flame retardants, polychlorinated biphenyls or PCBs, phthalates and bisphenol A or BPA). [1261] Smaller microplastic fragments (smaller than 130 µm) may be translocated into human body tissues and release toxic substances.
The examination of the microplastic exposure among Americans highlights a potential risk: [1262]

- Through the consumption of bottled water an average of 94 microplastics per litre are ingested (an average of 0.44 litres per day is considered) while through the water from the aqueduct a concentration of about 4 microparticles per litre is estimated. Therefore bottled water, with the same volume, contains at least 23 times more plastic than the water from the aqueduct. Alcoholic beverages may contain an average of 32 microplastics per litre.
- Between 97 and 170 microplastics are inhaled per day through breathing (between 35,000 and 62,000 micro-polymers per year).
- Through food and water consumption we are exposed to between 106 and 126 microplastics per day (between 39,000 and 52,000 micro-polymers per year). Consumption of fishery products along with beverages in plastic are important sources of exposure.

Overall, ingestion and inhalation, generate an exposure of Americans to between 74,000 and 114,000 particles as fibres and synthetic polymer fragments per year (depending on gender and age). [1262] These figures, although alarming, are an underestimate because the dietary intake is assessed for only a fraction of foods, i.e. 15% of caloric intake, and consumption of water and beverages in plastic bottles may be much higher than what is considered.
Unfortunately, microplastics are also a danger to the health of marine organisms, but even less information is available on this aspect: the ingestion of plastic promotes the transfer of resistance to antibiotics through the intake of microorganisms that adhere to polymers (microplastics are colonized by bacteria), it promotes oxidative stress, cell damage, and poisoning by various toxic substances. [1260]

These are just some of the negative aspects of plastic production; in reality, the externalities and consequences on the biosphere, the so-called hidden costs, are mostly unknown. The plastics management model is a striking example of how private interests are protected at the expense of the community. The problem is huge and affects us closely, and we can no longer ignore it. We need to abandon throw-away consumerism and embrace of refusing to throw-away philosophy: we need to deplasticize our lives.

THE ACIDIFICATION OF THE OCEANS

More or less 30% of carbon dioxide emissions end up in the oceans. Algae store at least three billion tons of it. Sea weeds alone, not counting other organisms, have a higher biomass than all terrestrial plants. [14] In general, the oceans contain enormous amounts of carbon, far greater than that contained in all terrestrial plants and animals (at least ten times more). The increase of carbon dioxide in the oceans increases the acidity level of the marine surface waters: between 1992 and 2007 the pH of the oceans dropped from 8.11 to 8.01, thus becoming more acidic (positive electrical charges are higher than negative ones). The current rate of ocean acidification is estimated to be higher than it has been in the last 20 million years, so the resulting effects are unpredictable and catastrophic. [5, 8] Many organisms are certainly sensitive to a variation in acidity, such as those that build shells and phytoplankton. Variation in acidity

and temperature in the seas generates coral death (a phenomenon known as "*coral bleaching*") and damages the phytoplankton.
Coral polyps host myriads of single-celled algae in their bodies: up to a million per square centimetre. [14] The algae carry out the photosynthesis (they synthesize glucose and release oxygen) and in return they receive carbon dioxide produced by the small animal: the coral polyp. With this symbiosis, coral polyps are able to build coral reefs ten times faster. The more than 700 known species of corals build structures that are natural works of art. If the algae leave the polyps, they fade and die. Ultraviolet rays, which are able to pass through the ozone hole, may also kill phytoplankton (microscopic algae) over vast areas of sea. [14]
Probably at least one in four ocean dwellers spend part of their lives in coral reefs. [642] Since 1980 we have destroyed at least 30% of the coral reefs in temperate waters. [10] If the current rate of damage is maintained, in one generation, we may be able to irreversibly alter all of the Planet's coral reefs. [8]
If marine ecosystems are destroyed (due, for example, to pollution and acidification), this could result in the release of huge amounts of carbon dioxide into the atmosphere.

UNSUSTAINABLE EXPLOITATION OF THE SEAS

The oceans (which contain an average of 35 g of sodium chloride per kilo of water) occupy about 70% of the Earth's surface (80% in the southern hemisphere and 61% in the northern) and have an average depth of 3.7 km (with a total volume estimated at 1.3 x 10^{18} m^3). [259] Despite the vastness of the seas, we have managed to compromise this ecosystem as well. At least one third of fish stocks are endangered and those of fish such as tuna, swordfish, cod, and plaice have been reduced by 90% in 50 years. [8]
The balance is in favour of humans even when it comes to predators as fearsome as sharks (at least 470 species are known). About ten humans die from sharks each year, but several million sharks are killed by humans. [14]
Fishing has become unsustainable by greatly exceeding the regeneration capacity, from a catch of at least 19 million tons per year in 1950 to over 87 million tons in 2005. [5] One-third of the marine life caught is used to feed farmed animals (forming meal for pigs, chickens, salmon raised in aquaculture), including domestic animals such as cats. [977]
In 2018, the global fish production reached about 179 million tons, with a total first-sale value estimated at $401 billion. Probably over 20% of the catch is bycatch, i.e. unwanted but unavoidable capture, such as turtles, seabirds or fish that cannot be used for human consumption; probably the number of dolphins, whales, and belugas victimized by fishing as bycatch is over 300,000 per year. [977] The current pace of industrial fishing may be considered a programmed massacre, an ecological holocaust so rapid and efficient that it is unprecedented: at least 1,000 billion fish caught every day. This is a programmed and perpetuated extinction on a planetary level. Also because of this self-destructive behaviour, from 1988 to 2015, the production of World fisheries has been reduced by more than 40%: in the Mediterranean Sea, captures, in general, have been reduced by more than one third. [987] It is difficult to keep up with the estimates of predation implemented in the seas as they are continually being updated negatively, but we are certain that the haul is proceeding at a rate much higher than the natural capacity for regeneration and reproduction. This is an unmistakable warning sign that we need to change course. Fishing must be regulated and restricted. The extension of protected marine and coastal areas must be increased.

Aquaculture products account for 46% of total production and 52% of fish for human consumption: compared to the 1960s, we have doubled the World's *per capita* consumption of fish to over 20 kg per year. [987] China is by far the leading producer in the aquaculture sector:

since 1991 it has produced more food from aquatic farming than the rest of the World. [9] Fish consumption accounts for one-sixth of the World population's animal protein intake and more than one half in countries such as Bangladesh, Cambodia, Gambia, Ghana, Indonesia, Sierra Leone, Sri Lanka, and several small island developing states. Some information on the state of World fisheries and aquaculture is given below: [9]

- global fish production in 2018: 179 million tons, and for human consumption it is about 156 million tons;
- aquaculture in 2018: 82.1 million tons, new all-time record.
- fish catch at sea: 84.4 million tons;
- freshwater fish catch: 12.0 million tons, an all-time record;
- first sale value of production from fisheries and aquaculture in 2018: $401 billion of which aquaculture's share is $250 billion;
- number of people employed in the primary sector of fisheries and aquaculture: 59.5 million, of which 14% are women;
- region with the greatest number of fishermen and fish farmers: Asia (85% of the total);
- number of fishing boats in the World: 4.6 million;
- greatest fleet by region: Asia (3.1 million boatls or 68% of the global fleet);
- rate of motor boats under 12 metres: 82%;
- percentage of global fish production covered by international trade: 38%;
- value of seafood exports: $164 billion;
- first producer and exporter of fish in the World: China;
- fish exporting regions (net values): Oceania, Latin America, the Caribbean, and developing countries in Asia;
- Africa is a net importer in terms of volume, but a net exporter in terms of value;
- places on the Planet that hold the record for the most unsustainable fishing: Mediterranean and Black Sea (62.5% overfished), South-East Pacific (54.5%), South-West Atlantic (53.3%);
- rivers with the highest production of inland capture fisheries: Mekong, Nile, Ayeyarwady and Yangtze.

Increased consumption of seafood today is supported by aquaculture, which is often fed with feed made from wild species and pollutes the sea, for example, with medicines such as antibiotics and pesticides. It is estimated that for every ton of fish produced by aquaculture, between 42 and 66 kg of nitrogen and between 7 and 11 kg of phosphorus are discharged into the environment. [5]

Fishing is an example of the successful economic model based on the free appropriation of common goods and the externalization of environmental damage. The exploitation of a common good enriches private individuals and creates an unpayable environmental debt for future generations. It is an exemplary economic model of "ecological injustice".

The causes of extinction or of altering the possibility of survival of a lot of marine species are not only linked to chemical pollution and fishing. Attention is drawn to another particular aspect: the noise pollution of the oceans. It is likely that many cetaceans are severely damaged by marine explosions, drilling noise and other human underwater activities, including boat engines. [14] In the Northern Seas hundreds of floating platforms generate noise that is fatal to some cetaceans. The damage generated by noise pollution explains some cetacean strandings: for example odontocetes orient themselves by echolocation, which becomes impossible if they are immersed in a bombardment of waves similar to those used naturally but produced by man (e.g. ships' sonar, explosions, and drilling).

NON-RENEWABLE USE OF WATER RESOURCES

Surface water and groundwater are renewable natural resources provided they are used sustainably:

1) withdrawals, implemented for different purposes, such as the supply of drinking water, industrial water, and water for agriculture, must not exceed the natural regeneration capacity of the resource;
2) water must not be contaminated, in particular if it is used for human or animal consumption.

Unfortunately, water is always returned to the environment more polluted than it was at the time of withdrawal, and this results, over time, in a deterioration in quality.
Demand for water consumption on the Planet is set to increase due to population growth and growth in the production of goods and food. Forecasts indicate that the availability of water (unsalted and unpolluted) will decrease, and the number of countries facing water scarcity will increase. Among the main causes of the reduction in water availability, there are climate change, deforestation, and pollution. [114] The number of inhabitants of the Planet who will have to live in environments with scarce water resources is set to increase: three billion by 2035.
Every American citizen consumes about 400 L of water per day for cooking, washing and other personal uses. If we add the water used to irrigate the food they consume, each American needs at least 5,700 L of water per day. [425] The FAO estimates a need for water to produce the food consumed daily, depending on the country, between 2,000 and 5,000 L of water. It may be considered that, in general, a vegetarian diet uses between 1,500 and 2,600 L per day, but for a diet rich in animal derivatives it exceeds 4,000 L per day. [880]
The average domestic water consumption (for drinking, cooking and washing) in Italy is 152 m^3/*per capita*/year. [880] In Italy, drinking water withdrawal is about 9 km^3 per year, of which 85.6% comes from groundwater and 14.3% from surface water. During the distribution of drinking water, between 33% and 55% of the water withdrawn is lost.
At least one litre of water is needed to produce one calorie from foods of plant origin, but at least ten litres are needed to produce one calorie from foods of animal origin. [882] To give some examples, the production of a litre of milk requires about 990 L of water, called virtual water, for a kilogram of coffee 18,900 L are used, for a kilogram of steak 15,400 L, for a kilogram of cotton textile 11,000 L, for a kilogram of eggs 3,300 L, for a kilogram of cheese 3,100 L, for a kilogram of rice 2,500 L, for a kilogram of pasta 1,800 L. [979]
In the agricultural sector, water consumption has more than tripled in 50 years: at least 70% of all unsalted water used is used in agriculture.
The problem of water scarcity is underestimated by estimates of potential increases in renewable energy and food production. Demands for food production and biomass energy are likely to increase in the future, so water consumption will grow at a faster rate than population growth. For example, trade in virtual water alone (the "invisible" water used to produce food and other goods) between the various states of the Planet, as a result of exports, has doubled in just 23 years. [880] Also in Italy the importation of virtual water exceeds that consumed by internal production.
Probably, for Italian agriculture, in 2050, due to several factors (lower rainfall, higher evaporation), there will be at least 19% less water available than the current situation. According to this forecast, in Italy, cereal production could be hit hard, so much so that it could be reduced by at least 17% due to water scarcity. [880]
Other examples can be given to focus our attention on the gravity of the situation. All cattle and horses bred in Piedmont (a northwestern Italian Region) need, just to drink, a consumption of drinking water equal to that more than sufficient for at least 80,000,000 people each year: that is 20 times the current population of Piedmont. The water used for animals and, in general, the

water used for breeding is returned to the environment, but with different contaminants: chemical and biological. It cannot be hoped that the dilution effect will be sufficient to protect our health for an indefinite period.
Humanity's water footprint has exceeded its natural renewal capacity: we are taking too much water, so we are reducing valuable supplies such as groundwater. According to some estimates, humanity is withdrawing water from groundwater at a rate of at least 3-4 times the natural capacity for regeneration. [985] As a result, the cleanest and most precious water is running out forever. More consideration should be given to these aspects and all useful strategies should be encouraged to reduce contamination, waste, leakage or misuse.

In general, in Piedmont, the costs to be sustained for water supply and wastewater management service are very low, therefore strategies for waste reduction and saving (e.g. wastewater recycling) are not encouraged. There is no incentive to reduce the waste of drinking water and to recover other types of water (e.g. rainwater for irrigation purposes). Another aspect worth highlighting is that the agricultural use of drinking water costs more than 4 times less than for domestic use, and livestock use costs more than 3 times less than the use for cultivation. [36] More attention could be paid to policies and strategies to limit waste and improper use, including a better modulation of the fee to be paid to receive drinking water and dispose the wastewater. Reductions could be envisaged for those who invest in saving and recycling, and increases for less important uses. It is recalled that the European legislation (Directive 2000/60, at the first "considering" sentence), reports:

"*Water is not a commercial product on a par with others; it is an asset that must be protected, defended and treated as such.*"

The economic principle of recovering the costs of water services should also consider the damage and negative repercussions generated to the environment and to water itself due to bad management and unsustainable use. Tariffs could be increased for productive uses, which generate income and, at the same time, return highly polluted water. For a more equitable modulation of tariffs, the following principles can be proposed: [36]

- for non-domestic users: those who consume more, pay more; that is, there should be scales based on the annual volume used, as happens in the case of domestic users;
- those who consume for non-primary utility purposes pay more per unit volume;
- whoever pollutes more, pays proportionately more;
- who wastes, pays more;
- those who save money and adopt strategies to improve the sustainability of water use pay less.

A better modulation of the tariff could encourage strategies to save and protect the water resources.

GENETICALLY MODIFIED PLANTS: A PROGRAMMED ENSLAVEMENT

GENETICALLY MODIFIED PLANTS

Genetically modified crops and their derivatives have now invaded the World. Genetic modifications mainly affect a few crops such as corn, soy, rape, cotton, and have two applications: resistance to herbicides and the independent production of insecticides; at least 10% of all cultivated land is used to cultivate genetically modified plants used for various purposes, such as animal and human nutrition. [159]

In 2018, 48% of all the World's corn, canola, soybean, and cotton acreage was occupied by genetically modified plants. [1241] Other genetically modified crops include sugarcane, papaya (virus resistant), apples (no browning) and potatoes (lower in asparagine). Some of the most cultivated genetic modifications on the Planet include: [1241]

- Resistance to herbicides such as glyphosate, glufosinate and, since 2016, 2,4 D and dicamba.
- Resistance to some insects through the production of bacterial toxins (Bt).

Already in 2001, more than 50 million hectares were occupied by genetically modified plants: 4 times the entire Italian agricultural area. [147, 155] In the USA, 70% of processed foods contain at least one ingredient derived from genetically modified organisms (GMOs). [8]

At least 50 different genetically modified crops are grown and marketed in countries such as the USA and Canada but, in 2010, only five species were allowed in Europe: three maize and two rape species. At least two types of genetically modified plants have been authorized for cultivation in Europe: maize and potatoes. In general, many restrictions were adopted in Europe because at least 19 countries chose to ban or restrict GMOs. These restrictions have not helped to prevent the invasion, on our tables, of foods containing genetically modified organisms or their derivatives. For example, genetic traces of laboratory-generated modifications have been found in all sweet potatoes intended for human consumption. [1076]

Foods containing ingredients derived from genetically modified organisms that may be marketed in the USA include: corn (at least 92% of corn crops grown in the USA are GMOs), soybeans (94% of crops in the USA are GMOs), cotton (94% of crops in the USA are GMOs; the modification of the composition of the oil is evident), sweet potatoes, rape, apples, papaya, sugar cane, courgettes, yellow squash, watermelons (seedless), beer, tea, bananas, blueberries, peanuts, walnuts, cheese (at least 80% is obtained through the use of enzymes produced by genetically modified bacteria). [1077, 1078, 1079, 1080] Over 75% of the processed products distributed in North American supermarkets contain at least one ingredient derived from genetically modified organisms. [1077, 1078] Surely the situation in Europe is not very different: how is it possible that more than two thirds of the food sold in America contains GMO derivatives or is GMO and in Europe none? Two-thirds of the protein contained in the feed used in Europe comes from soya, 70% of which is imported: at least 90% is made up of transgenic plants. [1081]

In Europe too, fermented products such as those of the dairy industry require the use of genetically modified micro-organisms.

Artificial characteristics conferred on genetically modified plants include: [127]

- resistance to herbicides (maize, tobacco, soybean, rape);
- flowers with sterile male (rape, chicory);
- resistance to insects (cotton and maize producing spores generated in nature by micro-organisms such as *Bacillus thuringiensis*);

- modification of the colours of the flowers (carnations);
- extension of the shelf life (carnations);
- modified starch structure (potato);
- delay in ripening (tomato).

The consequences for biodiversity and the spread of artificially created traits in nature are not yet clear. Genetically modified organisms may affect biodiversity through the spread of herbicide-resistant crops, which may become pests to other crops. [159] Thus, some negative effects are already recorded:

- the spontaneous spread of genetically modified herbicide-resistant plants that become pests on various crops;
- the increased use of certain herbicides (those for which GMO crops have been made resistant, such as glyphosate);
- the emergence of resistant insects and plants (e.g. insects resistant to toxins derived from soil bacteria but produced by transgenic plants).

The majority of genetically modified plants are cultivated in five countries: USA, Brazil, Argentina, Canada, and India. In 2011, at least 159 million hectares were cultivated with genetically modified plants, about 3% of the cultivated area of the Planet. [557]
As early as 2013, most of the soybeans and corn grown in the US were genetically modified and over 282 million hectares worldwide were cultivated with genetically modified plants. [175] For a measure of reference, it is useful to remember that, in 2016, European farms occupied 173 million hectares and Italian farms occupied 11-12 million hectares. [839] In some cases 100% or nearly 100% of the crops in a country are genetically modified such as cotton in India, soybeans in Argentina and the USA, and corn in the USA.
In Brazil (Mato Grosso) at least seven million hectares are cultivated with soybeans, 90% of which are genetically modified (e.g. resistant to herbicides such as glyphosate). [452] In 1950 this legume was cultivated on 17 million hectares, while today it occupies more than 250 million hectares (14 times more). Soy is used for various purposes such as feeding more than 700 million pigs in China. In the World, every year, at least 70 billion animals are killed for human consumption (excluding fish). [452] In order to feed animals, feeds travel around the World: the food supply system is extractive as it is not renewable and, therefore, has a very short time horizon. Biotechnology companies have created plants that are resistant to pesticides or that produce toxins thanks to genetic modification. This system is labour-intensive and uses machines, chemicals and much more energy than it produces in terms of food calories. [36, 159] In addition, most of the land under cultivation is managed by a minority of farmers who have become landowners. The result is a drastic reduction in biodiversity: the countryside is all the same so that it is alternately, in the different months of the year, deserts or monocultures (in mono-succession); these are artificial deserts.

The long-term effects of genetically modified plants on the environment and on humans are mostly unknown. However, some undesirable effects can also be observed in the World of pollinators. Some were easily foreseeable, such as the reduction of wild plants and, therefore, of flowers, due to the increased use of herbicides to which some plants are artificially resistant. The ability to resist the toxicity of herbicides (e.g. glyphosate) is one of the most cultivated genetic modifications in the World and concerns many crops. With this artifice, plants resist high concentrations of dangerous molecules, so that greater quantities can be used. Among the foreseeable undesirable effects, there is the appearance of plants resistant to the herbicide. [557] There is a risk of a disastrous spiral for the environment and for our health: the number of resistant plants that become weeds increases and, as a result, more herbicides will be used because the crops tolerate them. Other alternatives should probably be suggested, such as mechanical mowing, rotation, diversification of crops, and year-round cultivation of the soil so that it does not remain free of plants, for example in autumn or winter.

Genetically modified crops have been presented as an option to reduce pesticide use and increase yields. Some of the most widely grown GM crops in the World, such as corn, soybeans, and cotton, have received an increase in the use of pesticides (e.g. herbicides) and yields have not increased significantly.

The spread of genetically modified plants in places where agriculture is the main resource for survival also fails economically (e.g. in developing countries). [557] Studies show that the spread of herbicide-resistant GM plants is not economically sustainable even in developed countries and has not increased yields: e.g. cotton in southern USA. [557]

With these new technologies it is possible to alter the most intimate and important part of the characteristics of living beings. Along with the genes that regulate characteristics desired by entrepreneurs, such as the ability of plants not to be killed by herbicides or to produce poisons for insects (protein toxins originally produced by bacteria living in the soil, such as *Bacillus thuringiensis*), other alterations are generated. For example, genes are transferred that, besides not belonging to the plant, may confer resistance to antibiotics or may be promoters of the expression of characteristics that are genes of viral origin. Modifications are generated with effects on the genome and biodiversity that are largely unknown and predictably dangerous.

Another negative effect has been recorded in the case of the cultivation of plants that produce bacterial proteins with an insecticidal action. *Bacillus thuringiensis* (Bt) is a sporigenous bacterium that lives naturally in the soil and is capable of producing proteinic toxins. When the endotoxins produced by these microorganisms are ingested by some insects, they damage their digestive tract, as happens in the case of the larvae of diptera, such as mosquitoes, or cause a paralytic disease in the caterpillars of many lepidopterans (butterflies and moths). Adverse effects on the health of pollinating insects have been reported for some time: genetically modified soya plants damage bumblebees due to the presence of a protein (protease inhibitor). [361] The extensive use of herbicides also impoverishes biodiversity and reduces the flowers available to pollinators.

These toxins are used in the formulation of insecticides for organic farming and industrial agriculture, but also for the creation of transgenic plants (such as corn) able to produce the toxin themselves. The toxins of the bacterium *Bacillus thuringiensis* are also used in beekeeping, in the biological control of the beeswax moth. [204] Another negative aspect is that the insects have become resistant to the toxins produced by these genetically modified plants such as maize. [451] The development of resistance has been accelerated by the monocultures that follow one another every year in the same fields.

The use of genetically modified cotton plants, which produce bacterial toxins (proteins) with an insecticidal action (Bt), has led to a decrease in pollinating butterflies. [196] Moreover, in the case of genetically modified cotton grown in India, target insects (parasites of the cotton plant such as *Pectinophora*: red cotton moth or worm) resistant to the toxins have been recorded. Another enormous damage is due to the fact that genetically modified cotton seeds have to be bought every year by Indian farmers and cost up to eight thousand times more than the traditional ones, which in the meantime, due to the monopoly, have almost disappeared from the market. [205] Just in India it is interesting to remember that there are still hunters of honey produced by wild bees and that, unfortunately, the agricultural work of the manual pollinator had to be created due to the absence of the necessary insects (workers who brush the pollen on the flowers in the orchards). [196] In order to be able to implement manual pollination, for example of apple orchards in China, pollen must first be procured, so it must be collected, stored, marketed, and moved for pollination to the places where it is needed. [208] These are enormous and unsustainable costs: it is better to replace the crops with others that do not need pollinators. Even in other crops, such as tomatoes in Australia, instead of using the bumblebees a manual pollination is carried out with special sticks: in this continent the importation of bumblebees is prohibited (it is a foreign species). [688]

In conclusion, biotechnology has made it possible to concentrate food sovereignty in a few private companies. The food security of most of the inhabitants of the Planet depends on them and, in particular, of the richest ones. Economic rules have not considered biological and ecological laws, and they plan profits for a very limited period of time. This habit is unsustainable and dramatic because it favours the spread of poverty and is based on a fragile agro-chemical model, because it does not consider planetary limits.

THE PLANNED OBSOLESCENCE PLANTS

Technological innovation produced by research in the field of biotechnology allows the generation of real biological monsters, which could not exist in nature as they would become extinct. Genes modified in the laboratory to generate plants with sterile seeds are called *terminators*. Biotechnology allows the generation of sterile plants, or even plants that may require a chemical application to stimulate fertility each year: the grower must incur expenses to obtain the chemicals and reproduce the plants himself. Plants may be designed with sterility as a normal condition, but sterility may be converted to fertility with the application of an external stimulus that restores the plant's viability. To resurrect the seed (they are called *zombie* genes), the farmer or grower must use an external stimulus (such as chemicals under patent) to restore seed fertility. This is a new type of programmed obsolescence called reversible transgenic sterility. One can immediately see the economic power of such a technology, which allows the very dangerous possibility of control and monopoly by entrepreneurs. The community should have the ability to prevent science from becoming such a dangerous weapon. Such technology makes it possible to prevent farmers from cheating: it is a kind of protection against copying. Another worrying aspect is that farmers who buy genetically modified seeds, which produce fertile seeds, have to sign a contract committing them to buy them every year (even if the seeds are fertile).

A complication that is difficult to control could be the following: if a field is contaminated with pollen containing the *terminator* gene, the resulting plants will have sterile seeds, so the reproductive cycle ends in other fields. If the contamination occurs through the *zombie* gene, the resulting plants will require a chemical to make the otherwise sterile plants fertile again. The chemical substance is covered by a patent and, therefore, will have to be purchased: even if a farmer does not buy genetically modified seeds, he can have his harvest contaminated by crops in the vicinity. This condition of a monopoly that destroys biodiversity and subverts the rules of nature should horrify us and, therefore, spur us to act accordingly. It has been possible to apply the principles of programmed obsolescence, controlled by a few entrepreneurs, to food and nature. From the point of view of the entrepreneurs, this technology is a true masterpiece, permitting a complete and continuous control over food production and not only, as it happens with cotton. This type of biotechnological application is one of the highest expressions of the capacity of human ingenuity to destroy biodiversity and the local knowledge of farmers. It is a new expression of oppression, domination, and colonization. It is also a new form of warfare in which those who are defeated will die poor and starving. We allow, with apparent indifference, mankind's ability to feed itself to be monopolized and controlled by a few private companies throughout the World. It is a perfect but diabolical plan to control the ability of human beings to subsist and thus survive. In short, this plan involves having to buy seeds every year, forever: wheat, corn, soybeans, cotton to name a few. Patent protection regulations should be dismantled, because they allow a few entrepreneurs to control food and therefore peoples, and permit the devastation of natural balances. Still today, fortunately, more than a billion people depends on stored seeds as a primary source of food: they have not yet been colonized and, at the same time, they are resilient in one of the most important aspects during emergencies and crises, that of food self-sufficiency.

AN EXEMPLARY PROJECT OF PLANNED SLAVERY: THE HYBRID CORN

Seeds with sterility traits were popularized in the 1990s, but technologies to prevent farmers from using the seeds have been applied by seed companies for at least 70 years. Over the decades, ruthless entrepreneurs have invested heavily in developing hybrid seeds, especially for corn. Hybrid seeds have to be bought every year because successive generations gradually lose their original characteristics. We could say that they are automatically copy-protected and are part of the larger program of *planned obsolescence*. It is not worthwhile for the farmer to save the seeds and reseed them because he would have a lower quality crop, so he has to buy them every year. Planned obsolescence in this case generates *planned slavery*.

The introduction of hybrids into agriculture dates back to the first half of the last century and has allowed seed companies to secure huge profits. Many commercial varieties of major crops such as wheat, soybean or cotton are not hybrids and are wind-pollinated. A significant story of this new form of colonization and subjugation has been recorded in Italy since the end of World War II and concerns corn hybrids. We must remember that maize in Italy became established around 1530, imported from the New World. [737] From every quintal of seed it was possible to hope to obtain more than four quintals; a part was used and another part was kept for sowing the following year: the seeds of the best plants. In this way, plants with characteristics best suited to each particular environment were selected and self-sufficiency and territorial food sovereignty were guaranteed. Immediately after the Second World War, the United States helped with reconstruction in Europe and this also influenced the agricultural sector. From simple food aid, in the space of a few months after the war, reconstruction assistance was formally aimed at changing agricultural techniques. It was a time of crisis where hunger and unemployment were widespread throughout Europe. Help from the United States was therefore necessary in a post-war Europe gripped by hunger. Among the actions undertaken, there were:

- the economic and food aid plans of the United States (e.g. the Marshall Plan);
- the facilitation of imports from the United States into Europe (e.g. the reduction of duties on agricultural machinery).

Among the activities to support the Italian livestock industry, there was the import of maize. At the beginning of the Nineties the World corn market was dominated by the USA (at least 80%). In 1949 the imported hybrid seeds were covered by patents and cost more than twice those already on the market. More than 70 years ago colonization had begun by the American industry through its monopoly of the ability to produce food and feed based on maize. [711] Various strategies were used to encourage the entry of hybrid seeds into the market, replacing the fertile ones that had been present for five centuries. Government funding was provided so as to reduce the purchase price of hybrid seeds by farmers. In this first phase the price was made artificially similar to that of the fertile seeds present in the market, thanks to the financial intervention of public resources. Agronomic experimental stations were also financed, with the intention of trying out new varieties and demonstrating the potential advantages of these patented crops. The strategy, already in 1950, was very clear: convince the farmers to use the new plants and abandon those they had sown up to that moment. In a few years, farmers would become completely dependent on the seed industry, and that is what happened. [711] A few American entrepreneurs, in a very short time, secured the majority of the corn seed market. In order to favour the conversion from a self-sufficient agriculture in seed production to a completely subservient one, different social spheres were involved. In Italy at that time, politics, trade unions, and religious organizations were involved. In the 1950s, through parishes and agrarian consortia, seeds were distributed free of charge to over 25,000 small farmers living mainly in the Po valley. Capitalist and monopolized agriculture quickly took over. The anti-Americanism and anti-capitalism present in the post-war period and spread by the Italian left could not counteract this change. [711] The enterprise of colonization and deprivation of territorial food sovereignty was sealed by the blessing and active participation of the Church. In the first phase of colonization a part of the hybrid seeds was given as a gift and a part was sold at reduced prices, because it was supported by funding. To the experts of the time it was immediately evident that there was an imbalance between the funding allocated to national public research and the subsidies to encourage the sale of hybrid seeds by private companies from another continent. The artificial instrument of the subsidy favoured the diffusion of seeds which were not suitable for regeneration by farmers. The promoters of the diffusion of new seeds were well aware that with the passing of time the problem would be solved quickly because of the reduction in the germinability of local varieties. Seeds can be stored a few years, after which they can no longer be used to generate new seeds.

With the intent of promoting hybrid maize, a real advertising campaign was organized in the press and through the diffusion of videos designed for a non-literate public. It should also be remembered that in 1944 American troops brought the organochlorinated insecticide DDT (dichlorodiphenyltrichloroethane) which contributed to the eradication of malaria by killing the mosquitoes that spread it.

Hybrid seeds made it possible, in many cases, to record an increase in yields, but even then some defects were evident, such as being suitable mainly for animal rather than human consumption (polenta), and being more demanding in terms of chemical fertilization and pesticides. They required new machinery, such as seed drills, and needed irrigation. Most significantly, they forced farmers to incur a new cost, that of having to buy seeds every year. It was hoped that the increased cost of cultivation could be offset by the increased yields. Through the spread of non-reusable hybrid seeds, in effect, the population was being forced to reduce human consumption of maize to favour its use in animal husbandry. At the beginning of the 1950s, further factors of food insecurity were added in a period of serious crisis. In those years the lower nutritional value of the new hybrid seeds was already known, determined through biochemical analysis (for example a lower content of digestible proteins was identified: about 6.7% less).

The danger of the loss of local varieties capable of being produced autonomously came true. The local varieties were different and better adapted to the territorial environmental characteristics but the diabolical process of social engineering won. The uniformity of the landscape resulting from the reduction of biodiversity and the use of expensive technologies (machines, fertilizers, pesticides, biotech seeds, and irrigation) spread primarily in Northern Italy, where in 1971 most of the national maize cultivation was concentrated. In those years local varieties had almost completely disappeared: in 1981 hybridized maize covered about 92% of the national maize-growing area (in France, already in the '70s, it occupied more than 90% of the maize-growing area). [711]

Another negative aspect was the general trend of abandoning crop rotation in favour of monoculture. One consequence was that in the 1980s drinking water in the Po Valley became contaminated with the herbicide atrazine.

In Italy, in 2010, the majority of Italian farmers bought all maize seeds which are 99% hybrids. Corn may be found in animal feed or as oil, in dozens of products intended for human consumption: mayonnaise, snacks, frozen desserts, fruit in syrup, vitamin pills, etc. The sugary syrups derived from maize may be used as a feed for honeybees, replacing nectar from flowers. So maize is also cultivated to meet the needs of beekeepers. In the 2000s, corn cultivation was over-subsidized to generate an agrofuel: methane. The financing of biogas production, also called agro-methane, is another type of speculation that incredibly has not been hindered, generating an enormous waste of public resources. [741]

Other consequences of this colonization have been the involution of public research and the reduction of the capacity to contrast private interests. In Italy, at the same time, technological subordination and food dependence on foreign companies increased. The economic miracle of the 1970s generated an increase in *per capita* income that allowed people to eat more meat and less polenta and, therefore, Italians came to consume greater quantities of corn, but indirectly through animal feed. The increase in wealth allowed the disconnection from the precious and delicate ecological balances that were voluntarily compromised in an irreversible way. This temporary independence from biological rules was an illusion because, after years of blindness, the ecological disasters resulting from short-sighted choices are very evident even to those who have no interest in seeing them.

The monopolized technology package for corn today includes a further leap forward with genetically modified plants, such as corn that is resistant to herbicides like glyphosate. In this case, the farmer is also forced to buy certain pesticides subject to another form of monopoly, that of patenting. We have increased dependence on a few companies and increased profits for the sellers of this patented biotechnology. We have given away food sovereignty and devastated agricultural ecosystems without taking care of them as we should have done. The loss of biodiversity and the disappearance of the peasant culture that advocates self-sufficiency in food were irresponsibilities that will cost dearly.

In 2012 at least five European countries were cultivating genetically modified maize: Spain, Portugal, Czech Republic, Slovakia, and Romania. The other countries, such as Italy, could import it for zootechnical purposes (in Italy, cultivations of genetically modified plants, such as those of corn in the provinces of Udine and Padua, have been discovered several times). [987] This story tells us of the failure of the possibility of spreading a less polluting, more eco-sustainable, and self-sufficient agriculture.

Alarm bells that should have woken us up long ago include:

- the reduction in biodiversity, such as the extinction of pollinators and an increased mortality of honeybees;
- the reduction of soil fertility;
- water pollution;
- the appearance of super pests (pesticide-resistant vegetables and insects);
- the exaggerated use of fossil energy per calorie produced (a ratio of more than 10 calories invested for each one obtained in food).

These are some of the paradoxes of modern agriculture that can no longer be ignored. The condition that has become utopian, i.e. farmers keeping their own seeds for the following year, is the only one that is sustainably viable, but it is not currently possible. Probably the only way we can socialize the benefits of biotechnology is to give a serious encouragement to public research. Currently, biotechnological knowledge is often an oligopoly of big corporations whose primary goal is economic profit. Letting food sovereignty and security be monopolized by artificial economic rules is nothing short of self-defeating.

Unfortunately, we do not learn from our mistakes and even persevere by raising the level of negative pressure on ecosystems that are already collapsing. Genetically modified plants together with the use of pesticides (e.g. neonicotinoids in corn seeds or herbicides in biotech crops made resistant to these molecules) allow us to trace a history similar to this past but current one. Over time the destructive power of biotechnology applied in agriculture has increased enormously.

THE COLLATERAL DAMAGE TO THE COMMUNITY AND FUTURE GENERATIONS

Most agricultural systems are completely dependent on big companies, which have a monopoly or oligopoly on the supply of seeds and pesticides. In many cases farmers have no choice between, for example, traditional seeds and seeds from genetically modified plants. Biotechnological innovation favours the concentration of power over food sovereignty in a few large companies, making the system fragile and unsustainable. A negative consequence is that some monocultures are becoming predominantly planted with genetically modified vegetables, such as maize, soy, and rape. Undesirable effects generated by the widespread cultivation of genetically modified organisms include the following:

- The costs faced by farmers have increased.
- Territorial autonomy (what to cultivate and how to cultivate) and food security have decreased: alternative cultures to chemical agriculture are almost extinct.
- The influence of big companies on ethical, environmental and health choices has increased.
- Genetically modified crops can spread genes among wild species, leading to impoverished biodiversity, ecological problems, and insecurity (poverty and hunger). Genetic traits that confer herbicide resistance have already led to the emergence of plants with the potential to become super-weeds. Oilseed rape plants, the result of natural hybridization in Canada, have been recorded as being resistant to three herbicides at the same time: glyphosate, glufosinate, and imidazolinone. [694]
- Wild plants that receive pollen from genetically modified plants may become weaker.
- Pollen and nectar from genetically modified plants are the food of many insects with partly unpredictable effects. Through pollen and nectar both genetic modification and toxins may be moved through the environment and the food chain.
- Insects get resistant to toxins produced by genetically modified plants: caterpillars of butterflies feeding on plant tissue become resistant to toxins such as those of *Bacillus thuringiensis*. At least 500 species of pesticide-tolerant plant pests have been recorded.
- Toxins with insecticidal action - and in the tissues of genetically modified plants - are produced throughout the plant cycle. Pests, such as the corn borer, will be constantly exposed to a selective insecticide pressure and get resistant more easily. In order to reduce the selective pressure, which is counterproductive for agriculture, part of the adjacent agricultural area could be cultivated with plants that do not produce these insecticidal toxins. However, plants must be used that are not able to receive pollen from genetically modified plants. In fact, organic production could suffer serious economic damage.
- Toxins like those of *Bacillus thuringiensis* remain active in the soil even after 234 days. [694] Thus, both invertebrates and microbes in the soil are exposed to this toxin. An earthworm (*Lumbricus terrestris*) exposed for 200 days to this toxin exhibited sub-lethal effects such as weight reduction. From soil invertebrates, the toxin could move up the food chain through predators.
- The increased use of herbicides in crops of plants genetically modified to be resistant to high concentrations of certain molecules is causing an ecological disaster. Unavoidable side effects include the reduction of wild plants, pollinators, and birds. In addition, there are the health problems generated by residues in food and water. Herbicides destroy wild grasses and reduce the presence of insects that prey on crop pests. Thus a natural competition useful to farmers is diminished. Herbicides remain in the soil exposing microorganisms and invertebrates to potentially harmful effects. Persistence in the environment generates contamination of the food chain even among wild organisms.

The antibiotic effect of glyphosate (an herbicide) on soil micro-organisms that are important for mycorrhizal formation and nitrogen fixation has long been known.

- An increase in genetic homogeneity in the cultivated area, as genetically modified plants encourage intensive production systems using high amounts of energy and chemicals (fertilizers and pesticides). Resilience and biodiversity are reduced due to the simplification of the food production system.
- Some insectivorous insects are reared for biological control (e.g. *Chrysoperla carnea*). Sub-lethal effects have been recorded when these insects are reared by feeding on herbivorous insects, which are in turn fed with genetically modified plants capable of producing the *Bacillus thuringiensis* toxin. The toxin passes from the plant (genetically modified maize) to the herbivorous insect (larvae of the corn borer) and from this to the predatory insect (*Chrysoperla carnea*) increasing mortality and causing a delay in the life cycle. [694] *Chrysoperla carnea* is bred in some biofactories in America and Europe to be used in biological control, mainly in protected crops (greenhouses), but it is also very common in nature, in agrosystems where there is no massive use of pesticides. Contrary to other Chrysopidae, often predatory, the adults of *Chrysoperla carnea* feed on honeydew, nectar and pollen. The adults can move for distances of the order of some tens of kilometres. The larvae are zoophagous predators and feed on small arthropods or eggs. The preferred prey are aphids, but they can also prey on mites, psyllids, white flies, mealybugs or eggs of mites, lepidopterans and beetles. [625]

The most cultivated genetic modifications in the World, resistance to herbicides and the ability to produce toxins with an insecticidal action, generate direct and indirect environmental damage. The use of herbicides increases and toxins enter the food chain. The long-term effects of the spread of genetically modified plants on biodiversity and soil health remain largely undiscovered but, as far as we know, are likely to be negative. Applying the precautionary principle in this case would be a sign of foresight and love for future generations. It is risky, to say the least, to wait for the scientific evidence to be numerous, unquestionable, and accepted by the majority of the players involved (entrepreneurs, farmers and politicians). However, many dangers have been ascertained and are amply documented.

ENERGIES FROM BIOMASSES: AGRO-METHANE OR BIOGAS

MANY AGAINST A FEW

Oil, gas and coal were created in specific geological conditions that are not so easily repeated, especially not in times compatible with the current rates of extraction. For all non-renewable resources, it is clear that a peak is reached, after which production inexorably begins to decline. Consequently, it is necessary to prepare for the change in order to avoid it being sudden and, therefore, catastrophic. If we try to burn all available fossil fuels, the Planet will surely become very inhospitable and unlivable before they are exhausted. The known stocks of oil reserves, if extracted, could generate an increase in the concentration of climate-altering gases more than the double of the current one (e.g. carbon dioxide). Renewable energies, such as biomasses (e.g. firewood) or solar energy, must also be adapted to a system that has enormous limits. Biomasses take land and resources away from food production, and solar panels require materials that are available in finite quantities, and also occupy agricultural land and require oil to be built. Therefore, important choices are on the horizon that can only be reasonably made if aspects that are currently underestimated, such as ethical and environmental ones, are also considered.

Already in 2009, 3% of road transport fuels were agro-fuels: agro-ethanol (from sugar cane, maize, and wheat) and agro-diesel (from palm oil and soya). In 2009, 54% of agro-ethanol was produced in the USA (from corn) and 35% in Brazil (from sugar cane). [36] In the USA at least 10% of the liquid fuel used for cars is ethanol derived primarily from corn. On the Planet, in less than twenty years, the production of agro-fuels has increased almost 10 times (from 9 billion toe or tons of oil equivalent in 2000 to over 84 billion toe). [979]

The choice of whether to produce agro-fuels or food corresponds to having to choose between ensuring a lifestyle based on consumerism for a few rich people or avoiding extreme poverty. Just remember that in countries like the United States of America about 40% of the corn grown is converted into fuel (ethanol) for cars. [5] If the corn grown in the USA to generate agro-ethanol were destined for human consumption, hundreds of millions more people could be fed (at least 330 million according to some estimates). [985]

In 2010, World agriculture produced the equivalent of over 5,300 kcal *per capita*, more than enough to feed the entire World population. Unfortunately, a large part of this energy is used to feed farm animals or is wasted to produce agro-fuels and at least a third ends up in the trash. We would not need to produce more food if we were forward-looking and altruistic. The consumerist folly leads to an enormous waste such as the use of almost a third of the agricultural land to produce vegetables that will not be used for human consumption.

Agro-fuels derived from maize, wheat, and sugar cane, used to produce ethanol as a substitute for oil in road transport, will not be able to supplant fossil fuels, nor can they be a viable alternative even for a fraction of the energy consumption, unless we accept to increase the social and economic gap in the population and to foster negative environmental effects (e.g. water pollution and soil degradation). Furthermore, extending the waste of agricultural land to drive cars will increase the worsening of the climate change.

The story of agro-fuels effectively represents, in Italy, the inability of the community to defend itself from itself and from the selfish interests of a few. [954] This story highlights the struggle of a few against many, where temporarily a few are winners, but at the end of the game all will be losers. The following pages delve into the story of an enormous waste of opportunities, time and economic resources. It is about disproportionate squandering that has favoured the improper and uneconomic use of the territory. The sad story is recounted in detail in a publication of mine that was awarded, in 2017, the National University Publishing Award in Italy. The following is some information extracted from this book (only available in paper form for a fee and in Italian): *Energie rinnovabili da biomasse: rischi e opportunità. Coltivazioni, allevamenti, compost, biogas e agro-carburanti: analisi degli impatti ambientali, delle ricadute sulla salute e della sostenibilità* (EPC, 2016); the title translated into English: *Renewable energy from biomass: risks and opportunities. Crops, livestock, compost, biogas, and agrofuels: analysis of environmental impacts, health impacts, and sustainability* (EPC, 2016).

The incentives provided for the production of electricity from anaerobic fermentation have encouraged an unsustainable behaviour, such as the authorization in 2011, in the Province of Cremona (Italy) alone, of more than 120 plants implementing anaerobic fermentation of large quantities of cereals to produce methane. [954] This is an area with a high density of livestock farms and with insufficient availability of land to implement the agronomic use of digested products.

In Italy, in just a few years, thanks to public incentives, more than 1,100 plants producing methane using mostly cereals have been built. The design was not the result of environmental assessments, but was conditioned by the incentive mechanism. We are rewriting a story, summarizing it, with the hope that it will not be repeated, because it is a voluntary ecocide, as it was planned and supported by administrators and politicians, who, in fact, were enslaved to private and selfish interests.

THE PRODUCTION OF AGRO-METHANE

One of the technologies that can be used to produce electrical and thermal energy is represented by the production of the gaseous fuel methane, through anaerobic fermentation from biomasses such as: zootechnical dejections, by-products of the food industry (e.g. waste from the processing of vegetables, such as tomatoes), the solid fraction of urban waste and sludge derived from the depuration of urban waste water (water from the sewage system). [951]

For more than 35 years, waste water treatment plants and landfills in Italy have generated methane produced by anaerobic fermentation for energy purposes. Fermentation is carried out by microorganisms that are also contained in the digestive tract of animals such as cattle and pigs and that produce methane in anaerobic conditions, i.e. in the absence of oxygen (oxygen makes up at least 20% of the air we breathe, nitrogen 78% and carbon dioxide 0.04%). For this reason, fermentation is also called anaerobic digestion.

Anaerobic fermentation is a biochemical process based on the demolition of complex organic substances, such as lipids, proteins, and carbohydrates, contained in plants and matrices of animal origin (e.g. feces), carried out by different microbial groups. [951] It is possible to consider the fermenter as a container of a liquid substrate in which microorganisms are reared, which, like all living beings, require specific optimal conditions to live and reproduce. The advantage of this type of breeding is that, if certain conditions are met, the microorganisms produce methane and reduce the amount of organic matter, thus reducing the potentially polluting load of the matrices they ferment. This aspect is highly desired in a landfill of organic waste or for a sewage treatment plant. The plants operating in mesophilic conditions, i.e. between 30°C and 40°C, are the most widespread in Italy and use part of the energy produced to heat the fermenters, which will be insulated. The fermentation process in the liquid phase will last between 15 and 50 days.

A plant implementing anaerobic fermentation consists of a series of tanks for digestion and storage (tanks for the liquid fraction of the digested product), and an area where some matrices such as silage are stored (e.g. tanks for corn, for manure, for the solid fraction of the digested product, underground tanks for livestock slurry). Altogether, there will be several tanks of different sizes and some rooms for the management of the process (offices, electrical cabin, storage, toilets), for the engine that burns methane (the co-generator), for the storage of vehicles and equipment. There will also be a biogas tank, which can be located above the tanks and also in a dedicated area. The biogas is collected in the upper part of the tanks, through a gasometer dome cover and possibly in other storage systems. The gasometer dome is in the form of a semi-cylinder or spherical dome and can be made with three overlapping membranes.

Theoretically, any organic matrix can be used to feed the microorganisms that carry out anaerobic fermentation to produce methane. Like all living beings, the different microorganisms generally prefer certain nutrients, in well-defined concentrations and proportions. By suitably mixing different matrices at the fermenter inlet, nutrients may be guaranteed in the most suitable concentrations. For the production of agro-methane (biogas), matrices that can be used as fertilizers should be chosen: this criterion is very important.

Anaerobic fermentation produces biogas that contains methane in varying concentrations, along with carbon dioxide and other gases. The composition of biogas is similar to that of natural gas. The biogas produced by anaerobic fermentation is composed of:

- 50-65% methane (the percentage will increase if certain organic substances in the fermented materials such as lipids are increased);
- 35-45% carbon dioxide;
- 1-10% water vapour (that increases with temperature);
- 0-5% nitrogen;
- 0-2% oxygen and other gases.

The combustion of methane, which is the most abundant fraction in biogas, allows the production of electrical and thermal energy. The combustion takes place at temperatures above 850°C. Biogas has a density of 1.2 kg/m^3 and 1 m^3 corresponds to 0.6 L of oil or 2.7 kg of wood. The fuel (methane) and the comburent (air) will arrive at the burner which will produce heat through the flame. The heat produced with the combustion of methane represents more than 50% of the energy that can be obtained.

Biogas plants implement co-generation if they are able to produce both electricity and heat. The biggest problem is that heat cannot be transported or stored easily, so it must be used as soon as it is produced and close to the site. A plant working in mesophilic conditions uses between 11% and 20% of the thermal energy produced to maintain mesophilic conditions (depending on the climatic conditions).

Biogas, in order to be introduced into the methane distribution network (it is often also called bio-methane), must have a composition that is regulated. From biogas, with appropriate procedures, gases other than methane can be eliminated in order to reach concentrations of methane higher than 90%. Therefore, biogas obtained from the fermentation of livestock manure or landfill must be purified before it becomes bio-methane, which may be used for transport or domestic heating. Purification is an expensive and, therefore, uneconomical process, so this route is not competitive with producing electricity at the point of biogas production and feeding it immediately into the distribution network.

Energy production through anaerobic fermentation can be implemented during the period of time that manure is normally stored before it can be used as a fertilizer (usually between three and six months). Instead of temporary open storage, as is usually the case, manure could be stored in closed, insulated volumes with the recovery of the biogas produced.

Unfortunately, many plants (most of the plants financed in Italy) have implemented co-digestion, which means that specially cultivated vegetables, such as maize, sorghum, and triticale, are mixed with livestock manure. Therefore, cereal production such as corn, destined primarily for animal feed and secondarily for human consumption, is fed to microorganisms that come from the digestive tract of cattle or pigs and that, living in conditions of anoxia, are able to produce methane.

The digested output, i.e. the organic material in the liquid phase resulting from fermentation, can be mechanically separated into two fractions, one solid and one liquid. The liquid fraction will have a lower solids content (3-5% dry matter) and will make up most of the volume.

The final product coming out of the anaerobic fermentation plant is an organic material that has the potential to be used in agriculture as a fertilizer and, as already mentioned, is called digested. In the World, the digestate resulting from the anaerobic co-digestion of livestock effluents is used for the following purposes:

- Improvement of soil fertility (respecting certain health and agronomic criteria). The digested will be rich in nutrients such as nitrogen, which will be present mainly in the ammoniacal form that is easily used by plants, but is easily dispersed in water and the atmosphere.
- Drying and subsequent combustion for energy production.
- Aerobic fermentation for compost production (the solid fraction of the digested product is used).
- Production of house fly (*Musca domestica*) larvae for use as feed (larvae/digest conversion by weight can reach 22%). [892]
- Growth of algae used for various purposes by the chemical industry or for the production of feed and fertilizer. [893, 894]
- Integration of some nutrients in aquaculture. This application is exploited in Third World countries and China. [895]

In Italy, the prevalent use is agronomic, i.e. digestates are used as if they were livestock manure.

The production of electricity from agro-methane, obtained from the anaerobic fermentation of livestock manure and cereals, has been classified as a renewable energy source, on a par with wind, solar, geothermal, and hydroelectric power.

In some countries, such as Italy and Germany, the construction of plants implementing the anaerobic fermentation of cereals has been profitable thanks to the use of public economic resources by private individuals who, therefore, have simply taken the risk of anticipating the expenses. These costs were largely recovered, within a few years of the start-up of the plants, thanks to State funding. Regulation has not sufficiently selected the projects capable of ensuring the expected benefits of so-called renewable energies. In Italy, most of the plants, which produce methane from the anaerobic fermentation of zootechnical dejections and cereal crops, are located in Northern Italy, mainly in Lombardy, Veneto, Piedmont, and Emilia Romagna: the plants that managed to receive financing (all-inclusive tariff) had a power between 501 and 999 kWe, as the incentives favoured this size (Italian Law Decree no. 387 of 29th December 2003).

THE ADVANTAGES OF ANAEROBIC FERMENTATION

The declared intent, noble and shareable, was to encourage the production of electrical energy and heat favouring the reduction of carbon dioxide emissions into the atmosphere. By convention it has been established that 1 MW_e/h produced by the anaerobic fermentation of biomasses avoids the emission of 456 kg of carbon dioxide (CO_2) from fossil sources (Italian Law Decree no. 79/99); this quantity of carbon dioxide is that produced by a gas turbine with 55% efficiency, which produces 1 MW/h of electricity.

Anaerobic fermentation of livestock manure alone generates some advantages:

- It produces electricity and heat. Usually at least 10-20% of all the energy produced is needed to run the plant in mesophilic conditions (the temperatures suitable for the life of intestinal microorganisms). The percentage of energy needed to keep the temperature of the fermenters constant will be higher in winter and in colder climatic zones. Some of the energy may be used for other purposes such as electricity and heat generation.
- Emissions of climate-altering gases such as methane from manure storage (or sewage treatment) are reduced. [961]
- The product obtained at the end of the fermentation, called digested, presents a quantity of organic material inferior to the input one. Therefore, the biogas production process determines a reduction of the organic substance present in the zootechnical manure.
- It does not reduce the amounts of nitrogen and phosphorus, so the digestate can still be used as a fertilizer. The digestate can be used as a replacement for chemical fertilizers, similar to livestock manure.
- It transforms part of the organic nitrogen into ammonia nitrogen which is more easily assimilated by plants, but has the disadvantage of being odoriferous and more mobile in the environment. Ammonia will concentrate in the liquid fraction of the digested. The increase in ammonia may be an advantage or a disadvantage, depending on how the digested is used.

It must be clarified that the biogas production process of livestock manure alone does not solve the problem of the "disposal": the product resulting from biogas production, the digested, has a greater volume than that of the incoming matrices, due to the necessary dilution with water, especially of manure. With co-digestion, the volumes to be managed for storage and spreading will increase considerably, as the biogas plants will enter matrices that have a dry matter content of 20% for cattle manure and more than twice that for corn silage, and will produce a digested product that will have 6-10% dry matter. Therefore, the volumes to be handled will increase with the consequent costs for storage and transport.

Theoretically, in Italy, using only a part of the waste produced, it would be possible to obtain the equivalent of more than one and a half million tons of oil equivalent. The following potential productivity of biogas could be exploited: from the organic fraction of municipal solid waste, from wastewater treatment plants, from slaughterhouse waste.

Methane (CH_4) emissions from the waste (landfill) and sewage treatment sectors are estimated to account for 18% of all methane emissions from human activities. [940] If all the methane potentially available in landfills and sewage treatment plants in Europe were used, this would provide a significant advantage, including the reduction in the use of fossil fuels. Theoretically, an amount of energy (methane) equivalent to at least nine million tons of oil equivalent (9 Mtoe) could be obtained from waste and wastewater treatment in Europe. [941]

The agro-energy contribution to plants implementing anaerobic fermentation is preferably obtained from waste rather than from dedicated vegetables such as triticale, sorghum, and maize. Ethical and environmental aspects and competition with the food chain should be sufficient reasons to discard this possibility. The production of energy from waste biomass should be privileged, exploiting and using the most efficient collection and energy transformation techniques. However, it should be remembered that, almost always, materials such as straw, corn stalks, food company waste or slaughterhouse waste have been recycled, for example, to make animal feed (e.g. slaughterhouse waste is used to make pet food).

Not removing plant parts from the field means leaving organic and inorganic substances in the soil, so as to favour the maintenance of fertility and the reduction of the need for external inputs (e.g. chemical fertilizers). It happens, for example, with fruit or cereals, and it is considered a good agronomic practice, if well managed; in some cases, however, the practice of leaving part of the cultivated vegetables in the field has contraindications, such as that of favouring the spread of plant pests. Therefore, before using food industry waste for biogas production, it will be necessary to make thorough assessments, considering all aspects.

AGRO-METHANE FROM CEREALS AND LIVESTOCK MANURE

Biogas has a calorific value (lower calorific value)[I] of about 5,000-7,000 kcal/m^3 (equivalent to about 6-8 kWh/m^3) and a density of 1.2 kg/m^3; in comparison, methane has a density of 0.72 kg/m^3 and can provide about 9-12 kWh/m^3. Pure methane has an energy content of 8,380 kcal/m^3, while biogas has a lower energy content than methane, as it contains carbon dioxide and other gases. It can be considered that one thousand litres (1 m^3) of biogas is equivalent to about 0.6 L of diesel fuel.

Using special combustion engines, the biogas can be transformed into electrical energy (with a maximum efficiency of about 21%) and into thermal energy (with a maximum efficiency of 50%). [941] The high concentration of water and the presence of fibres are the main cause of the low production of methane from the anaerobic fermentation of manure: between 10 and 20 m^3 of methane per ton of manure treated. Thus, the digested manure will still have some organic matter potentially usable, for example, by plants through fertilization.

It is possible to obtain 230 m^3 of methane per ton of maize silage, compared to about 20 m^3/t from cattle manure without straw. [920, 942, 943] As one would expect, for the same weight, one gets ten times more biogas from corn. For comparison, it is possible to obtain 42 m^3 of biogas per ton from beet residues and 35 m^3/t from tomato residues. The vegetable matrices with the best energy yields are those with high concentrations of easily biodegradable carbohydrates (such as starch), and with proteins poor in hemicellulose and lignin that have a low biodegradability. [948]

From 20 m^3 of methane (theoretically obtainable from 1 m^3 or 2 m^3 of biomass such as livestock waste; biogas has a variable methane content, usually between 50 and 70%) one can hope to obtain an energy production of maximum 133 kWh. If we consider that 1 kWh corresponds to 860 kcal and that 1 litre of gasoline provides about 12 kWh, the 133 kWh (obtainable from 1 m^3 or 2 m^3 of biomass such as livestock waste) is equivalent to the energy provided by about 11 L of gasoline. [944] Thus, from 1 m^3 of biomass such as livestock manure it is still possible to extract a lot of energy equivalent to between 7 and 11 L of gasoline.

The amount of methane that can be obtained from one hectare of maize varies if you digest the whole maize plant rather than its fractions, such as just the grain (the seeds) or a mixture of cobs and seeds. For the same amount, the grain will produce more energy. The highest energy production per hectare, as is to be expected, comes from fermenting the whole plant. Silage maize, with the same volatile solids, produces higher amounts of methane, up to 25% more than non-dried maize, because it contains less water.

The maximum amount of dry matter that can be obtained from maize is 30,000 kg of dry matter and it can be considered that the amount of methane that can be obtained from the anaerobic fermentation of maize ranges between 5,000 and 10,000 m^3 per hectare per year. [945, 946] The

---

[I] The *upper calorific value* expresses the amount of heat that becomes available as a result of the complete combustion, at constant pressure, of the unit mass, when the products of combustion are returned to the initial temperature of the fuel and oxidizer. Usually, however, the products of combustion are released at a higher temperature than the starting temperature of the fuel and oxidizer. Thus, some of the theoretically available heat is lost in the form of hot flue gases (also consisting of water vapour).

The *lower calorific value* is conventionally defined as "the upper calorific value minus the heat of condensation of water vapour during combustion". This is the value usually referred to when discussing the calorific value of a fuel and the efficiency of a thermal machine. Thus, the lower calorific value will be lower than the higher calorific value and better represents the amount of usable heat. The *heat of combustion* indicates the energy content of fuels and is obtained from the product of the weight, or volume, by the lower calorific value.

methane productivity of the various biomasses depends on the quantity and quality of the organic matter contained and is therefore variable. [947]
The amount of energy that can be obtained from manure is less than that from maize, because during digestion in the intestine part of the organic substances are used (e.g. to produce milk).

Plants producing biogas from anaerobic fermentation, fed with zootechnical waste, in order to increase the energy yield, can co-digest with dedicated crops, such as maize, sorghum, and triticale silage. The methane yield is related to the chemical composition, i.e. the quantity and quality of organic substances. The anaerobic fermentation of maize will produce 9-10 times more energy than the same amount of cattle manure. This is one of the reasons why it seems energetically convenient to use cereals, since the energy and environmental costs necessary to produce them are not considered.
The quantity of methane between 5,000 and 10,000 m^3 per hectare per year from corn fermentation is that necessary to drive about 25,000 km in a year for three small cars (it has been considered that 1 m^3 of methane corresponds to 10 kWh, while 1 kg of oil equivalent corresponds to 11.63 kWh). In fact, this is a purely theoretical calculation that does not take into account the energy costs incurred to produce the methane. This estimate does not include indirect environmental costs, such as those for the production of corn, for the construction and dismantling of the plant after 15 years and many others.
The biogas may be used by a car engine only if it is cleaned from components other than methane (e.g. water, carbon dioxide) and it must be able to be compressed and stored, operations that have additional costs and that reduce the energy yield (about 6% of the energy contained is consumed). In addition, at least 8% of the electrical energy produced will be self-consumed for the operation of the biogas plant itself.
Some reference values for calculating the energy equivalence between 1 m^3 of biogas and other fuels may be as follows:

- gasoline 0.8 L;
- diesel fuel 0.6 L;
- ethyl alcohol 1.3 L;
- methane 0.6-0.7 m^3 (biogas contains methane and other gases such as carbon dioxide);
- charcoal 1.4 kg;
- wood 2.7 kg.

Biogas, considering only the value of public funding of 0.28 euro cents per kWh (all-inclusive tariff), has benefited from a production incentive that is equivalent to paying more than 3 euros a litre of gasoline.

THE SUPPLY OF A BIOGAS PLANT

To obtain the same amount of biogas that can be produced from one hectare of maize, alternatively, it is possible to use the manure of 30-40 cattle or 200 pigs (for fattening). If the plant (0.9 MW_e) ferments only maize, it needs between 500 ha (if the whole plant is fermented) and 830 ha per year (if only the grain is used), without considering the additional hectares needed to ensure crop rotation. If the plant is maintained by maize alone (the whole maize plant may be bought at 50 euro/t), one could waste 1,290,000 euros per year to buy this plant to ferment.

If the plant (0.9 MW_e) ferments only cattle manure, it needs the slurry from about 12,000 animals (between 7,000 and 20,000 cattle weighing between 200 and 600 kg) which in turn requires at least 4,000 hectares to produce feed (between 2,000 and 5,000 ha/year). If all the manure has to be purchased (3 to 5 euro/ton), between 360,000 and 600,000 euros per year may be spent on supply.

The handling of incoming biomass (cereals or manure) and outgoing biomass (the digested product: between 40,000 and 150,000 t per year) may require between 2,500 and 2,700 transport trips per year (one way with 30 t trailers) for plants fermenting only the whole maize plant, or more than 10,000 transport trips if cattle manure is fermented (one way). If all transport takes place within the maximum distance of 10 km it may affect between 4% and 15% of the incentive. If, on the other hand, the supply chain moves within 70 km, transport may affect between 8% and 47% of the incentive, i.e. of the all-inclusive tariff of 0.28 euros per kWh (the supply chain within 70 km is not sustainable if all the maize grain is purchased). These differences are generated by the various possibilities such as the complete self-production or the purchase of all the necessary biomass.

THE ENERGY BALANCE OF MAIZE PRODUCTION

The domestication of the maize plant probably began in 7000 b.C. in America, although the major changes came after the colonization by Europeans. Modern maize, compared to its ancestor (teosinte), generates a cob with a carbohydrate concentration three times higher and over 1,000 times bigger.

The maize plant can be pollinated by the wind and artificially crossing it is easier than with other species. One third of the World production of cereals is made up of maize, which is cultivated on an area of more than 160 million hectares (a part is cultivated with genetically modified plants, at least 50 million hectares): more than 14 times the entire agricultural area of Italy. [985] The main World producers of corn are the United States. It is cultivated in rotation with other crops (e.g. soy or wheat) or, unfortunately, continuously for tens of years. [949] An artificial and simplified ecosystem is perpetuated for tens of years, using great amounts of matter and energy.

In Italy, maize is grown mainly in the central and northern areas. On its own it represents more than the sum of all soft and hard wheat harvests and, despite this, it has to be imported.

To grow maize (*Zea mais* L.) in Italy, it is necessary to irrigate and use chemicals (e.g. herbicides and insecticides). It is sown when temperatures are stably above 10°C, at a density of 6-8 seeds per square metre, depending on the hybrid used. 10 t/ha of grain is obtained and the profit margins for the farmer can be very low.

For the cultivation of maize, used to produce agro-methane, through anaerobic fermentation it is necessary to spend a lot of energy: [741]

- To cultivate one hectare with maize, it may be necessary to spend between 1,300 and 2,200 euros per hectare per year (excluding certain expenses such as transport, ensiling, soil analysis, and the purchase of chemical fertilizers).
- Maize cultivation requires between 600 L/ha and up to 1,200 L/ha of diesel fuel per year. [950] It should be noted that various types of activities require different energy sources; for example, transport and pesticide production depend mainly on petroleum products, while fertilizer production depends on natural gas.
- Irrigated maize can require between 5 million and 14 million litres of water per hectare per year and, therefore, may require at least three times as much energy as is needed to produce the same amount with non-irrigated crops, which will be in wetter areas.
- In order to sustain the main expenses in equipment and insurance necessary to cultivate 500 hectares of corn destined to feed a 990 kW_e biogas plant, considering 5 farms of 100 hectares each, we can estimate to spend at least 5,266,350 euros, VAT included, in 15 years.

- Considering 5 farms of 100 hectares each, it will be necessary to purchase equipment with a total weight of at least 194,250 kg (about 389 kg/ha). [741] Therefore, it may be reasonably estimated, optimistically, to have to commit equipment with a total weight of about 400 kg/ha every 15 years, corresponding to about 27 kg of materials per hectare per year. If we assume that to build one kilo of material (e.g. steel, electrical and electronic components, plastic, rubber) it is necessary to invest three kilograms of diesel, it is equivalent to having to add a virtual consumption of diesel for the equipment between 50 g and 60 g of diesel for each kilo of maize (excluding transport; for some components the consumption could be even 10 times higher than that indicated).
- To transport maize within 70 km of the field (short supply chain), between 130 and 190 L of diesel per hectare per year will be used; this is an additional 12-18 g of diesel per kilo of grain (assuming a yield of 8,000 kg/ha).

Optimistically, it is possible to estimate a ratio between energy invested and energy obtained (for maize production) between 1.1:1 and 1.9:1, in the best conditions (supply chain within 70 km and not considering other costs such as cooking, packaging and transport to the consumer). The ratio is more advantageous if maize is used for animal feed (considering a caloric intake of 2,000 kcal/kg, instead of less than a half from seeds used for human food).
Therefore, to produce 1 kg of corn it is necessary to invest between 100 and 200 g of diesel, not considering all the real consumption. The real advantage obtained from this energy-inefficient system is given by the result that to obtain 1 kg of maize it is necessary to employ less than 2 minutes of human agricultural work in the fields per year (this does not include the labour necessary to build the machines, the buildings, the roads and the labour necessary to extract and produce the fuels; it does not even include the extra work of the health workers due to accidents, illnesses of the workers and of the population). To obtain about 14,000,000 kcal per hectare of maize it is necessary to invest less than 9,000 kcal/ha per year in human labour, which is the energy consumption estimated by calculating a physical effort of about 250 kcal/h for 35 hours/ha per year. If we consider only the work of the farmer in the field, the ratio between energy invested and energy obtained is more than 1:1,600, so it is a very advantageous ratio. However, the conclusion is that the cultivation of corn to produce methane consumes such a quantity of fuel that it is more convenient to use diesel directly, rather than building the biogas chain. [941]

THE ENERGY BALANCE IN THE BIOGAS CHAIN

For the estimation of the economic and environmental sustainability, it has happened to emphasize only the energy balance of some operations related to biogas production. These approximate and partial balances are incorrect and allow supporters of the diffusion of biogas plants to claim that the ratio between energy invested and energy obtained can be as high as 1:25 or 1:29. [943, 950, 952] In reality, biogas production to obtain electricity from cereal cultivation is unsuccessful, as it consumes more energy than is feasible. The production of maize to obtain electricity through anaerobic fermentation, even in the zero kilometre supply chain, consumes more energy than is achievable. In general, transport becomes uneconomical after just a few kilometres. Assuming that less than 30% of the funding from the all-inclusive tariff is spent on transport, manure has to be transported a maximum distance of 10 km and maize a few kilometres more.

A 0.9 MW_e biogas plant that ferments the manure of 12,000 dairy cattle produces an amount of energy that covers only a small part of needs to produce meat and milk. Theoretical calculations, which are a considerable underestimate, show that the energy spent to cultivate 4,000 hectares needed to feed 12,000 cattle, which with the manure feed a 0.9 MW_e biogas plant, is at least 14 times higher than the energy obtained. This estimate does not take into account various items: transport, milk and meat production, the cold chain, packaging production, and waste management, to name but a few.

To obtain energy (electricity and heat), if you burned the entire cereal plant you could hope to obtain a ratio of energy obtained to energy invested 2 to 4 times higher than that obtainable with anaerobic fermentation.

Also the combustion in a boiler of woody matrices, such as the one derived from poplar, could have originated more energy; 50% more than the use of the same surface for the cultivation of cereals. The use of multi-annual crops such as poplar, to produce thermal energy from combustion, generates several environmental advantages such as less soil erosion (in the case of cereals, the land often remains uncultivated in winter), and less use of fertilizers and pesticides. Direct combustion, instead of biogas production, generates also another effect that in the Po Valley could be considered positive: there is no production of digested manure (in this territory the availability of zootechnical dejections is higher than the needs of the crops present within a few kilometres from the farms).

In conclusion, energy from biogas cannot be considered a renewable energy source. The following table shows some data. [741]

Energy balance hypothesis for a 0.9 MW_e biogas plant using only corn (whole plant)

	0.9 MW_e plant using only corn (whole plant) [741]
Self-consumption of electricity produced	Between 10% and 20% of that produced
Consumption attributable to auxiliary services and losses up to the point of delivery of energy to the distribution network	11%
Consumption of thermal energy produced	Between 20% and 30%
Biogas obtainable per hectare	Between 6,000 and 10,000 m^3 (equivalent to between 3,600 and 6,000 L of diesel fuel)
Energy spent to grow 1 ha of maize (excluding transport)	20 Gcal
Electricity theoretically obtainable per hectare from anaerobic fermentation, with an efficiency of 21-22%.	Between 12,000 and 18,000 kWh/ha/year, corresponding to between 10,320,000 and 15,480,000 kcal/ha/year
Area required to power a 0.9 MW_e power plant producing 7,200 MWh of electricity per year (7,200 MWh x 860,000 kcal = 6,192 Gcal)	Minimum surface area: 516 ha (if the whole plant is used, without taking rotation into account; average productivity of 12 Gcal/ha)
Ratio of energy spent on growing maize to energy obtained in the form of electricity (20 Gcal/ha)/(12 Gcal/ha)	**1.7**
Electricity obtainable from the combustion (without fermentation) of the plant matrix obtained from one hectare (an efficiency of 20% is considered)	31,146,000 kcal/ha (55,000 kg x 572 kcal/kg)
Ratio between electrical energy obtainable from direct combustion of the plant (without fermentation) and that obtainable from anaerobic fermentation (per hectare/year, considering an efficiency of 22%)	**2.2** (31,146,000/14,000,000 kcal/ha)
Energy in heat theoretically obtainable per hectare from anaerobic fermentation, with 50% efficiency	Between 25,000,000 and 33,000,000 kcal/ha/year
Heat obtainable from the combustion (without fermentation) of the vegetable matrix obtained from a hectare cultivated with maize (a maximum efficiency of 50% is considered)	Between 60,000,000 and 75,000,000 kcal/ha/year (equivalent to 4,050 - 5,070 L of diesel fuel)
Ratio between thermal energy (heat) obtainable from direct combustion of the plant and that obtainable from anaerobic fermentation (per hectare/year, considering an efficiency of 50%)	**2.3** (70,000,000/30,000,000 kcal/ha/anno)

These estimates do not consider certain energy costs such as those for transport and plant construction, for which it is possible to estimate an energy consumption equal to the energy obtained from anaerobic fermentation conducted by a 1 MW plant in more than one year.

REFLECTIONS ON SOME ENVIRONMENTAL IMPACTS OF AGRO-METHANE FROM CEREALS

Encouraging the use of cereals to produce methane has favoured soil consumption and degradation. Co-digestion of cereals with manure increases the soil requirement for spreading. With co-digestion, i.e. the simultaneous use of livestock manure and dedicated crops such as maize, the amount of nitrogen to be managed, with the same number of animals raised, increases proportionally to the fraction of vegetables fermented in the plant. Co-digestion favours water pollution, eutrophication, and the emission of dangerous gases such as climate-altering ones. The anaerobic fermentation of manure and cereals does not modify the total quantities of nitrogen or even phosphorus. That is, the total amounts of nitrogen in the digested products (at the end of the fermentation process) will be the same as those contained in the incoming manure and cereals. The liquid fraction of the digestate will make up about 80% of the volume and will contain most of some substances such as nitrogen (ammonia).

The nitrogen contained in cereals will return to the environment after passing through anaerobic fermentation, which will transform its different forms into the inorganic, mainly ammoniacal one. Therefore, total nitrogen does not decrease but anaerobic fermentation increases its mobility in the environment. So a plant, if it ferments grain, generates an increase in pollutants to be managed by spreading, such as nitrogen compounds, compared to the situation before its construction.

Co-digestion involves the need to manage a quantity of nitrogen, organic and inorganic substances and volumes more than the double of those generated by livestock manure alone, with the same number of animals reared.

In the years when the plants producing biogas from cereals were promoted, 38% of the agricultural surface of Piedmont and 80% of that of Lombardy were classified as vulnerable, due to high concentrations of nitrogen in water (2010); if we consider only the surface in the plain (where the plants were built) the percentage is much higher.

With the diffusion of co-digestion plants, i.e. the fermentation of livestock manure and cereals to produce methane, there is an incentive to use agricultural land to grow cereals such as corn. To maintain a 1 MW_e power plant that produces biogas only with the fermentation of the whole corn plant, more than 516 ha are necessary and needs a direct investment per hectare between 600 and 1,300 L of gasoline, aqnd an amount that is higher if we consider the sum of transports, machine and equipment construction, production of fertilizers and pesticides, agricultural processes and others such as cooking, packaging production, and waste management; but the surface that will really have to be used will be higher because of the necessary crop rotation. While to obtain 1 MW of electricity with solar panels it is necessary to cover an area between 1.6 and 3.2 hectares: at least 160 times less (one hectare corresponds to 10,000 m^2). To obtain 1 MW with wind turbines, an even smaller area is sufficient.

In the case of co-digestion, the area needed to spread the digestate is larger than that needed to supply the cereals. [956]

Atmospheric emissions from the agricultural sector result from a variety of activities, such as fertilizer production and distribution, irrigation, soil disturbance, and pesticide use. The agricultural sector is responsible for 52-55% of all anthropogenic global methane emissions and 84% of all anthropogenic global nitrous oxide (N_2O) emissions, with the majority coming from the livestock sector. [53, 955] Overall, global livestock emissions, in carbon dioxide equivalents, amount to more than 20% of global emissions. In Europe, livestock manure is responsible for more than 80% of all ammonia emissions from agriculture.

Agricultural production in Europe is responsible for 10% of all ammonia (NH_3) and nitrogen oxide (NO) emissions. For example, flooded paddyfields are probably one of the largest sources of ammonia.
Regarding the advantage generated by the reduction of climate-altering gas emissions, the production of biogas from cereals is unsuccessful. [741] It can be considered that, in Europe and in Italy, the production of electric energy generates an emission of carbon dioxide between 400 and 500 g/kWh_e. Electricity is equated to:

- 0.23 toe/MWh if high or medium voltage;
- 0.25 toe/MWh if low voltage.

It follows that if 2.8 kg of carbon dioxide are generated by the combustion of 1 kg of diesel, 0.6 t of carbon dioxide are produced from 0.23 toe (tons of oil equivalent), while 0.5 t of carbon dioxide are produced from 1 MWh of electricity. The contributions in terms of emissions are very similar.

To sustain a 1 MW_e fermenter it is necessary to cultivate more than 516 ha with maize, which requires a direct investment per hectare between 600 and 1,300 L of diesel, and an amount that may be higher than the sum due to transport, construction of machinery and equipment, production of fertilizers and pesticides, and waste management. Thus, if a 1 MW_e fermenter produces at most 7,600 MWh of electricity in a year, this means, according to a superficial and erroneous assessment, that it has saved the emission of 3,466 t of carbon dioxide from other routes of electricity production (7,600,000 kWh x 456 g of CO_2 not emitted per kWh). This "non emitted" carbon dioxide, as indicated by the legislator, is the one that would be emitted with the electricity production normally used by the mix obtained from different sources: thermoelectric, i.e. the combustion mainly of natural gas and coal, and hydroelectric (in Italy most of the electricity is produced through the combustion of fossil fuels).
It may be considered that 1.8 kg of carbon dioxide are produced for every kg of methane and 2.4 kg for every litre of diesel burned. These values may be used to have an approximate idea of the emission balances.
The emission advantage (3,648 t) proposed by the legislator is cancelled out by the emissions summarised in the table below. [741]

Hypothetical emission balance in the biogas sector (in CO_2 equivalents per year) [741]

	Biogas from corn (**516 ha**)	**Biogas from manure** (**12,000 dairy cattle** fed by 4,000 ha)
Maximum energy in electricity from biogas production (0.9 MW_e plant)	8,000 MWh/year	8,000 MWh/year
Carbon dioxide saved from electricity production from biogas, rather than from the national electricity mix (456 g CO_2 per kWh)	**3,648 t** of CO_2 per year (8,000,000 kWh x 456 g of CO_2 per kWh)	**3,648 t** of CO_2 per year (8,000,000 kWh x 456 g of CO_2 per kWh)
Emissions produced by the combustion of methane contained in the biogas	**2,459** t of CO_2	**2,333** t of CO_2
Emissions from digestate storage without methane recovery (5% of total methane emission is considered)	**1,564 t** of CO_2	**1,495 t** of CO_2
Emissions generated to produce chemical fertilizers (assuming 3 kg of CO_2/kg of chemical fertilizer, and considering a use of 500 kg of chemical fertilizers per hectare per year)	**774 t** of CO_2 [(3 kg of CO_2/kg of chemical fertilizer) x 500 kg/ha x 516 ha]	**6,000 t** of CO_2 [(3 kg CO_2/kg of chemical fertilizer) x 500 kg/ha x 4,000 ha]
Emissions from pesticide use (5 kg CO_2/kg pesticide; 5 kg/ha pesticide use per year is considered)	**12.9 t** of CO_2 (5 kg CO_2 x 5 kg pesticide x 516 ha)	**100 t** of CO_2 (5 kg CO_2 x 5 kg pesticide x 4,000 ha)
Emissions produced by the consumption of fuels necessary for the cultivation of corn (600 L/ha x 2.4 kg CO_2/L; *in reality it could be more than the double*)	**743 t** of CO_2 (1.44 t CO_2/ha x 516 ha)	**5,760 t** of CO_2 (1.44 CO_2 /ha x 4,000 ha)
Nitrogen dioxide emissions from maize cultivation (in CO_2 equivalents)	**1,847 t** of CO_2	**14,304 t** of CO_2 (from 4,000 hectares)
Emissions from energy consumed in cattle breeding	--	**13,000 t** of CO_2
Emissions produced for the construction of the biogas plant *(this is an underestimate)*	**1,813 t** of CO_2	**1,813 t** of CO_2
Emissions produced for the construction of the machinery needed to grow maize (2 L of diesel for each kilo of equipment, for a total of 389 kg of machinery per hectare)	**964 t** of CO_2 (389 kg x 516 ha x 2 L diesel x 2.4 kg CO_2/L diesel)	**7,469 t** of CO_2 (389 kg x 4,000 ha x 2 L diesel x 2.4 kg CO_2/L diesel)
Emissions from diesel consumption to supply biomass within 70 km and to spread the digestate within 30 km	**541 t** of CO_2	**1,755 t** of CO_2
Emissions produced by the transport alone, per 100 km, of maize substituted for maize (as feed) removed from farms to produce biogas	**460 t** of CO_2	--
Ratio of emissions generated to emissions earned	**3.1** (11,178/3,648) t of CO_2	**14.8** (54,029/3,648) t of CO_2
To sum up: under all conditions, more climate-altering gases are generated than are potentially saved, although this estimate does not include all real emissions.		

As shown by the data reported, the biogas chain from cereals or from zootechnical dejections only (zero kilometres), considering only some emissions, is a failure as it consumes non-renewable resources such as diesel, soil, and materials (e.g. to build the machines) and generates more emissions than those gained. The estimates presented are a flawed representation of reality as, for example, consumption for transport, for the construction of machines and biogas plants are underestimated.

The water footprint of agro-methane production from cereals is another overlooked and at the same time underestimated aspect, especially in anticipation of the climate change that will make water more and more precious. It is necessary to premise that the water used is composed of three components:

- The green water that is rainwater.
- The blue water which is the surface or underground water.
- The grey water, which is the water needed to dilute the contained substances to concentrations below arbitrary threshold values (e.g. drinking water regulations). This fraction is the most abundant and is often not considered.

The sum of these three fractions is called *virtual water*.

An estimate of the amount of water needed to sustain the production of cereals or that of livestock manure used to power a 0.9 MW electric plant that produces agro-methane is called the water footprint. The main uses of water are those necessary for the cultivation of corn and those necessary to support the breeding of 12,000 dairy cattle. In the latter case, the use of water is shared with the production of meat and milk. The production of meat and milk, however, also requires the addition of other water consumption, such as that for slaughtering, pasteurization, packaging production, maintaining the cold chain as well as other transport and waste management. Another major water use is that required for plant construction. The estimates presented below underestimate some inputs such as water use for oil extraction and transport which may be over 100 times higher than those considered.

It is realistic to consider that at least 6 million tons of water per year are needed to support the production of a 0.9 MW plant that burns biogas for electricity from the corn plant. The water use increases by a factor of 7 if the manure of 12,000 dairy cattle is used (about 42 million tons of water), but some of this water is used to support milk and meat production. In conclusion, more than 795,000 L of water will be needed for each kWh of electricity produced from the fermentation of the whole corn plant, or 5,346,154 L/kWh in the case of biogas produced from cattle manure alone. [741] This amount of water represents only part of the water really necessary.

We often talk about water consumption even though the water used is actually returned to the environment but polluted, so we hope that no one has to drink it or use it for irrigation. So if you try to estimate how much more clean water needs to be used to dilute the contaminated water so that it can be reused with less risk, water consumption probably needs to be increased by a factor of at least 1,000; this dilution may be necessary for the grey water to turn into blue water i.e. low contaminated water. Assuming that the digestate contains 20 g/L of ammoniacal nitrogen (2%), it will need to be diluted by a factor of 40,000 to reach the concentration allowed in drinking water (0.5 mg/L). In order to reduce the risks to acceptable levels, much more water must be consumed to dilute the pollutants and, in any case, there is no return to the quality of the water originally used.

Unfortunately, there are no rules on the chemical characteristics of water that should be used for irrigation, for watering animals, for cleaning stables or for other industrial operations, but drinking water is set to become increasingly precious, especially in some areas, so we should take great care of it.

Overall, competition with the agri-food chain increases, energy is produced that is wrongly classified as renewable, increasing the consumption of non-renewable energy, and negative emissions into the atmosphere and the environment (eutrophication and acidification of water).

THE BIOLOGICAL RISK IS UNDERESTIMATED

The product leaving an anaerobic fermentation plant is, from a microbiological point of view, potentially polluted by a wide variety of microorganisms. Livestock manure may have very high concentrations of microorganisms (bacteria, parasites, and viruses). During anaerobic fermentation some potentially dangerous microorganisms increase in number (e.g. *Clostridium*). The improper use of digestates, such as manure, leads to a risk of microbiological contamination of workers (e.g. farmers), water, and crops. This risk is just as important as the chemical risk, although it is taken into less consideration.

Microorganisms are normally present in manure and concentrations increase in sick animals. The solid fraction of the digestate obtained from anaerobic fermentation has a higher microbial load than the liquid fraction, which will however constitute 70-80% of the total volume. In general, anaerobic fermentation has the effect of reducing some microbial loads (although specific categories of microorganisms will actually increase), but the digested product is still a good substrate for growth, even during storage. Some potentially dangerous bacteria, such as *Clostridium perfringens*, continue to be present at the end of the mesophilic anaerobic treatment. Spore-producing microorganisms are not reduced by the mesophilic anaerobic treatment. Concentrations of some microorganisms such as *Enterococci* (thermotolerant coliforms), *Enterobacteriaceae*, *Listeria monocytogenes*, and mesophilic bacterial load at 37°C may increase in the digested product during storage. [958, 959, 960] Thus, co-digestion actually increases the amount of microorganisms distributed in the environment, in proportion to the amount of grain fed for fermentation, compared to those distributed with livestock manure alone. The increased volume of material contaminated by micro-organisms, to be managed by co-digestion compared to livestock manure alone, increases the likelihood of contaminating water and fruit and vegetables. A new microbiological risk site, the biogas plant, will be created. This is also a reason to favour the installation of small plants on farms, therefore zero kilometres and sized for only the manure produced on the site.

Anaerobic fermentation may also be carried out with organic materials deriving from slaughtering and meat processing waste, which are much more problematic from a health point of view. Since these matrices are particularly risky, it is always advisable to carry out a thermal treatment, especially if the destination of the digested material is not the landfill but the spreading in agriculture.

In areas with a high density of livestock farming, such as some provinces of northern Italy, the amount of potentially dangerous microorganisms released into the environment is considerable. If in Piedmont it is assumed that we have to manage more than 17 million tons per year of livestock manure (deriving from the breeding of about 14 million animals, of which more than 10 million poultry, 1 million pigs, and 800,000 cattle), with a land availability for agricultural spreading of less than 500,000 hectares, it means theoretically distributing an average of 35 tons every 10,000 m^2. On average, we have about 3-4 kg of livestock manure per year for each square metre. In some territories the availability of 35 t per year is certainly an underestimate, as 35 t of manure can be produced by one or two dairy cows per year while, theoretically, in Piedmont there is a higher average density of animals (farms without land). These values are an underestimate also because the farms are concentrated in certain areas and it is not convenient to move the manure for more than 10-15 kilometres. In addition, chemical fertilizers, compost, sludge and water from surface canals that collect sewage (for irrigation purposes) are also distributed on the land. As an example, the pathogenic bacterium *Salmonella enterica* serotype *typhimurium* survives in soil for 203-231 days and was found on the surface of radishes and carrots, respectively 84 and 203 days after sowing, in soils fertilized by animal manure. [885, 896] If contaminated water (e.g. the liquid fraction of the digestate from anaerobic fermentation) is used for irrigation in the days before harvesting, the risk of spreading diseases (e.g. viral infections) by feeding fresh vegetables is very high. According to some studies, in order to reduce the risk of generating new infections to less than one new sick person per 10,000 people, irrigation with contaminated water should be suspended at least 14 days before harvesting vegetables to be eaten raw. [897] For some types of microbial contamination, 14 days is not a sufficient guarantee.

The construction of plants that produce methane using livestock manure and cereals makes the situation worse. There will be greater volumes of organic and inorganic substances to manage. The volumes, with the same number of animals raised, will increase in proportion to the cereals used and by at least two or three times, due to the dilution required during anaerobic fermentation. Consequently, the amount of microorganisms to be managed will also increase. The co-digestion digestate will have similar microbial concentrations to the digestate from manure alone, but it will have a much larger volume and therefore a higher amount of microorganisms.

Co-digestion of cereals (e.g. maize together with cattle manure) will decrease the availability of land for spreading, because organic and inorganic substances produced by the cereal crop are recycled to be used again to support cereals in the following year. Only part of some substances is used to produce methane. The remaining part enters the biogas plant (e.g. nitrogen and phosphorus) and returns to the field (the quantities do not vary if we exclude the losses to the atmosphere and water during the various passages). It will also be necessary to cultivate new areas to produce replacement feeds for those that are anaerobically fermented. Thus, co-digestion of cereals and manure increases the microbiological risks as the number of microorganisms distributed increases and the area available for spreading decreases, compared to the use of livestock manure alone.

Some microbiological hazards due to anaerobic fermentation of animal manure and cereals are summarized as follows.

1) The digestate produced from livestock manure with corn silage has an equal or slightly lower microbial load of microorganisms hazardous to human health than that of incoming livestock manure, although some categories of microorganisms increase.

2) The digested output still constitutes a good substrate for the growth of pathogenic microorganisms, so their concentration, during storage before spreading, may increase, cancelling out any reduction achieved (for some pathogenic species with mesophilic fermentation).

3) The microbial concentrations will be similar, but on a digested volume that will be greater than that of the incoming livestock manure, in proportion to the quantity of cereals introduced and the necessary dilution (we start from matrices with a quantity of dry substance even three or four times greater than that operating for the biogas plant). Moreover, the amount of nitrogen to be managed due to the use of cereals increases and, therefore, the extension of the surface of agricultural land necessary for spreading as well. The co-digestion in a biogas plant requires that the farm has more land available for spreading than previously needed to distribute manure. If this aspect is not well managed, the probability of polluting water, soil, and atmosphere increases.

4) The 0.5-1 MW_e plants encourage the concentration of livestock manure produced in several sites in the territory in a single point, with the consequent increase of related problems also of indirect type, such as transport that will generate air pollution, odours, dust and noise.

5) The site will present both fire and explosion hazards.

Regarding the chemical risk, the digested output has similar characteristics to those of sewage, but as already mentioned, it will have a greater volume and therefore this potential danger will also increase.

In conclusion, co-digestion increases microbial and chemical environmental contamination. Below are the percentages of the presence of some potentially pathogenic microorganisms measured on 12 monthly samples of cattle manure digest and silage. [957]

PATHOGENIC MICROORGANISMS	**Presence**
Salmonella spp.	9.1%
Escherichia coli O157	46%
Listeria spp.	64%
Yersinia spp.	82%

GENEROUS AND UNSUCCESSFUL INCENTIVES

Public funding for the production of electricity from the anaerobic fermentation of livestock manure and/or cereals was guaranteed to the value of EUR 0.28/kWh, and considering that one ton of manure through anaerobic fermentation can produce 20 kWh of electricity, this implies that up to 5.6 euro per ton of cattle manure (EUR 0.28/kWh x 20 kWh) could be available under the all-inclusive tariff; in this respect, it is important to recall that cattle manure was purchased at a price between 3 euros and 5 euros per ton.

In the case of fermenting corn, the theoretical energy yield is ten times higher than that obtainable from manure (200 kWh_e/t) and, therefore, the funding received with the all-inclusive tariff is equivalent to paying 56 euros a ton of corn plants (0.28 euros/kWh x 200 kWh). It should be remembered that the market price of the corn plant, during the years in which most of the biogas plants that received the all-inclusive tariff were built, was about 50 euros per ton.

Plants producing less than 1 MWh of electricity, built by 2012, were scheduled to receive an economic incentive equal to 28 cents per kWh, for at least 15 years; this was the all-inclusive tariff (Italian Law Decree no. 387/2003; art. 26 Law no. 222/2007; art. 2 Law no. 244/2007). This financing mechanism allowed, in fact, to amortize investments of 2 million euros in less than 5 years. As already pointed out, this incentive was equivalent to paying more than 3 euros for a litre of gasoline.

Different European countries have applied different incentives: 18.5 euro cents kWh in Austria, 26.7 euro cents kWh in Germany (2008) and, as already written, 28 euro cents kWh in Italy. The incentive mechanism has been rigid even if the different matrices used generate different costs or revenues. Some European countries, such as Germany, for many years have foreseen that a part of the contributions due to the operators of the biogas plants was destined to the cultivation of maize. In Germany the production of biogas in agriculture has spread rapidly thanks to these incentives that have financed the use of cereals with public resources.

In Italy this incentive (the all-inclusive tariff) has been supplemented by others such as those of the Regions. The Rural Development Plan of the Piedmont Region, approved on 20th November 2007 and valid for 5 years, provided economic support for farmers who used compost, of 180 euros per ha per year (the solid fraction of the digestate could be composted). Another aid was supported by the Community legislation encouraging the cultivation of cereals to produce electricity and heat; for example, Article 88 of the Regulation (EC) No. 1782/2003 provided for Community aid of EUR 45 per hectare per year for areas sown with crops used to produce energy (electricity) such as maize.

According to the incentive criteria provided by European and national standards, "*1 MWh of electricity, produced by wind energy or by the anaerobic fermentation of biomass, can produce a benefit equivalent to 456 kg of CO_2 not emitted by non-renewable sources.*". The legislation

(Commission Decision 2001/405/EC) reported that electricity fed into the distribution network in Europe, on average, produced 400 grams of carbon dioxide per kWh.
With a 1 MW_e fermenter one may choose to cultivate more than 500 ha on one's own and/or buy corn, and/or use the livestock manure that can be purchased. A 1 MW_e plant produces between 7,200 MWh and 7,660 MWh of electricity per year, if it works without interruption for 355 days a year (the maximum theoretical productivity is 8,760 MWh per year, if it works 365 days a year). The funding of 0.28 euros per kWh (as has been guaranteed for 15 years in Italy) means receiving an annual subsidy, only for the electricity fed into the distribution network, between 1,713,600 euros (if we consider the case in which 15% of the electricity is self-consumed) and 2,144,800 euros (in this second case the electricity not fed into the network is considered 10%). In reality, since the electricity sold is paid at a higher price than the electricity purchased, it is convenient not to self-consume the electricity produced.
In the best conditions, the financing of 0.28 euro per kWh allowed to maintain the biogas plant by buying all the necessary corn and/or manure. In reality there are other important costs such as transport, but also other possible grants, such as those for the cultivation of maize or for the construction of the fermentation plant. The most convenient form is where the owners of the fermenter are the farmers, as they have at least part of the necessary manure at their disposal free of charge and, often, part of the land to cultivate the maize and to be able to implement the spreading of the digested products. But the all-inclusive tariff has been so generous that it has allowed the purchase of the corn and manure.
It is important to note that the funding (of 0.28 euros per kWh) was guaranteed for 15 years, so each 1 MW_e plant at the end of this period will have received support of 33 million euros; a nice fortune for the owners of the biogas plants in operation in Italy but a waste for the community.
However, the energy produced by biogas is paid with an incentive equivalent to paying more than three euros for a litre of diesel, while agricultural diesel consumed for transport is paid less than one euro per litre. So, if one is careful in the management of transport, speculation is very convenient: 3,000,000 L of diesel equivalent in biogas, obtained from over 516 ha cultivated with corn, are paid as over 9,000,000 L of diesel derived from oil.
The incentives presented are only a fraction of those that have actually been available to entrepreneurs.
More than 1,000 biogas plants built in Italy have taken advantage of the mechanism of the all-inclusive tariff: 0.28 euros per kWh (data updated in 2015). Probably the category of farmers constitutes the longest-lived lobby on the Planet and it is also possible to argue that the fate of the Planet is, to a large extent, entrusted to farmers. Private interests and the selfish rules of economy have led to choices that benefit the few: the few against the many. The cardinal principle on which everything revolves is that of maximizing the profits of a few in the shortest possible time. The production of methane from anaerobic fermentation is one of the many speculative mechanisms that have followed in the agricultural sector, over decades. Real costs are reduced, such as those of energy production, plant cultivation and the production of meat and by-products, and the environmental assessments such as those of water and soil consumption are not carried out.
The design of new biogas plants was not the result of environmental assessments, but was conditioned by the incentive mechanism. In order to obtain the maximum possible speculation, it was necessary to build plants of 999 kW of installed power, ferment the silage, not recover the heat produced and not recover the methane from the digested product. In fact, this has been the case for most of the biogas plants financed. Incentives have encouraged failed choices such as:

- Growing cereals to produce biogas.

- Not using the thermal energy produced or using it for useless activities, such as evaporating the water of the digested product in order to reduce its volume; in this case it means using more than 50% of the energy produced to evaporate water, which is mostly added to dilute the dry matter provided by the silage: a huge waste. This strategy has been regulated by regional rules and voluntary standards such as UNI 10458 of 2011.
- Favour transport of raw materials (maize) and outgoing materials (digestate) over excessive distances (more than 15 km).
- Increase the organic and pollutant load, with the same number of animals reared, to be managed in territories where the impairment is already high due to the insufficient availability of soils compared to the biomass of animals reared. Contamination of water has been promoted (eutrophication, nitrates).
- Increasing the sealing of agricultural land and its concreting. A plant that produces 990 kW of electricity, with the anaerobic fermentation of manure and cereals, can occupy an area between 20,000 and 30,000 square metres, at least one third of which is covered or sealed.
- Speculation and waste of resources. One of the techniques proposed to be able to continue fermenting cereals to produce biogas and fertilizers (the digestates), in areas such as the Po Valley where surface waters are mostly already seriously compromised, is *stripping*. This is a technique for nitrogen reduction that requires a lot of energy and needs to use acids and a new device. So costs increase, energy consumption increases, while gains for speculation are reduced. The risks of this type of approach are underestimated: if the process of separation and production of (ammonium) salts is not carried out with proper precautions, ammonia is moved from the digestate into the atmosphere. However, even under the best conditions, the efficiency of ammonia removal never reaches 100% (it is at most 40% at 70°C). A lot of thermal and electrical energy has to be used. The thermal energy produced by co-generation may not be sufficient to maintain the temperature of the fermenters and, at the same time, provide the heat to perform the ammonia separation with the desired maximum efficiency. As a result, lower temperatures (60°C or less) will be used, resulting in an even lower removal efficiency. [918] This kind of economic speculation should be banned, because it passes off unnecessary investments as environmentally friendly and beneficial.
- Promoting unsustainable hypotheses, such as using the production of methane (biogas) from the cultivation of cereals to obtain the energy needed to produce mineral nitrogen fertilizers. [917] In this case a futile cycle is generated in which the only real business is public funding. Cereals are grown which require mineral fertilizers to be produced, which in turn are produced using energy derived from the methane fermented by the cereals themselves. Micro-organisms are bred that consume our food, our water and indirectly our soil.
- Send the digestate or its liquid fraction to a landfill or wastewater treatment plant and discharge it into surface water courses. [920]

Unsustainable solutions have been proposed that can be considered bureaucratic placebos, which imply a desire not to hinder speculation by entrepreneurs and a considerable superficiality, ignorance or collusion of those who authorize them and could, instead, impose limits and conditions. To make it easier to understand the extent of economic waste, we may compare the production of energy from wind turbines. A single propeller, with blades rotating at a height of 80 m above the ground (*onshore*), can have a power of 1,800 kW, which is equivalent to the power of two plants producing 900 kW of biogas and requiring over 1,000 ha of land cultivated with cereals.
No value is placed on how the energy produced is used. It is worth remembering that in order to set up this energy chain, electricity is paid at a price equal to at least 4-6 times the selling price in the market, thanks to public financing, but part of the energy produced can be used in an unconvincing way to heat swimming pools, dry corn for digestion or evaporate water from the

digested product. In short, public resources are used, the consumption of non-renewable energy is increased, hundreds of tons of biomass are moved by road, water is wasted, hundreds of hectares of land are cultivated with corn to feed intestinal microorganisms in fermenters, without having thought about it.

Biogas plants that do not use heat have been authorized and the indirect environmental and energy costs have not been assessed. Energy classified as renewable has been promoted through increased consumption of non-renewable energy (transport, crops) and increased consumption of limited resources such as agricultural soils and irrigation water. Incentives for renewables have not sufficiently considered very important indicators that should be used to modulate the promotion. To give an example, self-consumption of heat and electricity reduces losses due to energy transfers and transport, so it is more efficient and should be preferred; but incentive schemes necessarily require network distribution and sale at a guaranteed price, much higher than the market price. The energy from non-renewable sources consumed to run a biogas plant is not considered, although it cancels out the energy and emission benefits (in terms of climate-altering gases). The production of electricity and heat from co-digestion, i.e. from livestock manure and cereals, uses energy that is currently not evaluated in the energy account of these biogas plants.

The use of matrices such as livestock manure and waste, such as the organic fraction of municipal solid waste and sewage sludge, should be more strongly promoted for biogas production. With the caveat, however, that digestates obtained from waste (such as slaughterhouse waste or sewage sludge) are much more hazardous than manure and, therefore, their agricultural use as fertilizer should be avoided.

The European legislation has promoted the use of biomasses (e.g. cereals) to produce agro-fuels (Directive 2009/28/EC). In Europe, one of the objectives that was proposed was to produce, by 2020, at least 10% of the total fuel used for road transport using sources labelled as renewable (agro-ethanol, agro-methane, agro-diesel), but allocating agricultural land to the production of agro-fuels is a choice that is hardly acceptable from an ethical and environmental point of view.

Several official documents, which plan an increase in the saving of non-renewable energy and an increase in the use of energies passed off as renewable (e.g. agro-methane and agro-ethanol), provide for at least 5% of agricultural land to be allocated to energy production. For example, in Piedmont in 2009, it was planned to allocate 5% of cultivated land to produce agro-fuels (DGR of 28 September 2009, n. 30-12221, which approved the Programmatic Report on Energy). These intentions are not very intelligent. It would have been much more far-sighted to use these economic resources for objectives useful for the transition needed to obtain food in a sustainable way. Instead of wasting land to produce agro-fuels, which are a non-renewable energy source, farmers could have been helped to dedicate at least 10% of their agricultural land to re-naturalization. The increase in biodiversity also brings significant benefits to food production. The agricultural system can no longer afford to lose soil fertility and must embrace the saving of fossil fuels. Public resources must be managed in a more far-sighted way and with a less energy-intensive vision of the future.

In 2015 (September) about 1,340.4 million euros were financed with public resources for biogas plants thanks to the all-inclusive tariff, 83.4 million euros for plants producing biogas thanks to the mechanism of green certificates, 43 million euros for small plants exploiting the tariffs provided after 2012 (www.gse.it). About 5,767 million euros were spent on direct incentives for all renewable energies, of which about 1,500 million euros for plants producing biogas; as already described at length, there were also other forms of indirect incentives for agro-methane.

The only real advantage is for the investors who, in a few years, will have recovered the investment made and will collect a consistent income for at least ten more years.

Biogas generation from manure cannot even compensate for part of the emissions generated by livestock farming. Therefore, the biogas chain could have been framed among the technologies that may be used to reduce some of the negative environmental impacts generated by the production of meat, milk and dairy products. In conclusion, the biogas chain produces non-renewable energy.

THE BUREAUCRATIC MACHINE DID NOT PROTECT THE COMMUNITY

The bureaucratic machine can no longer be easily managed even by the best administrators. It has become too complex and often incomprehensible, with strong contradictions, and the traceability of the sector's rules is not ensured. It is not easy to identify the compulsory fulfilments for any activity or initiative. One strategy used by local administrators, such as municipal ones, to hinder the construction of biogas plants has been to appeal to formal quibbles. This strategy, if politicians are determined, can easily lead to victory for the simple fact that there are thousands of rules, and knowing them all and at the same time respecting them is impossible.

Despite the fact that the bureaucratic apparatus lends itself very well to slowing down or even inhibiting any entrepreneurial action, such as those in favour of biogas production, over 1,000 plants have been built in a few years, without having guaranteed the necessary environmental protection that at least common sense might have suggested.

For the initial administrative process, necessary to obtain the authorization for the construction of a plant implementing anaerobic fermentation (0.9 MW_e) and financing (all-inclusive tariff for 15 years), it was necessary to spend a total of less than 10,000 euros. The payment of the work carried out by the public institutions such as, in Italy, the health service (ASL) and the Province has a negligible impact on the expenses that the entrepreneurs had to anticipate; even if, in some contexts, the workers of the public institutions have certainly dedicated a lot of resources and energy to the evaluation of the preliminary documentation. As a matter of fact, this expense was distributed on all taxpayers, that is, on the population, in an apparently invisible way. This was another indirect economic benefit.

We must reflect on an important aspect: can a small community be free to choose what to do with the territory in which it lives, especially if the main aim is environmental protection and collective well-being? Can one defend oneself against unjust rules? Communities should be given full authority to defend their territory if local choices go in the direction of a greater environmental protection, independently of national policies.

Any proposal to manage the defence of the territory and the community, and at the same time the prudent planning of public expenditure, will certainly benefit from better a management of certain aspects such as:

- increase the cultural level (training and information) of the whole system (administrators and population);
- ensure a healthier meritocracy in the selection of stewards of the public good;
- exclude political decision-makers from technical evaluations, especially if there is doubt about a possible conflict of interest or lack of impartiality;
- simplify the rules;
- ensure certainty in the application of sanctions, which should be better modulated, especially for environmental crimes.

One of the most important objectives for the future could be to design and organize the entire public system in such a way as to have greater professionalism in providing indications for the protection of health and a greater ability to condition choices that safeguard the principle of protection of the collective interests.

THE CONSEQUENCES OF AN EXAMPLE OF PLANNED ECOCIDE

To replace all the gasoline consumed on the Planet with agro-fuels, it is necessary to occupy an agricultural surface at least four times bigger than the existing one, therefore it is necessary to colonize four more planets similar to the Earth (in reality it is not necessary because more energy is used, in terms of equivalent oil, than that obtained). The diffusion of the plants that produce biogas has been possible only and exclusively thanks to public incentives, theoretically destined to favour the safeguard of the environment. If one carefully evaluates the energy balance and the environmental impacts of all the required operations, the biogas plant is inefficient and actually consumes a lot of non-renewable energy and does not ensure the expected environmental benefit such as the reduction of climate change. The negative impacts increase while the self-sufficiency decreases in the supply of the matrices to be fermented, as well as the availability of agricultural land for spreading near the plant, whereas the use of cereals increases. Paradoxically, public resources have been used to incentivize an unsuccessful behaviour from an energy and environmental point of view, such as the use of cereals to generate methane and electricity, passing the whole operation off as ecological and a priority for the community.

The failure to place constraints on the use of vegetables such as cereals to produce agro-energy will result in:

- Encouraging the reduction of biodiversity.
- Decreasing the availability of food.
- Decreasing feed availability.
- Increasing soil and water degradation (pollution and eutrophication).
- Increasing irrigation water consumption and promoting contamination.
- Importing cereals such as maize and other vegetables important for human and animal nutrition. The energy costs of transport and the ethical aspects (exploitation of poverty conditions in distant territories) increase considerably, and they are unsustainable and unacceptable.
- Encouraging deforestation and other environmental problems in distant countries.
- Encouraging the cultivation of genetically modified organisms (e.g. maize and rape).
- Increasing the prices of cereals, that is, of food and feed. For some time now, studies have shown that 75% of the increase in food prices recorded in the World, in the years 2002-2008, was also favoured by the increase in the production of agro-fuels. [953]
- Increasing rental and sales prices for agricultural land.
- Increasing the consumption of non-renewable energy to produce energy labeled as renewable.
- Increasing the use of pesticides (herbicides, fungicides, and insecticides).
- Increasing the number of hazardous substances to be managed and therefore of pollutants per unit area (e.g. metals, nitrogen compounds).
- Increasing vehicular traffic and the impacts generated (e.g. air emissions).
- Promoting climate change also through the increased emission of climate-altering gases, the reduction of biodiversity, water pollution, and soil alteration.
- Wasting public resources to grow crops aimed at producing waste used for less efficient energy recovery than other alternatives, such as burning dedicated crops for this purpose or installing wind turbines.

If the area cultivated with the grains needed to feed the microorganisms in a 1 MW_e fermenter were used for human food, the equivalent of the caloric requirements for several thousand people for a year (at least 2,000) would be available. The energy obtained for animal feed would be even higher, as the whole vegetable can be used (40-60 million kcal/ha per year).

In order to effectively carry out the main function for which they should be conceived, i.e. to recover energy from waste matrices that are in any case produced for another primary objective, i.e. to feed the human species, this type of plant must be managed differently. Excessive financial aid from public resources and the absence of binding rules (e.g. on the consumption of non-renewable energy, on the use of cereals, on the use of soil and water) has favoured unacceptable conditions from an ethical, environmental, health and, therefore, economic point of view. If negative environmental impacts (e.g. pollution) continue to be given no value and ethical aspects are not considered, then, paradoxically, according to some superficial and short-sighted lines of thought, the incentivization of the agro-fuel production with public resources may lead to the misinterpretation in a positive way of some economic wealth indicators such as GDP or Gross Domestic Product.

The estimates given in this chapter probably have many inaccuracies, but the order of magnitude is realistic. Economic and environmental balances, such as those of emissions, show that the biogas chain is in fact not sustainable and not renewable, both in the case where only cereals are used and, in the case where only livestock manure is used. In fact, the latter are produced by intensive systems, which inefficiently transform oil into food. Therefore, the biogas chain, even the most efficient one, is not able to compensate even a part of the emission balance produced by livestock. The biogas chain, applied only to livestock manure and zero kilometres, should be considered a useful way to reduce the amount of pollutants distributed in agricultural soils and in the environment, and as a supplement to the energy needs of energy-hungry production systems, such as beef production. It cannot be called renewable energy because the energy invested is greater than the energy obtained, and it is a strategy that is only possible by increasing the consumption of fossil fuels. The desired emission advantage does not really exist either, as it is cancelled out by the preliminary activities required to produce the biogas. The legislator wrongly advertises these energies as renewable energies, since it refers only to the activity of biogas combustion, without examining the emissions produced throughout the chain, such as those generated by cultivation, the production of meat and by-products, and the management of digested products.

The general principle that should be applied in all sectors should be not to provide subsidies and facilities to all activities that have impacts on the environment and in particular on food production, water and air quality, and soil consumption. On the contrary, ecological taxes should be increased for all activities that compromise our future.

AN ECOLOGICAL DISASTER: THE IRREVERSIBLE DESTRUCTION OF BIODIVERSITY

PARADISE IS A GARDEN

When we ask high school students why trees are important, or try to explain why it is essential to plant them instead of cutting them down, you realize how widespread environmental ignorance is and how ineffective the current school education programmes are on certain ecological issues. Environmental illiteracy is so widespread that trees in the city are cut down because they make the cars dirty or because they are home to birds whose songs disturb us and whose droppings dirty us (again). In Paradise, which has always been depicted as a lush garden, man was an integral part of nature, but for some time now man and nature have been irretrievably separated. This premise has been functional in wanting to remind, above all to the over 50% of the planetary population that lives in the cities (at least 67% of Italian inhabitants live in cities), why trees are a biological, environmental, energetic, and social resource:

- Trees are indispensable to our life, without them we could not live: they produce the oxygen we breathe.

- Trees and green areas increase the ecological value and biodiversity of the environment such as the urban environment.

- Trees contribute to mitigate the negative effects on the climate caused by man, counterbalancing the greenhouse effect through the production of oxygen and the absorption of carbon dioxide. As far as carbon dioxide is concerned, an evergreen tree (with a foliage of at least 30-40 m^3) absorbs a few kilos of carbon dioxide per year. To absorb all the carbon dioxide produced by a small diesel car that travels 20,000 km per year, 4-5 hectares of mature forest (with trees over 20 years old) are needed. The moment the trees are burned the carbon dioxide returns to the atmosphere.

- Trees reduce air temperature, which is very useful in the warmer months. They have a thermoregulating function, thanks to the effect of increased evapotranspiration (urban areas record higher temperatures, compared to green areas, on average between 0.5 and 3 degrees Celsius). In Chicago (USA) it has been estimated that if tree cover were increased by 10%, or if three trees were planted per building, it would save 50 to 90 dollars per housing unit (per year) for heating and cooling. [963]

- Trees improve air quality: they absorb carbon dioxide and other substances. Trees have a hygienic-sanitary function, linked to the chemical purification of the atmosphere, to the fixation of toxic gases, to the bacteriological purification, to the absorption of fine dust and other polluting agents. Some plants have demonstrated that they are able to absorb from the air even 2 milligrams of formaldehyde (*Fatasia japonica* and

Nephrolepis exaltata, an evergreen fern) or half a milligram of xylene every hour. [986] Other substances that can be absorbed are benzene (e.g. *Crassula portulacea*), ammonia (e.g. *Nephrolepis exaltata*) or volatile organic compounds.

- Urban greenery and the presence of trees decrease the occurrence of asthma in children which is one of the important diseases of childhood. [721, 964]
  The number of deaths related to motor vehicle pollution exceeds that of road accidents (in Italy, in 2018, there were 172,553 road accidents with injuries to people, generating 3,334 victims and 242,919 injured). [829]

- Trees prevent erosion (they make the ground more stable) and reduced fertility of the underlying soil. Trees improve the absorption of rainwater during rainfalls (they avoid stagnation) and reduce the violence of the rain. It is estimated that trees can retain between 7% and 22% of the rainwater that could accumulate on sealed city soils. The amount of water that plants can extract from the soil is considerable. A leaf can transpire a quantity of water equal to about 0.2 litres per day, a tree about 200 litres per day and a hectare of forest more than 10,000 litres per day. [963] The circulation of water, through plants and ecosystems, represents the greatest chemical-mediated energy transfer in the biosphere.

- Trees reduce the growth of grass and other plants below the canopy: the need to have to cut grass or use herbicides is reduced.

- Trees increase biodiversity as they are a refuge and food source for urban wildlife (e.g. tits, robins, redstarts, passerines, and numerous insects). Hundreds of species of beetles have been collected from a single species of tropical tree (*Luehea seemannii*), of which about one fifth depends on this tree, which can be 30 m tall. [699] Even some bees, in the wild, are wont to make nests in the hollow trunks of trees.

- Trees have an aesthetic and ornamental value (e.g. they reduce visual contact with neighbouring apartment blocks). They improve the quality of urban spaces in terms of visual perception, generating beneficial effects.

- Trees improve the decoration and value of nearby homes.

- Trees improve the quality of urban spaces and are also beneficial in psychological terms. There is a link between the number of trees in the place where one lives or is cured and health. To confirm this, it is useful to highlight that horticulture has proved to be a valid therapy for many health problems. [721]

- Trees have an educational value, especially for children.

- Trees reduce noise pollution, thanks to their natural sound-absorbing capacity; they can reduce even more than 50% of the sound perceived in the city, which is the cause of discomfort, disturbances (e.g. sleeplessness), and increased incidence of certain diseases.

- Trees can provide energy (combustion): in much of the Planet they are the most important source of combustible energy.

- The trees provide important fruits for human nutrition and essential medicines.

- Trees can help prevent crop diseases. Around cultivated areas, it is useful to plant tree species that are attractive to pests of the species of agricultural interest. In Brazil, it was possible to reduce the density of a citrus pest beetle (*Cratosomus flavofasciatus*) by planting a small tree (*Cordia verbenacea*) at a distance of 100-150 m from cultivated fields.
  Some plants can promote pest repulsion or the attraction of natural enemies of the crop pest: this strategy has been applied in maize cultivation in Africa.
  The restoration of semi-natural areas increases the productivity of, for example, the mango plant by about 1-5 kg of fruit per tree. Crops that benefit from the service provided by beekeepers such as cherry (*Prunus*) are benefited by the presence of semi-natural areas that provide refuge zones for wild pollinating insects. Increasing the proportion of the area occupied by wild species or semi-natural environments (within a radius of less than 1 km) by between 20% and 50% increases cherry production by 150%, regardless of the density of honey bee colonies raised. [616]

- Trees make physical activity enjoyable and help reduce the risk of obesity and other lifestyle diseases such as diabetes. People who live more than a kilometre away from green areas report more health problems than those who live closer.

- Trees reduce stress. Contact with nature regenerates the body and reduces stress, so that even a short break from work spent in a green area brings benefits to physical and mental well-being: walking among the trees reduces stress with physiological effects that can be measured after 15 minutes. [965] Living in contact with nature improves our creativity by up to 50%. [966]

- Trees help the growth of young people. Attendance of green areas by youngsters (8-10 years old) significantly improves their attention span, which prompts consideration of implications within education policies and school zone design. [967]

- Trees promote social cohesion: sharing public green spaces encourages people to get involved, also through voluntary organizations. Social relations are strengthened and a strong sense of community spreads, with consequent reductions in isolation and marginalization.

- Trees and green spaces have a considerable recreational and health value. In the days of children who live in the city, solitary activities (often in front of a screen) and structured activities (rigidly framed by parents) are taking more and more place, to the detriment of free play and time spent independently in freedom. Natural spaces are becoming increasingly rare, while abnormal and pre-packaged excitatory stimuli are on the rise. These stimuli are often absorbed passively and uncritically through a screen. In this context, digital tools, which on the one hand allow information to be acquired and shared on an unprecedented level, induce a sedentary lifestyle and disconnection from the real world. Increasing the availability of open space can help improve important factors for physical, mental, and developmental balance.

Trees can be very long-lived, even more than 3,000 years, as is the case with the giant sequoias of North America, so they can provide very useful and free services for millennia. Some plants are able to survive in conditions of extreme drought, such as that of the Namibian desert where it rains less than 19 mm of water per year (in Italy it falls between 700 and 900 mm per year). Plants adapted to these extreme conditions are able to accumulate water in the short periods when it is present or are able to extract it from the fog: even the equivalent of 35 mm of rain in a year. For example, a 2 m tall grass (*Stipagrostis sabulicola*) is able to collect 5 L of water from the air for every square metre of leaves. [986]
Trees are a social resource and improve the quality of life. A phrase attributed to a tribal chief of the American Indians, may help us reflect on this.

"*When you have cut down the last tree, when you have caught the last fish, when you have polluted the last river then you will realize that you cannot eat money*".

Here are some data that may provide a measure of the benefits generated by public green. It has been estimated that the 692,892 trees surveyed in the city of New York (in the United States of America) produce the following economic benefits: [968]

- removing carbon dioxide from the atmosphere generates a benefit of over $4 million;
- the removal of air pollutants generates an economic benefit of over $6.5 million;
- rainwater absorption generates an economic benefit of approximately $10.6 million.

On the whole, in the city of New York some environmental benefits, generated by the presence of just under 700,000 trees, are estimated at over one hundred million dollars: 140 dollars per year per tree. [968] Probably for every euro invested in trees in the city (planting and maintenance), there are economic benefits, in terms of ecosystem services, estimated at between four and six times more. [969] Dramatic pruning (e.g. 50% reduction in tree canopy size) substantially diminishes these benefits.
The positive effects on human health of the trees in the United States of America are estimated at $6.8 billion (range: $1.5 to $13 billion). It is worth noting that among the estimated benefits, there is the prevention of two hundred thousand lost school days. [970]

In support of what has just been written, it is important to emphasize that the Italian national legislation provides for the planting, by the municipality, of a tree for every new born. In addition, the Green Regulations of many municipalities require the planting of a new tree to replace every tree cut down. Probably, if we imagined that *trees provided free wifi (internet) we would plant them everywhere. It is a shame that they only provide the oxygen we breathe*.

Among the ambitious but achievable goals, the European Community highlighted:

> "*...the Union needs to increase the extent of its forests, improve their quality and make them more resilient, in particular against fires, drought, pests, diseases and other threats that are becoming more imminent with climate change. In light of these findings, the Commission will propose in 2021 a specific EU Forestry Strategy in line with our broader ambitions for biodiversity and climate neutrality. The proposal will include a roadmap for planting at least 3 billion additional trees in the EU by 2030, in full respect of ecological principles*." [1182]

In order to improve our ability to safeguard the environment, it is vital to involve stakeholders, such as the more than 50% of the World's population who live in urban centres.

[1183] Urban ecosystems need to be regenerated with a view to safeguarding the environment, promoting sustainability and improving liveability. The ecosystem services provided by nature are manifold:

- the supply of products (e.g. timber, fibre, and food);
- the protection of genetic diversity;
- cultural services (e.g. tourism, recreation);
- services essential to life (e.g. regulation of the climate, water cycle, pollination).

Urban ecosystems also contribute to well-being and resilience. Citizens' participation and involvement is essential to deal with the uncertainties and changes ahead.
Unfortunately, farmers, who are the most important gardeners on the Planet, usually do not like trees unless they are directly useful according to objective criteria. Ecological consciousness, in some cases, emerges only as a good cosmetic move, as for politicians to be filmed while planting a tree. We have succeeded in estimating the number of trees on the Planet at about three trillion, but we are unable to stop the destruction of the biodiversity of the oldest and most important forests. The illusion of omnipotence distances us from nature and lets us believe that if we destroy it we can remedy somehow.

WE HAVE ENTERED THE AGE OF LONELINESS

It is estimated that there are between 2 million and 100 million living species on the Planet: perhaps only eukaryotic species are over 8 million. They are probably many more: only bacterial species could be in the order of billions, therefore we have classified very few prokaryotic microorganisms, perhaps less than 0.01%. For 99% of the species discovered (less than 3% of those that probably exist) we know where they were found, they have been given a name, a few specimens are preserved in a museum and some anatomical descriptions have been published in scientific journals. [639]

No one knows exactly how many different animal and plant species exist on the Planet, but anyone can easily measure the rate of destruction of ecosystems and the reduction of biodiversity. From prehistoric times to the present, the main causes of extinction and, therefore, of reduction in biodiversity have been:

- hunting;
- the destruction of ecosystems (e.g. due to pollution or deforestation, and we can also add climate change generated by human activities);
- the introduction of foreign animals (e.g. rats and goats);
- diseases and problems created by transplanted "outsiders".

After the risk of a planetary scale war with weapons of mass destruction, the reduction of biodiversity, climate change, and the degradation of fertile soil are the most serious environmental threats facing humanity.
Human activities have altered at least 75% of the Earth's surface and 85% of wetlands (e.g. marshes) have been destroyed. [339] Since 1870 more than 50% of coral reefs have been destroyed, or irreversibly altered, and the rate of degradation is increasing. [339] Coral reefs occupy less than 0.1% of the marine surface (which occupies 70% of the Planet's surface) but are essential for at least 25% of all marine fish species; unfortunately, three quarters of coral reefs today are at risk. [345]
Among the services performed by marine and terrestrial ecosystems, there is the mitigation of the effects of climate change as they sequester several billion tons of carbon each year (at least 5.6 Gt or giga-tons). [339] The services provided by nature are essential to achieve the environmental sustainability goals programmed by the United Nations, summarized in 17 strategic themes, to avoid the collapse. [340]

The loss of biodiversity in the last 50 years is unprecedented in human history: species have become extinct at a rate of hundred or thousand times the natural rate. Despite awareness of the gravity of the situation, the rate of disappearance of thousands of species is increasing. [277] Previously no creature has altered so much the life on the Planet. Following the great extinctions of the past it was discovered that it took such a long time, millions of years, to regenerate biodiversity that there is no interest in what might be human expectations. At least 5 major episodes of mass extinctions have happened in the past. The first occurred about 450 million years ago, when most life forms were aquatic. The most serious one in the past probably happened 250 million years ago and the most famous one extinguished the dinosaurs, about 65 million years ago. However, these 5 events occurred over a time span of hundreds of millions of years and, therefore, may be considered extremely rare.

The study of past fossils has made it possible to estimate the natural extinction rate, that is, the rate at which species become extinct in the absence of human dominance over nature. For mammals (there are about 5,500 classified species), probably the best studied group, the background extinction rate could be one species every hundred years. [699] An amphibian species could have gone extinct roughly every thousand years. These data suggest that during the lifetime of a human being, the extinction of an animal would have been very unlikely to be recorded. Unfortunately, as we now know, this is no longer the case: amphibians are among the most endangered animals, with a rate of extinction forty-five thousand times higher than naturally expected.

In the past, during the existence of a human being, it would have been impossible to record the generation of a new species, whereas thanks to ingeniousness and biotechnology the creation of new living beings can take place very quickly. Genetically modified microorganisms that do not exist in nature can be designed and generated in the laboratory in a matter of days. For animals and plants, a few months or years may be enough. Therefore, this increase in the speed of creation and dissemination on the Planet of species created with biotechnology, the result of human creativity, is also unprecedented. The manipulation of genetic biodiversity is a worrying factor as it allows potentially horrible applications, because they are useful to satisfy war aims or objectives of economic domination.

The planetary ecosystem due to anthropogenic factors is irreversibly slipping into a new state, with dramatic and partly unknown effects. The continuous manipulation operated in the structure of the composition of plants on the Planet is destructive. Unfortunately, as the number of species that are disappearing or on the verge of extinction increases, the rate of extinction of the survivors accelerates. [693] The reduction of a species beyond a certain safety threshold, called *minimum viable population*, leads to its extinction due to several factors, such as genetic impoverishment and the inability to adapt to new environmental factors; as it happens in zoos that host the last surviving specimens of some animal species, which no longer exist in nature, as their ecosystems have been irreversibly altered, for example, due to agriculture. The phenomenon is even more rapid when entire ecosystems are destroyed. As extinctions increase, at some point biodiversity reaches a critical point and the ecosystem collapses. Unfortunately, we do not know what thresholds of biodiversity reduction that ecosystems, such as agricultural ones, can withstand before catastrophe occurs. We can reasonably see that at the current rate of biodiversity destruction we have entered a new era that can be called the *Eremocene* or *Era of Solitude*, that is, one in which the human species and the few domesticated species dominate the biosphere. As far as we know an Era with these requirements cannot last long, so it could turn out to be one of the shortest periods in human history.

Institutions such as those of the European Community also stress the importance of safeguarding biodiversity: [1182]

> "*...The protection of biodiversity has inescapable economic justifications. Genes, species and ecosystem services are indispensable inputs for industry and business, especially for the production of medicines.... The overall benefit-to-cost ratio of an effective global programme for nature conservation still in the wild is estimated to be at least 100 to 1*."

No one knows exactly how many species are there on the Planet, and very little information is available for most of those known. In 2015, the number of classified species exceeded two million, including 32,000 species of fish, 10,000 species of birds and at least 5,500 species of mammals. [693, 962] In addition, at least 270,000 plant species and 900,000 insect species were classified. These data show that for every species with hairs and mammary glands there are at least 160 species with compound eyes and antennas, although there are probably more insects to be discovered than classified, and than mammals to be discovered.

If we assume that today the general extinction rate is between 0.1% and 0.01%, i.e. very low, and if we assume that there are at least another 2 million living beings different from the human species, it means that between 200 and 2,000 species are getting definitively extinct every year. However, if we assume that there are 100 million species present, with the same rate of extinction, we are eliminating between 10,000 and 100,000 species from the Planet every year. Some estimates report an extinction rate of 150 species per day. The rate of extinction may vary greatly; for example, the loss of life forms in fresh waters is at least 4-6 times faster than on land. [5] Scientific reports recommend that the rate of biodiversity loss should not exceed 10 species per million species per year, so the current rate of extinction is much higher than this threshold value by at least 100 times.

Geological history has taught us that we are the most destructive species in the history of life. Until the arrival of mankind, that is, about 200,000 years ago, according to experts the extinction rate was between one and ten extinct species per million per year. [693] Today this extinction rate has increased on average between 100 and 1,000 times, but in some ecosystems it is even higher: between 3 and 40 species go extinct every day. Unfortunately, there is a continuous increase in the rate at which living things are disappearing. The loss of so many species so quickly should despair everyone and not only conservationists, as it generates enormous economic damage due to the loss of functionality of the ecosystems.

What percentage of current survivors will there be at the end of this century? No one can predict exactly what will happen in the future but at the current rate of extinction probably half or less than a quarter of the species known today will have survived by the end of the century. How long can we go on not dealing seriously with this catastrophe without suffering its disastrous consequences? The mass extinction that humankind is causing, and with it the extinction of ecosystems and genes, is along with World wars, pandemics and climate change among the deadliest threats humanity has ever imposed on itself. We are driving the *Anthropocene* towards the *Eremocene,* the era during which, for a very short time, we will remain with the few species domesticated for selfish purposes. It will be a very short Era because it precedes the collapse. Overcoming the ever narrowing bottleneck has become an imperative, also because it is really difficult to hope for peace and health for the World population that, by the end of this century, could include between 10 and 12 billion individuals. The final game on the fate of biodiversity is being played out in the next few years, probably in less time than a human generation.

The science that studies the interactions between organisms in an ecosystem or in the entire biosphere is still very young. Knowledge on these issues is very scarce, so we are not able to give decisive answers, and all that remains is to interrupt the negative pressure on the environment. At present this hypothesis is a utopia. Probably on the Planet there is a wild species approximately every 1,000 - 2,000 human beings (excluding unknown species and microorganisms, which could be numerically superior to those classified) and, therefore, it could be hypothesized to find a sponsorship to protect them. In order to reach a balance for the preservation of the biodiversity necessary for the survival of the human species, it would probably be necessary to dedicate most of the surface of the Planet to wild species.

Despite the fact that what can be called the sixth mass extinction is now underway, as at least five others have been recorded, we continue to discover new species. Every year at least other 13,000 new species are described on the Planet including: 130 - 160 species of fish, 25 - 30 of land mammals, 6 - 7 of birds. [5]

SOME CAUSES OF NON-HUMAN EXTINCTION

Among the main causes of biodiversity reduction it is useful to mention:

- The destruction of natural ecosystems: agriculture is one of the main causes.
- The spread of alien species, i.e. the introduction of non-native species.
- Pollution of air, water and soil. The massive use of chemicals contributes to damage biodiversity: it was discovered that the Bengal vulture (*Gyps bengalensis*), in India, was killed by an anti-inflammatory medicine widely used in medicine and veterinary medicine, due to the exposure to the active ingredient present in the corpses of cattle (it was diclofenac which is an anti-inflammatory, non-steroidal analgesic; this medicine is easily found in the waters of rivers throughout the Planet at high concentrations). [978, 979]
- Population growth (a very unpopular aspect but probably a theme that is well present in the survival strategies devised by the war powers and which is at the same time very distressing).
- Overharvesting such as hunting and fishing.
- The illegal and the legal trade: there are more tigers captive in the USA than free in the rest of the World (in Europe you can eat in restaurants offering zebra steaks, crocodile sausages, meatballs of marsupials, camels and pythons). [1276]
- Climate change.
- The loss of soil fertility.

According to some researchers, these are the main causes of the irreversible reduction of biodiversity. Here are some details that help to understand the seriousness of the situation: in Europe at least 393 aquatic plants have been classified (270 live also in Italy) of which 63 are endemic. The major causes of extinction for aquatic plants, in decreasing order are:

- irreversible modification of ecosystems (e.g. wetland reclamation) and cementification (e.g. tourism, city expansion);
- water pollution caused by agriculture;
- intensive agriculture;
- intensive livestock farming;
- water pollution due to civil and industrial sewage.

It is a sad fact that one of the main causes of the destruction of biodiversity is fire. In the year 2000 alone, more than 350 million hectares went up in smoke. The frequency of fires increases with climate change, although most are the result of business interests. Our ancestors probably deliberately used fire, for a use other than cooking meat and vegetables, over 160,000 years ago. [703] Pollen and charcoal indicate that people in Colombia used *slash and burn* as early as 5,000 years ago to allow maize and cassava to be grown in forested areas. The ancient *slash and burn* technique is still used by more than 300 million farmers in Africa or Central America. Fires are also set by Italian farmers, for example, to burn stubble and straw, even though this type of intervention is forbidden because it damages the soil and the atmosphere and, therefore, our health. In the course of evolution many plants have adapted themselves to fires to the extent that some, in certain conditions, are advantaged by them.

Various studies show that the abundance of species has decreased over the years as a result of changes brought about by man. In England, land-use change has also had negative effects on bees and wasps over 80 years. [472] Bee and wasp abundance declined at 75% of the sites surveyed. The reduction in both abundance and biodiversity is influenced by the expansion of crops and practices such as ploughing, insecticide and herbicide use. The spread of monocultures, often in mono-succession, reduces the presence of wild pollinators. This reduction may be partly countered by increasing biodiversity. Conservative management of natural areas close to crops benefits the latter. Increasing biodiversity in agricultural areas has resulted in economic benefits from improved pollination services for the following crops: cherries, strawberries, beans and oilseed rape. [472]

Soil biodiversity is being destroyed by the use of pesticides. Nitrogen-fixing micro-organisms, such as those living in symbiosis with certain plants such as legumes, are damaged by pesticides. A consequent negative effect is the reduction of harvests due to the decrease in soil fertility.

Studies show that climate change in Europe could cause a reduction in species numbers between 27% and 42% by 2080. Nature is our greatest ally in solving this emergency. One species can only thrive if all the others are healthy; we must espouse this principle. It is not about saving the Planet but ourselves. Nature will regain our space after the collapse. When we try to hypothesize the future by looking at the past, we can imagine what will remain of the present. All that we count among the great works of our species will be, in millions of years, compressed into a layer of sediment that may be less than a centimetre thick.

LOST PARADISES: DEFORESTATION AND BIODIVERSITY

Forests are estimated to occupy about 30% of the Earth's land surface (most are tropical forests, followed by boreal forests and then temperate forests) but are home to at least 80% of all species on land. Tropical forests alone occupy a small portion of the Earth's surface (probably 6%) but contain over 70% of the known species. Most of the Planet's forests are found in five states: Russia, Brazil, Canada, USA, and China. [36]

In 2018, one-third of the World's population used wood as their main energy source for cooking and heating. Forests provide roughly 40% of the renewable energy consumed on the Planet, in the form of wood fuels: an amount equal to hydro, solar, and wind combined. [977]

Overall, there were about 5 billion hectares of forest surveyed in 1900; this area was reduced to less than 4 billion hectares in 2010. Therefore, the estimated worldwide forest area is approximately 4 billion hectares which, compared to the 2010 population estimates, makes an availability of about 0.6 ha of forest per person. But if we consider only primary forests, those which have never been cut by man and in which there are no signs of degradation caused by human activities, then the percentage goes down a lot because only 36% of the forest area is today considered primary forest: in the last 10 years more than 40 million hectares of it have been lost.

Between 1990 and 2000 the loss of the World forest area averaged about 8.3 million hectares per year: an area almost equivalent to Italy's agricultural land. [15] The deforestation of the Planet is proceeding at the rate of about 4,000 square metres per second. [28] Every year a surface covered by forests equivalent to two thirds of the Italian surface (200,000 km^2, equal to 20,000,000 hectares) is destroyed. [10] According to other, more optimistic estimates, deforestation is proceeding at the rate of 13,000,000 hectares per year, thus every year at least

1% of the Planet's forests disappear. [37] For the construction and maintenance of biodiversity, the extension of an area is very important. Scholars believe that as the size of an area increases tenfold, the number of species can double. [639]
Deforestation has generated a serious and irreversible loss of biodiversity and an increase in desertification. In the areas of the Planet still covered by forests, choices already made in other countries are being repeated, such as the deforestation of Europe, which in the past was completely covered by forests. Each territory is a different case, for example, in New Guinea 80% of the 21,000 species of plants recorded are endemic, so it is enough to destroy small areas to generate a great loss of biodiversity.
Unfortunately, there is no single definition of a forest or of the area covered by a forest. What should the minimum vegetation cover be? According to some interpretations 10%, according to others 20%. [7] Reforestation cannot be considered equivalent to a forest that has never been damaged by humans, so data on the level of forestation should also provide other information. This is one of the reasons why it is possible to find very different estimates. South America and Africa have recorded (2000-2010) the highest rate of loss of forest area, while in Europe the area covered by forests has increased; but it is sad to discover that, in some cases, this is a consequence of the fact that it is profitable to import timber from the tropics.
The fraction of natural (non-human generated) forests that are being supplanted, in some cases, by human-planted forests, often consisting of non-native species, continues to be reduced very rapidly and irreversibly.
Forest vegetation and soils, after the oceans, are the largest reservoir of carbon. We are destroying at least 12-15 million hectares per year, probably causing more than 20% of the Planet's greenhouse gas emissions into the atmosphere. Forest biomass alone probably contains 289 Gt of carbon. [15] If action is not taken now and current trends continue, over 230 million hectares of forest will disappear before 2050.
An ambitious goal is reported as follows:

> "*By 2020, at least 17% of terrestrial and inland water areas and 10% of marine and coastal areas, especially areas of particular importance for biodiversity and environmental services, shall be conserved, through effective and equitable management, of an ecologically representative and well-connected system of protected areas, with other effective area-wide conservation measures and integrated with wider terrestrial and marine landscapes*"; (10th Conference of the Parties to the Convention held in 2010 in Aichi-Nagoya, Japan).

From the point of view of other living species, a Nature Rights Tribunal would rank us among the worst environmental criminals ever existed on Earth: super-efficient, short-sighted killers capable of extinguishing different species at a rate never recorded before and rapidly accelerating in the last 10,000 years. In conclusion, it can be realistically predicted that today's children, as adults, will not be able to visit the most important remaining wilderness sites because they will have disappeared. We need wisdom; we are the only species capable of imagining our future. We need to build the perfect home.

INSECT BIODIVERSITY IS IN DECLINE

Globally, at least 41% of insect species are in decline and 2.5% of the biomass is lost annually. [344] In Europe 44% and in the USA 51% of insect species are declining rapidly. If mankind suddenly disappeared, the Earth would probably return to the way it was several thousand years ago, on the contrary, if insects disappeared, the biosphere would collapse. According to the Red List of the International Union for Conservation of Nature, 9% of bees and butterflies in Europe are threatened. After the beetles, butterflies and moths (nocturnal lepidoptera) are the biggest family of insects, with at least 200,000 species classified. It has been found that flowers visited by butterflies contain more nectar than pollen and that those visited by moths open at night, when these insects are most active. [869]

In 2017, a 27-year survey (1989-2016) in Germany recorded a 76.7% reduction in the biomass of flying insects: monitoring was carried out in 63 protected natural areas. [344, 346] During the summer, the greatest reduction was recorded: 82%. 94% of the insect trapping points were located near cultivated areas. This rate of reduction is higher than that recorded for wild vertebrates in 42 years up to 2012, which was 58% (total abundance). [346] In recent decades, some scientific works report an annual rate of reduction of insects and, in general, of arthropods between 2.2% and 2.8% per year; the artificial environment of chemical agriculture is the most important cause of this alarming reduction.

A study measured the biomass of insects by catching them using the same system from 1989 to 2013 in a nature reserve in northeast Germany. [278] In 24 years the biomass of insects caught decreased by 78%. The traps used collected mainly flying insects. In 1989, the traps had collected 17,291 flying insects belonging to 143 species but, at the same site and with the same method, 2,737 individuals belonging to 104 species were captured in 2014. [278] A similar result was obtained in the south of Scotland. Between 1970 and 2002 the biomass of individuals captured in Scottish traps decreased by more than two thirds. [278]

Between 1989 and 2013, in some areas of Europe, pesticides and land use change were responsible for the disappearance of 78% of the insects and 86% of the pollinators such as bees. [276] Among the factors that promote the decline of some animals such as pollinating insects is the use of herbicides, as they decrease the possibility of feeding. [483] These molecules, along with other categories of pesticides such as fungicides, manifest harmful effects on insects even though they are apparently not designed to kill them (as opposed to insecticides).

The decline in flying insects is accompanied by the disappearance of other animals such as birds and reptiles. Between 1970 and 2012, the World population of birds, fish, mammals and amphibians declined by 58%. The estimate was obtained by monitoring 3,706 species of vertebrates belonging to 14,152 populations and confirms an average reduction of about 2% per year. [345] The percentage of decline is lower in terrestrial ecosystems (1.1% per year) and higher in freshwater ecosystems (3.9% per year).

In Britain, between 1980 and 2013, there was a strong reduction in the abundance of 353 species of flying insects. [348] Wild pollinating insects, in 33 years, experienced a 33% reduction: in the mountains a 52% decrease was observed. At each monitoring point (1 km^2 over 33 years, approximately 11 species got extinct, of which 4 were wild bees. [348]

About 59 species of butterflies have been classified in Britain and more than 400 in Europe, at least a third of which are definitely in decline. [848] In European grasslands, between 1990 and 2011, butterfly abundance declined by 50%. [346] Monitoring the abundance of 17 species of grassland butterflies, conducted between 1990 and 2017 in 16 European nations, records a reduction for 6 species (35%). [347] In Europe, in 27 years, the abundance of butterflies decreased on average by 39%: this figure shows a dramatic decrease in biodiversity; in some areas of Europe the reduction recorded was much higher. [347] In England, the abundance of butterflies in

agricultural areas fell by 58% between 2000 and 2009, despite the fact that expenditure on nature conservation has doubled and climate change should have encouraged an increase. [353]
According to the Authors, one of the factors that has favoured the reduction in butterflies is the presence of neonicotinoid insecticides (for 15 of the 17 butterfly species studied). [353]
Even in the United States there are frightening rates of reduction: by 80% for the monarch butterfly (*Danaus plexippus*), between 1985 and 2017. [350] Among the causes of the reduction of the monarch butterfly and other butterflies in North America, the use of insecticides emerges such as neonicotinoids. [351, 352] Many pesticides produce sub-lethal effects on the metabolism, reproduction (they can behave as hormone analogues), and behaviour (e.g. neonicotinoids) of butterflies, just as they do for bees. [584]
With regard to butterflies (lepidopterous insects) it must be remembered that they are useful pollinators and can be used as indicators of the state of health of the environment. At the same time, it should be pointed out that most crop pests are probably butterflies, mainly moths.
Insecticides simplify ecosystems by reducing their biodiversity. In Europe, the much-feared nocturnal butterfly in maize cultivation (the corn borer) has been favoured by the use of insecticides such as imidacloprid as it has extinguished its natural enemies. [682]

An increasing number of publications show that the use of seeds treated with insecticides (e.g. neonicotinoids) does not increase yields and harms beneficial insects such as ladybirds. Insectivorous beetles, such as *Hippodamia undecimnotata*, are damaged by sub-lethal doses of imidacloprid and carbofuran (reducing their fecundity). [682] *Adalia bipunctata* is also damaged by sub-lethal doses of neonicotinoids. [679] The ladybird *Adalia bipunctata*[I] is used in biological control. A paradoxical situation is that for farmers, in some contexts, it has become difficult to purchase seeds, such as those of maize, not treated with pesticides such as insecticides (e.g. neonicotinoids), as has happened in Italy. [682]

The continuous and massive presence of insecticides has favoured the evolution of resistance mechanisms. Some examples of artificial selection of pesticide tolerance mechanisms in butterflies are given below: [584]

- a reduced body permeability to insecticides such as organochlorines (DDT);
- the production of new molecules (e.g. proteins or lipids) that can bind pesticides on the outside of the body to prevent their penetration;
- the modification of proteins that bind to neurotransmitters (this is the case of dieldrin resistance generated by a genetic modification that favoured the selection of different receptor proteins).

As it happened with antibiotic resistance developed by bacteria, the intense use of insecticides may favour the generation of genetic factors for multi-resistance, i.e. the ability to be less damaged by the simultaneous presence of different active molecules. The artificial selection operated by insecticides favours the appearance of super-parasitic insects.

The corn rootworm (*Diabrotica virgifera*) is an insect of American origin belonging to the order of beetles. [684] Damage is caused by both larvae and adults, although it is the former that cause the greatest yield losses as they feed on the root system. There have been several reports of insects becoming resistant to insecticides used on maize. [682] Diabrotica can cause

[I] In some populations of *Adalia bipunctata* (ladybird), the majority of beetles are female. [679] In these populations, 80-90% of the progeny of a female is female. The cause of this anomaly is the presence of symbiotic bacteria which live inside the gametes of the female beetles. The bacteria are too big to live in the male gametes (spermatozoa), so the bacteria can only be passed on to the next generation through the female gametes. When these bacteria end up in a male, they die along with the insect. Therefore, the bacteria kill most of the newly laid male embryos. The dead male embryos become food for their sisters when they emerge from the eggs. This characteristic reproductive behaviour is determined by different bacteria (*Wolbachia, Rickettsia, Spiroplasma*), which are present in up to 20% of the females.

serious damage to maize if the crop is repeated two or more years in a row on the same plot, so alternating cultivation may generate greater benefits than using pesticides.

Pesticides such as insecticides, but also fungi and the bacterium *Bacillus thuringiensis*, which produces toxins that are poisonous to lepidopteran larvae, are used to control the growth of butterflies that damage crops. For several years, genetically modified plants capable of independently producing toxins from the *Bacillus thuringiensis* bacterium have been cultivated with the aim of protecting crops such as maize. These toxins are found in pollen and eggs: they can generate trans-generational effects. [584] This new biotechnological strategy to defend plants from pests, such as moth larvae, has led to the appearance of insects resistant to toxins produced in genetically modified plants, as has occurred in migratory moths (*Helicoverpa armigera*). [694] These moths attack various crops such as tomatoes, maize, chickpeas, alfalfa, tobacco, and above all the cotton plant, from which the common Anglo-Saxon name *the cotton bollworm* derives. The larvae of this nocturnal butterfly can feed on at least 120 different plant species. [796] The toxins produced by the genetically modified plants (those of the bacterium *Bacillus thuringiensis*) are harmful to non-target butterflies too.

The corn borer (*Ostrinia nubilalis*) is considered one of the main pests of maize but it attacks over 250 species of plants including sorghum, peppers, eggplant, beans, string beans, apple trees, pear trees, and some ornamental flowering plants. [683] It is an extremely polyphagous nocturnal moth, whose larvae dig tunnels in the stems of maize and other plants. Agronomic practices implemented to control it include the use of insecticides (e.g. neonicotinoids on seeds) and varieties genetically modified for their ability to produce toxins from the soil bacterium (*Bacillus thuringiensis*). This toxin is active in the gastrointestinal tract of some insects, including the order of lepidoptera (butterflies and moths), so it kills *Ostrinia* caterpillars which, feeding on the tissues of the transgenic plant, ingest the toxin. These maize seeds are called *Bt-corn*.

In Europe, of the 68 species of bumblebees surveyed in 2014, 30 are considered to be in decline and 12 threatened with extinction. [417] So for at least 62% of the bumblebee species in Europe have been determined to be in serious danger. It is puzzling that among the factors responsible for this reduction in biodiversity climate change is in evidence but little emphasis is placed on pesticides. [417] In the UK more than 50% of the bumblebee species have become rare or are in decline. [418] Some insecticides such as neonicotinoids, at doses 10 times lower than those to which insects are exposed with pollen and nectar, reduce the fertility of bumblebees. [418] In Europe, the reduction in the diversity and numbers of bumblebees (*Bombus* spp.) has been associated with agricultural practices and the loss of important ecosystems for feeding and nesting. It should be remembered that, in England, three species of bumblebees have been declared extinct and 22 are threatened. In the United Kingdom, the planting of plant species that attract pollinators, such as bumblebees, on the edges of cultivations has led to an increase in the presence of bumblebee species that are considered rare (*Bombus ruderatus* and *Bombus muscorum*). [612] Among the plant species useful for this function, there are some leguminous plants and some flowers able to provide the nectar needed by the insects (annual or multiannual plants). Therefore, to counter the decline of pollinating insects such as bumblebees, sowing flowers at the edges of cultivated fields can also be a useful strategy for farmers. The choice of plants to be sown in soil strips intended to restore semi-natural areas is very important, as it may affect insect numbers, even by hundreds of times. [612] In this study of the 40 species sown and visited by bumblebees to obtain nectar and pollen, four species held 92% of the visits by these insects. [612]

An examination of the variation in the occurrence of bumblebee species in North America, carried out by comparing data from a census conducted in 1971-1973 and after 35 years, in 2004-2006, reveals a drastic reduction in both quality and quantity. [389] In the years 2004-2006, 11 species of bumblebees were identified while there were 14 in the 1970s. In the same sites monitored after 35 years an important decline is recorded, underlining a reduction in diversity but also in abundance of 50% of the species surveyed. Several studies demonstrate the decline of bumblebees in North America: over 20 years, a 96% reduction in the relative abundance of 4 bumblebee species was recorded, and the range was reduced by between 23% and 87%. [361, 392] In addition to the decline, an increase in the prevalence of infections (e.g. *Nosema bombi*) and a reduction in genetic diversity was recorded. The decrease in genetic variability, together with the reduction and fragmentation of habitats, has deleterious consequences.

All these studies confirm that a true apocalypse is underway. It is necessary to become aware of the planetary limits and, in particular, of the physical and biological ones. This very rapid mass extinction will have very serious repercussions because insects, in over 400 million years of evolution, have engineered with plants and other living beings some fundamental balances for the survival of ecosystems.

BIODIVERSITY AND FOOD SAFETY

Many official documents stress the importance of biodiversity for food safety (Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee, and the Committee of the Regions; 2020): [1182]

> *"...Biodiversity is also fundamental to safeguarding the food security of the EU and the Planet as a whole, and its depletion poses a threat to food systems, endangering our food security as well as our nutrition. Biodiversity also underpins healthy, nutritious diets and improves both rural livelihoods and agricultural productivity: more than 75% of the World's food crops depend on animal pollination. Despite the urgency of this moral, economic and environmental imperative, nature is in a critical state. The five main direct causes of biodiversity loss (land and sea use change, over-exploitation of resources, climate change, pollution and invasive alien species) are rapidly causing the natural environment to disappear... Over the past 40 years, the Planet's wildlife has been reduced by 60 percent due to human activities, and nearly three-quarters of the Earth's surface has undergone alterations that have relegated nature to an ever-shrinking corner.*"

We have classified about two million species on the Planet, but we think there may be at least other eight million yet to be discovered, most of them consisting of plants, invertebrates, and microorganisms. More than 270,000 species of plants are known, but 90% of our diet is based on about fifteen of these, including: wheat, rice, corn, barley, soy, and millet. Three species namely rice, wheat, and corn probably provide over 50% of the calories consumed by the human species. Animal nutrition is also based on a few existing species, less than 10 including cattle, sheep, goats, pigs, chickens, and ducks. Currently 90% of the food used by humans is derived from 15 plant species and 8 animal species (75% from 12 crops and 5 animal species). [985] If we accept the estimate of the existence of at least 10 million other species, we can conclude that we have been able to exploit very few species for food and that there is still an enormous potential. In the history of humanity, it is estimated that at least 20,000 species have been used for food and that more than 80,000 could potentially be used today. Unfortunately, it is likely that at least half of the species that exist today will be extinct within this century due to human activities.

The first signs of domestication of plants and animals go back a long way: wheat and sheep were used for agriculture and breeding in the East as early as 10,500 years ago. In the Indus Valley there are traces of cattle domestication dating back 9,000 years. [208] Rice was used by the first Chinese farmers probably already 9,500 years ago, while maize was planted in Mesomerica already 5,500 years ago. The cat was domesticated in Egypt in 6000 b.C. [208]

The selection of the varieties or breeds most "useful" to man and their artificial diffusion through agriculture has given rise to a very dangerous phenomenon: genetic erosion. Rice and pork were used by the first Chinese farmers probably already 9,500 years ago. In just a few years China has gone from cultivating over 10,000 varieties of rice (in 1949) to just a few hundred. Local varieties have been reduced to small extensions. [187] In India, too, it went from growing over 30,000 varieties of rice to a few dozens. It is underestimated that an ecosystem poor in biodiversity is much more vulnerable to change and disturbance.

We have only been able to use a small fraction of the biodiversity that nature and fate have made known to us. The exploitation of such a small part of plant wealth is unsustainable, especially because at the same time we are permanently destroying the possibility of using other species and varieties. To give a few examples, 96% of the more than 7,000 varieties of apples classified in the USA at the beginning of the 20th century have been lost forever. The vast majority of varieties of cabbage, rice, corn, peas, and tomatoes have been lost forever too. [175]

The irreversible reduction of biodiversity also compromises global food security. [338] At least 559 of the 6,190 species of mammals domesticated by humans are extinct and more than 1,000 are at risk. [339] Rapid and massive extinction is homogenizing the Planet's diverse biological communities into man-made environments managed by humans. How much longer can we not deal with this apocalypse before it overwhelms us? In less than 50 years the population has doubled, but the World economy has increased fourfold and the market has increased tenfold, generating predation on limited and therefore precious resources.

Most of the World's industrial production of chickens for fattening is managed by a few companies capable of obtaining tens of millions of offspring from a single male. This model of control and impoverishment of biodiversity is also repeated for cattle: a single bull can have hundreds of thousands of descendants. [985] Genetic homologation in livestock breeding is a spectacular and at the same time distressing manifestation of humanity's dominion over nature. The concentration of animals bred in large numbers and in small spaces generates various consequences such as the abuse of antibiotics: farms in the USA consume at least eight times the amount used in hospitals. More than half of the World market for patented seeds is in the hands of a few big companies. A few companies control the genetics of the animals and plants that make up most of the biomass of living beings on the Earth. Probably the most numerous terrestrial fossil bones, which our paleontological descendants will be able to study, will be chicken bones: animals created by humans to satisfy selfish needs. By the time we decide to act, it will be too late.

Much of agro-biodiversity has been lost to industrial monocultures and this is naively underestimated. Pollinators are important for food production but also for biodiversity conservation: they contribute to maintaining the health of ecosystems providing essential services. [863] In fact, a pollinator rarely visits a single plant species and, at the same time, the time period in which insects need pollen and/or nectar is much longer than the flowering period of a single species. Therefore, most pollinators cannot survive among monocultures, except under artificial conditions such as feeding the reared insects.

At least 50% of the plants that are sources of vitamin A depend on insect pollination.[740] The loss of this biodiversity could increase some nutritional deficiencies and diseases: according to some (optimistic) estimates, the loss of 50% of the pollinators could increase the number of deaths up to 7 million per year. [736] This reduction could be generated by pesticides or climate change: these two factors, together, could cause an apocalypse among pollinators such as insects. Currently, in order to meet the nutritional needs of the World's population, it could be useful to increase plant production that requires pollination from 10 to 20%. [736]

The value of global agricultural production, in 2016, was about $2.6 trillion while that of pollinator-dependent production was estimated at between $235 and $577 billion. [339, 341] In the last 50 years, the volume of agricultural production dependent on pollination by animals has increased by 300%: wild bees contribute to the production of at least 20% of these crops. [341, 344] Probably at least one third of the World's agricultural land is dependent on pollinators. In addition, it is estimated that about 90% of wildflowers (310,000 known species) depend on animal pollination. [341] For these reasons, too, pollinator density should be increased by increasing the presence of wildflowers to boost yields by 20% or more. [342] A survey of the 54 most important crops in France shows that the intensification of agriculture has not led to an increase in pollinator-dependent crop yields in the last 20 years. [343] Industrial agriculture, characterized by high mechanization, large areas cultivated with monocultures repeated over the years, and the use of chemicals, has generated the reduction of pollinators and of crops dependent on them.

Agriculture promotes a reduction in plant, insect, and bird biodiversity. [1240] Crops that need insects to be pollinated would benefit from a different ecological management, i.e. more biodiversity. Increases in the use of agro-chemicals, over 20 years, have not generated the expected improvement in yields.

An interesting fact is that for at least two billion people, insects are part of the food culture: 1,900 species of insects are used for human nutrition. [736]

That part of biodiversity which is considered useful even to farmers is being destroyed before it can be exploited commercially. Important pollinating insects include stingless bees, of which hundreds of species in at least 21 genera have been classified. The hymenoptera of the family Apidae Meliponini, which are a group that includes more than 500 species, are worth mentioning. [730] These insects (the Meliponini) have stings, but they are extremely small and cannot be used for defence. The genus *Melipona* is found in tropical and subtropical regions, such as Australia, and includes about 50 species. Another important genus is *Trigona* and includes about 130 species, also widespread in tropical regions. [729, 731] Some of these insects can pollinate tens of species of different plants, they can move within a few kilometres (*Meliponia panamica* up to 2.1 km), some form colonies with thousands of individuals (*Trigona carbonaria* forms colonies of 10,000 individuals, which make over 20,000 flights a day); they have characteristics similar to those of the domesticated honey bees and, like these, they have the potential to be bred in artificial nests. These insects could be of fundamental importance for pollinating tropical crops in areas where *Apis mellifera* does not exist in nature. Among the crops pollinated by these stingless social bees, there are some that are commercially important such as strawberries, peaches, pears, coffee, cherries, coconuts, mangoes, and onions. [729] Coffee is a self-fertilizing plant but benefits from the presence of wild pollinating insects (they improve both crop quality and quantity). [867] Also for crops such as avocado and mango, several wild species important for the pollination service have been identified (belonging to 11 families in the case of avocado and 8 families in the case of mango). [1186] Unfortunately, there is often little and insufficient information on the service performed by wild species, so the importance for food security may be underestimated. A diverse pollinator community is certainly more resilient and improves agricultural production. In order to ensure a greater biodiversity within pollinators, it is necessary to move away from the non-conservative principles on which the industrial chemical agriculture is based. In some crops the effectiveness and efficiency of pollination by these insects is higher than that of domesticated honey bees, as the latter either do not visit the flowers or collect only nectar or are less efficient. Maintaining semi-natural or natural areas near crops increases the probability of survival of these insects and the resilience of the agricultural system. Risking to reduce the pollination service to a few, exclusively bred, insect species is a hazard as it increases uncertainty and instability to an unacceptable degree.

Probably, the companies producing seeds in the World, in 1981, were more than seven thousand, after thirty years six multinational companies control about 63% of the market. The same companies manage at least 75% of the global pesticide market. [196] A monopoly has been created that deprives a large part of the World's population of food sovereignty and self-sufficiency: self-sufficiency in the ability to reproduce and choose the species and varieties to cultivate and breed is thus seriously compromised. Unsustainable economic constraints and food systems, which a forward-looking society should have avoided, have been fostered. Unfortunately, food insecurity is irreversibly increasing. The right of peoples and states to decide their own food policy is increasingly undermined by abuses by the powerful.

POLLINATORS AND FOOD SAFETY

Agricultural production depends on the resources used (e.g. fossil fuels, fertilizers, pesticides, machinery, irrigation) and on the services provided by the ecosystems: soil fertility, rainfall, climate, pollinators, natural enemies of crop pests. Unfortunately, the contribution to the pollination service is known or partially known for only a small fraction of the potential wild pollinators. Some researchers suggest that we have information on the benefits generated by pollination for about 12% of the bee species studied. [1176] Thus, for most species we have no knowledge or insufficient information. Most pollinating insects, like the thousands of bee species, are at risk of disappearing forever before we know what their potential benefit might be to food security (and more).

A crop is considered very pollinator-dependent when in the absence of pollinators production is reduced by as much as 95% (e.g. almonds and pumpkins). A crop is classified as moderately pollinator-dependent when in the absence of pollinators production is reduced by 65%. In 45 years (1961-2006), the planetary production of highly insect-dependent crops has increased less than that of crops that are little or not at all pollinator-dependent. [478] Food production directly dependent on pollinators probably accounts for between 5% and 8% of global food production. [481, 484]

The continuous and systematic use of insecticides generates a decrease in production by plants that require pollination, including those cultivated by humans. [450] In just a few decades it has been possible to record a drastic reduction in plant biodiversity and in the food supply. With the reduction in the supply of plants, the possibility of following a balanced diet decrease. In this regard, it is useful to remember that at least 2 billion human beings suffer from malnutrition due to a lack of nutrients such as vitamins and certain minerals; another 750 million people suffer from malnutrition and 2 billion from overweight or obesity. [415, 985] The reduction in pollinators also decreases the ability to produce medicines, plant fibres, and other materials.

Pollinators and their essential service to agriculture are severely compromised. More than 90 plant species important for human nutrition depend on pollinators and, in particular, on domestic bees. At the same time, unfortunately, at least one in three bee colonies dies every year due to pesticides and the irreversible reduction of plant biodiversity. To compensate for the loss of wild pollinators, fruit and vegetable producers around the World use, in addition to honey bees, at least 48 other species of pollinating insects managed for this purpose. [338]

The shortage of pollinators for food production can be confirmed by the cultivation of almonds in North America. In the United States more than 1.5 million colonies of domesticated honey bees (making up about 75% of the colonies present) are moved to pollinate California almonds each year (at least 4 colonies per hectare). It is one of the greatest artificial migrations on the Planet, occurring by road transport. In the USA more than 90% of honey bee colonies are moved to favour the pollination of a few crops: almonds, apples, melons, alfalfa (from seed), plums, avocados, blueberries, cherries, cucumbers, pears, sunflowers, pumpkins, and legumes. [974]

In 50 years (1961-2012), the global pollinator-dependent production increased fourfold, while non-pollinator-dependent production increased twofold. Over 50 years, agricultural production has increased its dependence on pollination even though the quantities produced are smaller than for crops not dependent on pollinators. [481]

If bees were to become extinct, the food supply available to the human species would be greatly reduced. It is estimated that between 15% and 30% of human food depends, to varying degrees, on pollinators. [415] Of the 124 plant species most important for human nutrition, 87 (70%) depend on pollinating animals. [415] These 87 species account for about 35% of the total amount of plant food produced. For example, the presence of bred and wild bees increases the production of cotton (*Gossypium hirsutum*) and sesame (*Sesamum indicum*) crops (in Burkina Faso, Africa, where, for example, cotton cultivation is an important source of income: 60% of the exported volume). Experiments in the field have shown that the absence of a pollination service causes a reduction in the yields of 37% in the case of cotton, and 59% in the case of sesame (cotton flowers, which usually bloom one at a time per day are visited by at least 30 different species including *Apis mellifera*). [1188] Many crops depend on both bred and wild pollinators, the benefits of which are often underestimated.

The shortage of pollinators (bees, but also bumblebees, butterflies, beetles, birds, and bats), at a planetary level, could generate the loss of 23% of fruit, 16% of vegetables, and 22% of nuts and seeds (partial and optimistic estimates). [676] Most crop species require pollination and although they make up one-tenth of all the agricultural production, they contribute about one-third of the market value. [676] In fact, non-pollinated crops (e.g. corn, wheat) have a lower market value, per unit weight, than pollinated crops (e.g. fruit). Pollinated crops often have a higher nutritional value and help provide essential nutrients such as vitamins, antioxidants, minerals, and fibre. [676] Some optimistic estimates calculate that the loss of pollinators in Europe could reduce the food supply by at least 7%. [415] In Europe, at least 12% of the agricultural land is dependent on pollinators such as the honeybee and bumblebees. Pollinator-dependent crops in Europe economically represent 31% of the market value of the agricultural production, and the value of the pollination service is estimated to be between 10% and 12% of the value of the agricultural production. [415]

In Italy, at least 600,000 hectares, in 2017, were cultivated with plants dependent on pollinators; in 2016, more than 40,000 hectares were cultivated for the multiplication of vegetable seeds. It may be considered that at least 10% of the Italian agricultural area depends on pollinators. [196, 199] The capacity of the pollination service present in Italy covers only 60% of the real demand, so there is a need for pollinators also in Italy. [199] The absence of this service generates an economic damage measurable in millions of euros. Due to the decrease in the pollination service, in the worst-case scenario, the number of people who will die of hunger or suffer from malnutrition will increase.

It should also be noted that at least twenty thousand species of pollinators are classified and most wildflowers depend on these insects. [196] It is estimated that 85% of wildflower plants depend on pollinators: at least 299,200 species. [415]

Rather than asking whether humans will be able to survive without pollinators, it would be more correct to shift the focus to whether we can withstand the changes that cause a reduction in the survivability of wild insects, but also of bred insects.

The increase in the number of plant species in the vicinity of cultivations generates benefits both to pollinators and to other useful organisms such as natural enemies of plant pests: it is a natural biological control. [471] Some economic estimates, although they have many limitations, value the service provided by the forest in the vicinity of coffee plantations higher than the value of the forest itself, because the pollinators of this crop are advantaged. Services such as pollination, offered by a wild environment to a crop, may encourage the conservation of natural areas. In the vicinity of crops, among the services offered by a complex environment rich in biodiversity that can be monetized are pollination (by wild animals) and the presence of natural enemies of crop pests. The latter limit the economic damage to crops. In an agricultural environment with a greater biodiversity both pollinators and natural enemies of plant pests are more abundant. This is also the case in polyculture compared to monoculture. Information regarding these two types of services provided by nature to crops is scarce and fragmentary. According to some indications from the watermelon cultivation, the pollination by wild bees occurs with a sufficient efficiency when 30% of the land within 1,200 m of the crop is covered with wild plants. [471] Increasing the complexity of agricultural ecosystems has positive effects on both the abundance and richness of bees, spiders, herbaceous and tree plants. However, this is a very anthropocentric view and, therefore, partial, as it is based exclusively on the quantification, in the short term, of an economic benefit for the farmer. If the services offered by nature were inventoried, one would have the advantage of having arguments on which economists and farmers are very sensitive and receptive. Therefore, conservation strategies, such as dedicating a percentage of the cultivated area to wild plants for the protection of natural biodiversity, could be more easily proposed.

Approximately 70% of the 1,330 tropical crops and 85% of the 264 crops in Europe benefit to some extent from the pollination service provided by animals. [615] In economic terms, pollinators may probably increase the productivity of at least 115 crops by 75%. Consequently, an increase in food insecurity may be expected as a result of the pollinators' decline. The information available to us is not reassuring:

- Biodiversity is rapidly declining, wild habitats are being destroyed and the number of wild pollinators is decreasing.
- The stability of agricultural production also depends on the presence of wild pollinators, which, however, are rapidly decreasing.
- The use of artificial breeding of pollinators, such as honeybees and bumblebees, cannot compensate for the loss of biodiversity. In many countries, the number of honeybees kept is declining and, in any case, is not sufficient to meet the increased demand for pollination at the global level. The demand for the pollination service from farmers is increasing faster than the availability of bred insects.
- The increase in the demand for food is achieved through the increased use of destructive agricultural methods (e.g. pesticides, monoculture, ploughing) and the increased degradation of natural areas to establish plant crops and livestock. This trend, in the short term, may generate an increase in the agricultural production but will be counterproductive in the long term.

All these elements put together suggest that it will be difficult to hope for an increase in the agricultural production of pollinator-dependent species. On the contrary, it is more realistic to foresee a reduction in the productivity of plant species dependent on pollinators, but it must be stressed that crops that depend on pollinators allow, on average, higher earnings, even five times higher. [615] To compensate for the reduction in pollinators, the economic rules have devised unsustainable remedies:

- The breeding of honey bees to pollinate, for example, apples, cherries, almonds, watermelons, pumpkins, and melons.
- Breeding bumblebees to pollinate tomatoes.
- Pollination is done by hand by vanilla workers and in some orchards, in areas of the Planet where chemistry has destroyed pollinators and where labour, under ethically questionable conditions, is considered cheap. Pollen is either collected by hand and

stored in refrigerators for the following year, or it is collected in areas where the climate allows early blooms.

In conclusion, pollination-dependent crops are more unstable and heavily and negatively affected by ongoing changes. One consequence is that, between 1961 and 2008, about 70% of the increase in the cultivated area involved the spread of non-pollination-dependent species. [615, 616]

Many predictions are based primarily on the economic assessments and do not consider or underestimate the fact that the conditions that generate the disappearance of pollinators will compromise the ability of ecosystems to support a human population that expects to grow numerically and wishes to increase the consumption of resources that will no longer be available.

THE LOSS OF FOOD SOVEREIGNTY INCREASES INSECURITY

With the affirmation of economic rules that favour monopolies and oligopolies, food sovereignty is diminishing. The inability of the States, but also of local communities, to decide autonomously and freely what to produce, how to produce it, also from an ecological and social point of view, and to decide what markets to direct their products towards, constitutes a devastating and unsustainable source of insecurity and slavery. The loss of food sovereignty may also easily lead to the impossibility of meeting the nutritional needs to ensure a good state of health of local populations. The reduction of food security and food sovereignty is very dangerous and should be opposed with more energy than we are doing. We have given the keys to our pantries to a few big companies, which operate according to completely artificial market logics, detached from ecology and social security. At the same time, the ability of future generations to secure the necessary food resources themselves has been severely compromised. The availability of food is a fundamental civil, economic, and social right that promotes equality, freedom, and dignity for all human beings. We have severely compromised this right by giving the control of our freedom to a few entrepreneurs who are so powerful that they can influence the life and health of the entire Planet. To promote greater justice, it is important to increase the transparency, reconnect the producers with the consumers, and promote ecological and sustainable practices.

The reduction of independence in food production and agro-diversity is very dangerous. 63% of the seed market and 75% of the pesticide market, worldwide, are controlled by a few multinational companies. [196] This concentration of power confers a risky economic and political strength and, at the same time, is a manifestation of the delirium of omnipotence. Let us remember that the global food production is responsible for 60% of the reduction in biodiversity and at least 24% of all climate-altering gas emissions. [980]

SUB-LETHAL EFFECTS OF PESTICIDES ON WILDLIFE

Insecticides, such as neonicotinoids and fipronil, are associated with an increase in infectious diseases in wildlife. [442] These pesticides weaken fish, amphibians, and birds by inhibiting their immune defences. Thus, the inhibition of the immune system caused by the use of neonicotinoids and fipronil promotes the occurrence of epidemics in wild animals.

The LD_{50} of imidacloprid is 40 ng per bee (much lower than that of other insecticides) but the daily ingestion of 1 pg (a 4,000-fold lower dose) kills bees in less than 10 days. In addition, imidacloprid slowly degrades into at least six metabolites, some of which are more toxic than the starting molecule. Concentrations between 2 ppb and 50 ppb (e.g. in pollen) are sufficient to

generate severe sub-lethal effects in bees; 5 ppb (parts per billion or 0.005 mg/kg) are sufficient to promote the emergence of parasites such as the pathogenic fungus *Nosema ceranea*.
Bats are harmed both by the disappearance of their prey (insects) and by the fact that they contain insecticides in harmful doses. [442]
Acetamiprid and clothianidin alter the immune system in rats and therefore could have the same effect in other mammals, including humans.
Neonicotinoid insecticides are found in the pollen and nectar of treated plants, but also in plants in nearby fields. These molecules contaminate water to the extent that they are found in more than 50% of the rivers monitored in the USA. [483] Neonicotinoid insecticides have been shown to inhibit the immune defences of mussels (molluscs) and chickens. [483] The use of insecticides in rice fields has been responsible for an increased fish mortality due to the simultaneous presence of parasites (*Trichodinia* in the medaka fish). [483] Some neonicotinoids can remain for a long time in water: the half-life of clothianidin is more than 500 days in water, and between one and two years in the soil. This persistence harms the aquatic fauna such as fish exposed to treatments in rice fields (e.g. medaka, the rice fish or *Oryzias latipes*). [442]

In Europe (Germany and France) and Australia (Victoria), pesticides are implicated in the reduction of invertebrate biodiversity. At sites most contaminated by pesticide use, invertebrate biodiversity is reduced by up to 42% in Europe (France and Germany) and up to 27% in Australia. [276, 277] The presence of pesticides is negatively correlated with the observed species richness and abundance. Decreases in species numbers are recorded significantly even when concentrations of toxic substances are very low, i.e. they are considered of no concern. Reductions in biodiversity are recorded at pesticide concentrations between 100 and 10,000 times lower than those that are considered lethal to 50% of the individuals of an aquatic organism (LC_{50} or lethal concentration for 50% of the exposed individuals): *Daphnia magna* or the water flea, a small aquatic crustacean. [277] Very dramatic effects are recorded at concentrations considered safe by European standards for pesticides used in agriculture. The current risk assessment has resulted in a European legislation that tolerates and considers as non-hazardous concentrations of pesticides that actually cause an irreversible damage to invertebrates. It is wrongly considered that no adverse effects should be recorded below a dose of less than one hundredth of that capable of killing 50% of the crustacean *Daphnia magna* in the laboratory; this also applies to fish. The use of pesticides is therefore responsible for the reduction of biodiversity: the correlation has been demonstrated for 21 pesticides used in Germany, 10 in France, and 97 in Australia (of the more than 400 active molecules used: so they are used before they have guarantees). The demonstration of the existence of a correlation between the reduction of biodiversity and the use of pesticides is strong even when only some of the active molecules used are examined and even when the effects of metabolites and other molecules present in commercial preparations are not considered. [277] Unfortunately, the use of these toxic molecules is increasing on a global scale, partly due to climate change.

Fungicides can favour harmful insects as they can kill the fungi that parasitize them. To give an example, benomyl, which is a fungicide, favours the spread of some insect pests of soybean because it kills entomophagous fungi that would help to limit their growth. [502] The costs resulting from the destruction of the natural enemies of insects that damage crops, generated by the use of pesticides, may be estimated between 25% and 50% of the costs generated by the use of insecticides (e.g. in cotton, apples, peaches, oranges). [502] In agro-ecosystems, the natural enemies of crop pests are responsible for at least 50% of the pathogen growth control.

Pesticides also have direct and indirect negative effects on wild vertebrates. [412] An indirect effect of the reduction of insects is the reduction of animals that feed on them, such as birds, reptiles (e.g. lizards), fish, and amphibians. [552] Insects may constitute between 40% and 90% of the diet of freshwater fish, 75% of that of amphibians and lizards. Sub-lethal effects generated by the exposure to small doses of pesticides are known. Recorded sub-lethal effects include genotoxic and cytotoxic effects, immune system alterations, reduced reproductive success (e.g. recorded in the yellowhammer in the UK). All these effects are generated in wild vertebrates at doses much lower than those necessary to record a mortality in a short time (that of the acute intoxication). The ingestion of a few seeds treated with clothianidin and fipronil has been shown to be sufficient to kill small birds or to cause severe reproductive problems. [412] Insecticides can reduce the ability to fly in sparrows, and can alter the immune system and damage the DNA of quails and partridges.

Imidacloprid, clothianidin, and fipronil (three insecticides) are toxic to birds and fish. Clothianidin may be present at doses between 4 g and 7.5 g per kilogram of rape seed and imidacloprid between 2.1 and 6.6 µg/kg of maize seed. Higher imidacloprid concentrations may be recorded in sugar beet leaves from treated seeds (1-12.4 mg/kg). Thus, both granivorous animals (e.g. birds, small mammals) and herbivorous animals (e.g. insects, small mammals) are exposed to significant doses.

Rapeseed and cottonseed treated with clothianidin reduce the chance of survival of small mammals and birds. For sparrows and partridges, it is sufficient to ingest between 1.5 and 6 beet seeds treated with imidacloprid to reach the dose capable of killing them, with a 50% probability, but the amount of imidacloprid contained in a quarter of a seed is sufficient to generate sub-lethal effects.

In soil cultivated with wheat, imidacloprid concentrations between 18 and 60 µg/kg have been recorded, while after sowing treated maize seeds, concentrations up to 652 µg/kg are recorded in the soil (this concentration drops to 11 µg/kg 30 days after the maize harvest); this means that soil-dwelling organisms (e.g. ants, termites, earthworms) are exposed to high concentrations too. [412] In this respect, it is interesting to remember that most wild pollinating insects spend part of their lives in the soil (e.g. laying eggs). In the natural vegetation around maize fields, whose seeds have been treated with clothianidin, imidacloprid concentrations of up to 9 µg/kg have been recorded. [412]

Insecticides reduce the biodiversity of the aquatic ecosystem. In rice fields, neonicotinoids are used to kill insects such as the rice weevil (*Lissorhoptrus oryzophilus*) that eats the leaves of the plant. [415] Aquaculture is also adversely affected by pesticides. Fish stressed by widespread chemical contamination are more likely to be susceptible to parasites (e.g. medaka fish are more susceptible to the parasite *Trichodina ectoparasite* if imidacloprid is present). [415] Pesticides, at very low doses, generate alterations measurable by changes in enzyme activities in the liver of fish (indicators of liver damage that are sub-lethal effects). Also molluscs such as mussels (*Mytilus galloprovincialis*) show negative effects due to the exposure to very low concentrations of insecticides (e.g. imidacloprid).

Pesticides can be persistent in the environment and can therefore cause problems long after their distribution and far from where they have been used. This may be highlighted by recalling the finding of organochlorine insecticides and PCBs in cetaceans. [299]

We are the cause of an ecological disaster and we seem to have forgotten that we need worms in the soil, flies, beetles, microorganisms, and fungi to decompose dung, ladybirds and hoverflies to eat aphids, bumblebees and butterflies to pollinate plants, microorganisms to help plants fix the atmospheric nitrogen. We are heading towards the Eremocene which, let us not forget, can only exist for a very short time because the collapse of the services rendered by nature immediately generates the collapse of the human society. We have the illusion of having freed ourselves from the evolutionary constraints of natural selection, when instead we continue to depend on the biological and geological systems of the Planet that we are altering at an unsustainable rate.

BIRDS AND POLLINATORS SHARE THE SAME FATAL DESTINY

Cases of poisoning of birds by pesticides, for example by organochlorines and organophosphates in Argentina and Venezuela in the 1960s, have been recorded all over the World for several decades. [431] The disappearance of insects is closely related to both a quantitative and a qualitative reduction in bird numbers. It is estimated that the average number of birds in Europe decreased by 56% between 1980 and 2015 (at least 500 million fewer birds). [307] In Europe, 37 species of farmland birds (sparrows, larks, linnets, meadow pipits, yellowhammers, etc.) have been reduced numerically, on average, by more than 52% in 30 years. Many birds survive by eating insects, so these two groups of animals are closely related.

In wildlife such as birds, cases of pesticide poisoning are recorded in Europe; birds, if not killed by hunting, are poisoned by the lead of the bullets. [429]

In the French countryside, bird populations have been reduced by at least a third in 20 years. [421] Species such as skylarks, sparrows, and whitethroats have lost at least one in three individuals in less than 20 years. For some species the reduction is more dramatic: eight out of ten individuals have disappeared in the case of partridges. Skylarks have recorded a reduction of at least 35%. Unfortunately, in recent years (2016 and 2017) the extinction rate has increased. The decline of these species is linked to that recorded in insects. Many birds feed on insects and even granivorous birds can, over a lifetime, feed on them. Among the factors accelerating the disappearance of birds, there is the treatment of seeds with insecticides and the use of herbicides. For small birds, such as sparrows, it is enough to eat two or three seeds (e.g. wheat or corn) treated with neonicotinoids to die. Other factors that reduce the possibility of bird reproduction are the lack of refuge areas where to nest and the working of the soil: many birds nest on the ground. Examples include the yellowhammer (*Emberiza citrinella*) that nests on the ground in grass or hedges, and the female whitethroat (*Sylvia communis*) that builds nests hidden in stubble a short distance above the ground; these birds are migratory, coming to Europe from North Africa. [797, 798] The constitution of semi-natural and uncultivated areas with trees provides the possibility of feeding and nesting to many species of birds that also feed on insects. [962]

In France, the analysis of the causes of 3,130 incidents involving the death of birds due to suspected poisoning, between 1995 and 2014, reveals that 103 incidents were definitely related to the use of seeds treated with pesticides, in particular with imidacloprid. [422] Of these 103 poisoning events, 101 occurred in conjunction with the sowing of cereals (wheat, barley, maize) and resulted from the use of imidacloprid (neonicotinoid insecticide found in liver and seeds contained in the digestive tract). [422] A total of 101 imidacloprid poisoning events resulted in the death of eleven bird species and one mammal (common hare or *Lepus europaeus*); together they caused the death of at least 734 animals. On average 7 dead animals were collected per incident. The birds most affected included the grey partridge (*Perdix perdix*), wood pigeon (*Columba palumbus*), and pigeon (*Columba livia*). [426, 427, 428] Pigeons were involved in 51% of the incidents and partridges in 38%. Of the dead animals, 81% were columbids. For most birds it is sufficient to ingest a few insecticide-treated seeds to reach the dose capable of killing them, with a 50% probability (LD_{50}). [422] The sowing of wheat involves burying between 150 and 450 seeds per square metre, of which a fraction (about 40 seeds per square metre, more or less 9%) remains on the surface and is therefore easily usable by the birds (each seed contains between 0.028 and 0.042 mg of imidacloprid). Cereals such as wheat are mainly sown in autumn on 6 million hectares. Behavioural disturbances were recorded in 39.6% of the incidents: sudden falling in flight, paralysis or paresis, disorientation, apathy, and impaired alertness. The researchers conclude the article by pointing out that the concentrations of imidacloprid found in the liver of dead birds may be so low (in the order of billionths of a gram per gram) that they

cannot be easily measured analytically. Therefore, lethal effects can be generated by very low doses.
Some studies have recorded up to 9 birds killed per acre due to pesticide exposure. [425] It is estimated that the USA distribution of about 500,000 kg of pesticides causes the death of over 72,000 adult birds and damages over half a million colonies of bees and other wild pollinators every year. [425] These are probably underestimates, but they provide a measure of the severity of the problem and suggest we need to take action before it's too late.

Pesticides can accumulate and are capable of damaging bird reproduction, as it has been demonstrated since the 1960s for organochlorine insecticides such as DDT. In Italy, residues of organochlorine pesticides and their derivatives are found in the eggs of birds (e.g. kestrels: a small migratory bird of prey that feeds on mice and other rodents, small birds, insects, and earthworms) in quantities sufficient to damage the shell thickness seriously. [26, 430] Dangerous concentrations of organochlorine insecticides are also found in the liver of birds of prey in Greece. [432]

A study carried out in the Netherlands shows a strong correlation between the presence of imidacloprid in surface water (at concentrations of the order of billionths of a gram per litre: 20 ng/L) and the decline of birds, amounting to 3.5% per year. [434] The researchers extrapolated this result by studying 15 species of birds, 9 of which are insectivorous but all 15 feed their chicks on invertebrates (some, like passerines and starlings, are also granivorous). Neonicotinoids can remain in water for long periods (imidacloprid, in water, can have a concentration half-life between 30 and 162 days). [434]

A study conducted in Switzerland during the spring of 2015 demonstrated the presence of neonicotinoid insecticides in all 146 feather samples of house sparrows (*Passer domesticus*), which feed on both insects and seeds. [441] Each sample consisted of the feathers of three birds caught at the same time and site. Thiacloprid was present in 99% of the feather samples and clothianidin, which was detected in 75% of the samples, reached the highest concentrations (131.4 ng/g). Imidacloprid was found in 40% of feather samples, acetamiprid in 37%, and thiamethoxam in 24%. Less than 9% of the samples contained only one neonicotinoid but 5.5% of the samples contained all the 5 neonicotinoids searched. Birds living near fields where organic farming is practiced recorded lower concentrations (maximum 23.6 ppb) than those detected in bird feathers from areas where conventional farming is practiced (maximum 140.5 ppb). [441] These data confirm the ubiquitous presence of neonicotinoids in the environment and the exposure of birds to complex mixtures whose synergistic, additive, and sub-lethal effects are mostly unknown. In birds, sub-lethal effects of neonicotinoids include thyroid hormone destruction, and adverse effects on reproduction and embryonic development. Censuses conducted in North America, between 1966 and 2013, recorded a 74% reduction in bird species living in agricultural areas (57 of 77 species surveyed). [444] The group of birds with the most noticeable decline were insectivores. The most important causes of the reduction in bird biodiversity highlighted in this work were pesticides (42%) and, in second place, habitat alteration and destruction (27%). In 2012, 16% of the pesticides used on the Planet were distributed in the USA where they were estimated to be responsible for the death of between 67 and 72 million birds per year. [444] In the same years, in Canada, pesticides were killing at least 2.7 million birds. These are probably underestimates but it would be essential to listen to these alarming signs.

Insecticides that have been associated with an increased bird mortality include carbamates and organophosphates. In Canada, carbofuran (a carbamate insecticide), used in canola seed, is believed to be responsible for between 109,000 and 958,000 bird deaths, annually. [444] In the USA, the same active ingredient, used in the 1980s in corn crops, was thought to be responsible for the deaths of between 17 and 91 million birds each year. Sub-lethal effects of pesticides on birds include the loss of orientation ability necessary for

migration. In passerines (*Zonotrichia leucophrys*), the ingestion of a few seeds containing imidacloprid also resulted in a decrease in body weight. The ingestion of low doses of organophosphate insecticides, such as acephate or chlorpyrifos, impairs the orientation ability, while carbofuran impairs the thermoregulation ability, generates a loss of movement control, paralysis, and spasms. [444] The use of the insecticides azinphos-methyl, dichrotophos (organophosphates), and carbaryl (a carbamate) causes a reduction in the number of trips that birds make to feed their offspring in the nest. These effects, which decrease the ability to survive and resist predators, have also been recorded in bees.
It is useful to remember that many species of birds build their nests on the ground, therefore the lack of grass or operations such as ploughing decrease their reproductive success. The presence of uncultivated areas with spontaneous vegetation increases the biomass of insects by at least 12 times compared to monocultures. Therefore, agronomic practices also greatly influence ornithological and entomological biodiversity. If at least 10% of the agricultural surface were dedicated to uncultivated areas with spontaneous vegetation, also agriculture would benefit greatly.
Following the review of the scientific literature on the decline of birds on the Planet, it is possible to state that the current system of food production, which has its foundations in industrial chemical agriculture, constitutes the most important threat to the survival capacity of hundreds of arthropod species, birds, and other animals.

THE DOMESTICATION OF THE NATURAL LANDSCAPE IN ITALY

Since 1990, at least 239 million hectares of natural forests on the Planet have been destroyed. [345] The artificial reforestation produced by man to compensate for this loss is useful, but in any case biodiversity is lost and genetic homologation of forests is generated; the forests planted by man today occupy about 7% of the Earth's surface. [345] Probably, before 2050, one billion hectares occupied by natural ecosystems will be converted for agricultural use and to meet the increased demand for food. [604]
At least two billion people derive their energy needs primarily from burning wood, at least four billion people derive their medicines directly from plants and animals, about 70% of the medicines used to treat cancer are of natural origin or are synthetic molecules inspired by natural molecules. [339]

In Italy, unfortunately, there is no place like a forest that may in any way be considered natural, i.e. with the appearance and components that it would have without man's modifying intervention. [737, 748] The national territory has been totally modified, transformed, and degraded by the work of a few hundred generations. Centuries of deforestation, ploughing, land reclamation, fires, hunting, pastoralism, urbanization, and industrialization have destroyed the natural heritage that existed before man's dominion over nature. In 6500 b.C., in Italy, the population was probably less than 60,000 and at that time the territory was completely covered by forests. In 200 a.C. (after Christ) there were already 8.5 million inhabitants, but they were reduced to less than 4 million in 700 a.C., because of the plague epidemic, imported from Egypt in 543 a.C., and of devastating floods; in 900 the population was still around 4.5 million and in 1200 it returned to the number of 200 a.C., that is about 8.5 million. [737] Subsequently the population increased again but, because of hunger and epidemics, in 1400 it had fallen to around 8 million, as it had been 1000 years before. In 1650, 11.5 million were estimated and, in 1850, 25 million. For centuries the population did not increase significantly but, despite the low population, the territory was nevertheless profoundly modified. Like the agrarian landscape, the forests can be considered an artefact, having been entirely modelled by the work of man. In the

sixteenth century, the Italian wooded territory had already been reduced from 90% in prehistoric times to 50%. [737]
In Italy today, there are no longer natural forests, but rather forests that have grown back on abandoned land or forests where the management has been interrupted. Recent phenomena of natural or artificial reforestation have triggered new processes, not always desirable from the ecological point of view, such as the spread of alien species or plants artificially selected by the technological innovation. This loss is irreversible, it dries up the cultural sphere and impoverishes the social one: a historical memory is also lost.
For some millennia the natural harmony of the forest has been modified by man, so that in the first centuries after Christ it would already have been very difficult to find virgin forests in Italy. [748] Paleobotanical studies have shown that in central Italy, around 1000 b.C., there was a presence of chestnut pollen equal to 8% of the total tree flora. Due to human intervention, at the beginning of the Christian era, this percentage increased to 48%: in the 15th century, chestnuts were still used as a form of payment, similar to wheat. This information confirms the role of the Roman civilization in shaping nature.
Until the Second World War, most of the rural landscape also consisted of forests. Farms could not exist without the forest and were based on polyculture and rotation on small plots. The transition, which took place mainly in the last century, from solar-powered agriculture, i.e. tending to be self-sufficient in energy consumption but with a relatively low productivity, to agriculture using external energy, mostly fossil fuels, marked the decline of the forest and promiscuous agriculture in the plains.
The woods in Italy, in the last century, have increased due to land abandonment: they have an extension of about 0.21% of the forests of the Planet; about 35% of the national territory is mountainous and 23% is occupied by plains. [748] Italy is not self-sufficient in food (most of it is imported) and not even in timber, since 85% comes from abroad. Italy does not have enough land to meet national needs: for each inhabitant there are about 5,000 square metres of which only 1,500 are cultivated; a similar extension is made up of forests and the rest are more or less urbanized or degraded areas. If we try to estimate the current trend for the future, a very worrying situation emerges: the demand for soil increases but the available surface area decreases.

The estimates have several weaknesses because, for example, it is not clear what is meant by a *forest*, since not all types are equivalent with respect to the services they can provide, such as the protection of biodiversity: can even-aged plants, of the same variety artificially selected by man, and with a homogeneous level of cover and density, grown exclusively in the mountains, be considered a forest? What is the minimum level of land cover and the level of biodiversity that a group of trees and other plants to be considered a forest? Probably many areas covered with trees cannot be included in the concept of a forest (e.g. poplar cultivation, which, among other things, could be carried out with genetically modified plants; for example, those that have been modified so as not to produce seeds with an airborne diffusion system, which are considered troublesome).
The forest has been altered for various purposes: to obtain timber for fire, to have building material, to obtain food (e.g. from chestnut trees) or to be able to raise animals for grazing. The need for construction timber has altered the natural proportion between conifers and deciduous trees in favour of the former. Fires set to create pastures have also favoured fire-resistant plants such as the strawberry tree and some conifers (called pyrophytes). For centuries the forest was synonymous with grazing, but for less than 100 years grazing has become an enemy of the forest. The combination of fire and grazing has, over time, favoured plants that are more resistant to fire and the bites of herbivores. In the past, some types of sparse woodland were used for grazing by hogs feeding on acorns (e.g. oaks).

Wood was used to produce charcoal, which allowed for the high temperatures needed for various processes such as smelting metals and ceramics. The process is energetically unfavourable as it takes at least 5 kg of wood (about 17,500 kcal) to obtain one kilogram of coal (capable of providing 7,500 kcal). The advantage is that coal is a more concentrated form of energy and easier to transport; it also allows higher combustion temperatures to be reached, which are essential for certain types of processing such as metals.
Deforestation for military purposes has a long history. In 256 b.C. a war between the Romans and the Carthaginians is documented in which the Romans used 160 ships. In a later battle they used 330 ships. In order to have a large naval fleet, it was necessary to manage forests from which the necessary wood material could be obtained. Therefore, since ancient times the need to protect the forests for various reasons such as military, charcoal production or construction has emerged.
Even cities depended on forests: it has been calculated that the foundations supporting Venice originated from at least twenty thousand hectares of forest. [737]
The deforestation of hills and mountains causes serious hydrogeological disruptions with enormous social costs. The forest is the most effective means of consolidation and protection for land exposed to the action of meteoric erosion.
In 1530, in Italy, measures were taken to reduce deforestation and to reconstitute the forest heritage; for example, it was obligatory to reforest at least 8% of the land cleared in the previous 40 years. Between 1600 and 1700, in order to protect some Italian forests, the death penalty was envisaged. Probably, in order to protect biodiversity today, measures of equal magnitude should be adopted. In order to reduce the current rate of degradation, it would be useful to organize the free distribution of seedlings for reforestation and to involve the population and schools. As in the past, agricultural regulations and subsidies could encourage the planting of trees on farms, such as those in the plains, and in cities.

HUNTING IN ITALY

Humans began manipulating and altering ecosystems in a way that was very apparent to paleontology over 10,000 years ago. 50,000 years ago there were at least 150 genera of animals belonging to the mega-fauna, i.e. animals weighing more than 44 kg. 10,000 years ago, when the colonization of the Continents by man had ended and the climate stabilized on milder regimes, 100 of these genera had disappeared, and at least 180 animal species were extinct. [693]
Heavier animals were extinct much earlier, for instance in Australia, 40.000 years ago the fauna weighing more than 100 kg was already completely extinct. In North America, mammals weighing over 1,000 kg disappeared completely over 11,500 years ago. [703] In South America, about 12,000 years ago, 70% of the animals weighing more than 40 kg disappeared with the arrival of humans. In Madagascar, after the colonization which took place about 1,500 years ago, all animals weighing more than 44 kg disappeared forever. [977]
Probably in the epochs in which the first extinctions due to human beings began to be recorded, the number of people on the Planet was at most a few million. Paleontology has documented that the disappearance of big mammals immediately followed the appearance of man. Especially in the islands, the coincidence is very evident and indisputable. In a very short geological time, the human species has monopolized the biomass and energy of the Planet. The capacity to intervene in natural dynamics has expanded by a factor that is simply astonishing.
Among the animals that, in Italy, our ancestors could have observed, there were freshwater crustaceans and molluscs, beavers and otters in lakes and rivers, elks, lynxes, bisons, bears, pelicans, cranes, Egyptian vultures, bearded vultures, griffins, monk vultures, bustards, black and white storks, hazel grouses, sultan chickens, hermit ibises, three-toed quails, and monk

seals and tortoises in the sea. In spite of the destruction of most of the wilderness, Italy currently has more than half of the plant species (at least 5,500 species) recorded in Europe and a number of bird species that is not found in any other European country.

Hunting has also generated many problems. According to ISTAT data, licensed hunters in Italy currently represent 1.2% of the population, reaching 760,000 in all. [830] Probably the record number of hunters on the national territory was reached in 1973: 2,370,024 licenses, with a density of 7.3 hunters per square kilometre, one every 17 hectares. In those years cartridge manufacturers declared that in our country at least one billion shots were fired per year, which probably meant the killing of at least 100 million animals every year. Among the Italian victims of hunting are the Italian partridge, the sultan chicken, the griffon vulture, the bearded vulture, the sea eagle, the osprey, the rusty hunchback, the Egyptian vulture, Bonelli's eagle, the Corsican seagull, the monk seal, the sturgeon, the crayfish, the fallow deer, the otter, the mouflon, the Sardinian deer, the lynx, the wolf, and the bear. There are 48 huntable species in Italy and each hunter has the possibility to hunt a certain number of animals per hunting day and a certain number per season. Based on the number of hunters, which in Italy are about 700,000, and on the register of the number of animals killed during a hunting trip of the Regions Veneto, Lombardy, Sicily, and Tuscany, 464 million animals may be legally killed every year. [831] Only the migratory birds killed every year by Italian hunters are at least 150 million (against 80 million in France, 50 in Spain, and one million in Denmark). [833] These are estimates and do not consider poaching and other factors such as hunting in derogation. At least 25 million wild birds are killed illegally every year in the countries bordering the Mediterranean: Italy also records 8 million victims (every year) among passerines and birds of prey. [832] They are killed by gunfire, caught in nets or glued to branches. At the same time, thousands of tons of lead are dispersed into the environment (every year) in the form of bullets: a flood of poisonous fragments; not to mention the plastic of the cartridges that are not collected by hunters, who would be obliged to do so. In Italy, if the 700,000 registered hunters were to deploy all together over the entire national territory, including cities, lakes, rivers and mountains, every square kilometre would host six of them.

Massacres of wild animals have been carried out also in the past and hunting has been fatal for many species. We remember some representative events: the emperor Gordian I (157-238 a.C.) offered to the Roman population, having them slaughtered during the shows in the Colosseum, 1,000 bears, 150 wild boars, 100 wild goats, 200 deer, 200 ibex, and 200 roe deers (besides many African animals). Carnages were also carried out in small towns during festivals: in 249 a.C. in a town in central Italy, during a festival, 40 bears and 16 deers and roe deers were killed. [737]

In Valle d'Aosta there is the castle of Sarre, which dominates the access to the upper valley from a hill overlooking the Aosta basin. In the Gallery of hunting trophies and in the Great Game Room there are compositions of hunting trophies of the Savoy family: walls and ceilings are entirely covered with 3,612 horns, from a thousand ibexes and almost eight hundred chamois (killed in the second half of the 1800s). [1279]

POLLINATORS IN ITALY

In Europe, at least 85 species of amphibians, 151 species of reptiles, 489 species of birds, 546 species of freshwater fish, 25,000 species of plants (vascular plants), and 100,000 species of invertebrates (including 482 species of butterflies, of which 264 species are also present in Italy) have been classified. [424] Overall, 57,468 species have been censused in Italy. [5] The 97.8% of the faunistic richness of our Country is composed of invertebrates (e.g. 45,888 arthropods and 2,141 molluscs).

A census of wild pollinating insects, conducted in 52 sites located in 8 Italian Regions, in the years 1997-2000, recorded the presence of 355 species, three of which were recorded for the first time. [685] 75% of the species captured and classified were collected in agro-ecosystems. This biodiversity is very important for the agricultural ecosystem. Species that are also bred include the bumblebee (*Bombus terrestris*), used to pollinate tomatoes in greenhouses, the solitary bee (*Megachile rotundata*), which plays an important role as a pollinator of alfalfa crops (*Medicago sativa*) and other species of solitary bees (of the genus *Osmia*) used in the USA to pollinate some fruit plants. In this Italian census, 13 species of the genus *Bombus* were recorded, including *Bombus terrestris*. Another census conducted in Italy, between 2001 and 2006 in Val Susa, recorded the presence (in the catchment basin of the Po River, located in Piedmont and partly in France) of 30 different species of bumblebees (in the Planet 240 have been classified, of which 43 in Italy). [689] Therefore in this area, the Susa Valley, 70% of the species of bumblebees known in Italy have been recorded. Eight of the thirty species of bumblebees surveyed were dominant, each one representing more than 5% of the catches. An interesting result reported in this publication is that the different species of bumblebees show preferences regarding the plants they visit. Some plants are visited by only one of the eight most frequent species (e.g. plants of the Rosaceae, Caryophyllaceae, and Geraniaceae families), while others are pollinated by all eight dominant species (e.g. plants of the genus *Trifolium*). Three species of bumblebees that had been recorded in previous surveys were not recorded in this research and the Authors conclude that changes, such as the climate change, could reduce the presence of plants important for some of these insects. [689]

The reduction of bumblebee species has also been recorded in other countries in Europe. Probably in the United Kingdom, in 1988, a specimen of *Bombus subterraneus* was last recorded, which was extinct due to the disappearance of habitats and flowers. [688] This insect, however, had been artificially introduced into New Zealand, in areas where forests unsuitable for these bumblebees were extinct. This story is a reminder that the destruction of the original ecosystem in the UK caused the extinction of a bumblebee, but the artificial transport to another place (New Zealand), where the original natural ecosystem was also destroyed, saved this species of bumblebee. Unfortunately, Planet-wide extinction has been documented for some bumblebee species (e.g. *Bombus franklini*, *Bombus rubriventris* and *Bombus melanopoda*). [688]

The reduction in biodiversity is a very serious phenomenon and not only among pollinators. One aspect that is widely underestimated is that our fate is linked to that of other animals and, in general, to that of other living beings. Today it is estimated that due to human activities the rate of extinction is, in general, from 100 to 1,000 times faster than what the history of evolution has recorded over time, that is, over the last 10 million years: at least one species every 20 minutes is destroyed forever by human activities (between 50 and 200 species per day). To have an idea of the extermination in progress we can say that between the year 2000 and before the end of the century more species will have disappeared than those extinct in the tens of millions of previous years. The peculiarity of this mass extinction is that it is very fast and is generated by a single species, which has become a super efficient parasite, and will consequently become the victim of this depopulation: the human species.

ARTIFICIAL COMPETITIONS BETWEEN POLLINATORS

A little studied aspect is that of competition between pollinators. The sudden arrival of millions of bees in the vicinity of an orchard, in the city or in a forest produces imbalances. Bees are artificially aided by man so they are more competitive and the few natural resources, in terms of pollen and nectar, are taken at the expense of wild species: a reduction in the growth and reproduction of species of bumblebees and other wild bees (e.g. *Bombus impatiens* and *Osmia bicornis*) due to beekeeping has been recorded. [1171] There is little information on this competition with native pollinator species: there are tens of thousands of wild bees, and waiting for scientific research to provide definitive answers for a huge number of possible combinations is unfeasible. In addition, there are also non-competitive interactions, such as altering floral composition over time. Predicting how bred insects modify plant biodiversity is not straightforward, especially in environments with a low level of anthropization. Another issue is the possibility of reared insects spreading diseases to other insects and plants. In turn, the reared insects could receive new pathogens. In the long term, precaution will safeguard wild species, but also beekeepers and farmers.

An interesting exercise is to try to estimate how much honey bees can affect the food resources that wild insects also need: pollen and nectar. This involves estimating how many animals, which feed on pollen and nectar, can be supported by a cultivated field, a natural area or a semi-natural area. We know that during the short flowering period one hectare of orchard could safely support between 3 and 5 hives. This sustaining capacity lasts only for a few weeks a year and is possible thanks to the artificial intervention of the beekeeper and the farmer; the blooms of monocultures are synchronized over wide areas. We also know that the utilized agricultural area in Italy is about 10-12 million hectares, depending on the year, and that the area covered by forests is about the same size, about 11 million hectares. Orchards, in Italy, occupy a surface area of less than 300,000 hectares, cereals occupy at least 2.5 million hectares, 500,000 hectares are occupied by oil plants (soybean, rape), 4 million hectares are meadows and pastures, vines occupy at least 700,000 hectares, and olives about 1.2 million hectares. [838] As can be seen, very wide areas are of little relevance for pollinators such as bees (olive trees, vines, wheat), or constitute a source of food for insects only for a few weeks a year (orchards). The same applies to forests and natural areas. Therefore, the flowerings available in Italy, assuming that they can be efficiently and constantly exploited by beekeepers, to the detriment of wild animals, are not able to sustain honey production, which in Italy is carried out by at least 1.4 million hives (these are the registered ones). The national surface covered with flowers, available to bees between the end of winter and the beginning of autumn, varies between less than a third and less than a fifteenth of the cultivated surface, depending on the period, the place and the season. For this reason, too, it is necessary to feed the bees artificially with sugar-based feeds and alternative sources of pollen proteins, and to carry out nomadism. It is not possible to maintain the number of insects reared with the flowers potentially available, without implementing nomadism and without providing feed.

We can try to calculate how much pressure from bred bees affects animals such as wild insects. A hive in spring can host more than 60,000 bees of which 20,000 could be foragers, i.e. bees that look for pollen and nectar. Let's assume for simplicity's sake that there are 10,000 forager bees in each of the more than 1.4 million hives in the spring and summer. Each of these worker bees can visit over 3,000 flowers a day, but to make the calculations more realistic let's assume that they are able to empty of nectar 100 flowers a day. From this theoretical calculation it results that each hive could deplete, of nectar and pollen, over 1 million flowers per day (100 flowers/bee/day x 10,000 forager bees/hive), depriving other insects of them. If we assume a

density of 10 flowers per square metre (100,000 flowers with available nectar and pollen per hectare), this means that each hive is able to take possession of the nourishment present in over 10 hectares, every day.
Assuming a density of 10 flowers per square metre (100,000 flowers with nectar and pollen available every hectare), this means that a flowered area of at least 14 million hectares must be available every day (in spring and summer) to feed all the bees kept in Italy. Two apiaries with 20 hives each, placed in a forest with a theoretical average density of 10 flowers per square metre, deplete 200 hectares of nectar every day. These data are imprecise but certainly an underestimate, since forager bees are much more numerous, some flowers do not produce nectar or are not attractive for other reasons (e.g. they are not suitable for the length of the honey bee's tongue), the density of flowers for most of the year is much lower than estimated (10 flowers per square metre, every day during the 6 - 7 months of greatest bee activity). In conclusion, in order to feed all the honey bee farms in Italy without the use of feed, but by nomadic beekeeping, a surface area much wider than that available would be needed, at least three times. Without the support of feed and without nomadism, the Italian agricultural and semi-natural areas could support a number of beehives more than 10 times lower than the present one. It is understood that the high number of bred bees contributes to reduce the possibility of feeding for wild insects, as resources such as nectar are limited. This reasoning, theoretical and provocative, is however helpful as a premise to the conclusion that it is impossible, despite the possibility offered by nomadism, to produce the current quantities of honey without using feed. It must be remembered that honey is the food support of the colony during the winter months, when the colony does not leave the hive. This food reserve, which is essential for survival, is taken by the beekeepers, and this is one of the reasons why the use of feed is becoming an essential part of the business.
It should be considered that flower resources must also be available to wild insects. At this point we might ask ourselves what percentage of the harvest carried out by bred bees can be sustained, by a more or less artificial system, without irreversibly damaging other pollinators and animals that use pollen, nectar, and honeydew. How much do bred bees influence, in natural and semi-natural ecosystems, the reshape of the balance among plants, favouring the species they pollinate? Whenever hives are placed, especially when areas with wild or spontaneous plants are present, one should try to answer these questions. The artificial food competition that beekeeping generates against wild pollinators favours their reduction and extinction. In addition, bees carry and spread diseases to plants and wild insects. Unfortunately, we do not have conclusive answers, but the application of the precautionary principle should prevent us from waiting until we have the desired knowledge when there is no turning back.
We need to know what is the maximum level of food competition that especially natural and semi-natural areas or areas of importance for biodiversity can tolerate without undergoing irreversible changes. Beekeeping introduces into natural ecosystems an artificial breeding system that is alien: therefore apiaries are alien, suddenly transplanted and continuously moved from one habitat to another without caring about the effects. Nomadism and the free movement of the beekeepers, regardless of their density in the territory, are tolerated today. Although nomadism generates potential ecological damage in natural and semi-natural areas, it is not sufficient to avoid the use of feed by beekeepers. The fraction of honey derived from feed is probably much higher than we imagine. As already mentioned, honey is the feed that the colony uses during the long winter months, when it does not leave the hive. Therefore, the subtraction of honey must be compensated by the use of feed, however, even if flowers are present in abundance. In conclusion, honey bees live in an artificial home, they no longer have to produce wax with the special glands they have because the beekeeper regenerates it by building artificial cells, they move around in vehicles even for several kilometres a week, they must necessarily be fed with feed and are treated with the support of veterinarians who prescribe acaricides,

fungicides, and other medicines; queen bees can be selected and reproduced by artificial insemination and can be purchased by consulting a sort of family tree (there is a trade in seminal fluid for assisted fertilization). [670] The technique for artificial insemination was invented in 1926 but was perfected in 1940; the spermatozoa of drones may be conserved at room temperature (15°C - 25°C, they are seriously damaged by temperatures below 10°C) for two weeks, therefore they can easily travel around the World, even if with cryopreservation (at -196°C with the addition of cryoprotectant substances) they may be conserved much longer (queen bees can be inseminated in a time window of a few days: between the fifth and tenth day the best results are obtained). [974]

The feed is based on sugar, which may be derived from sugar cane, beet, but also syrups made from corn, rice or other plants. Modern beekeeping is therefore in no way inferior to intensive livestock farming, as it also requires the cultivation of nectar replacement feed. Beekeeping also requires the rearing of animals as a source of essential nutrients, such as pollen replacement protein, and limits on economic exploitation should be accepted and the protection of natural biodiversity should be regulated more consciously.

Information on alien species can be found on many dedicated websites. [566] For most alien species, it is not easy to estimate the detrimental effects on the ecosystem, let alone the consequent costs. It is reasonable to assume that the introduction of alien pollinating insects, such as honeybees and bumblebees, into new ecosystems, as has been the case for decades, may generate various problems:

1) Reduction in the availability of honeydew, pollen, and nectar as a food source for native species. In some areas of the World, native insects as well as birds and other animals feed on these substances. Beekeeping creates unnatural and unthinkable densities of bred insects, generating competition and artificial pressure that are harmful to wild insects and others. As a demonstration of what has just been written, it is known that bumblebees tend to be smaller, in the presence of apiaries, due to the competition with food resources. [688]
2) Reduced pollination and reproduction of native plants because, for example, bumblebees can steal nectar through a hole instead of entering the flower; they do this when they cannot reach the nectar because the tongue is too short and not adapted for that type of flower. The flower loses its attractiveness to wild species adapted to enter the flower and, as a result, loses nectar without being pollinated.
3) Favour the pollination and spread of non-native flowers that may originate from Europe. The bumblebees with pollination will promote the spread of the plants they like best (e.g. lupine, rape, beans). This is the natural objective of the symbiosis between plant and pollinator. Each pollinator, with its activity, will promote the spread of the plants for which it carries pollen.
4) Promoting the spread of a disease. The spread may occur in both directions, from alien domesticated bees to native wild bees and vice versa. Thus, with nomadic beekeeping, pathogens travel around the World sooner. Domesticated bees may also promote the spread of diseases among plants.

With the uncontrolled spread of species useful to man, such as bred bees, biodiversity is diminishing and problems are increasing, also for beekeepers. Nomadism has favoured the diffusion of the most important diseases for the bees, all over the Planet.

An interesting aspect to underline regarding beekeeping is that some of the most important mono-floral honeys are obtained from species imported to Italy, such as citrus fruits, eucalyptus, black locust or acacia, sunflowers and medlar. For example, the eucalyptus is a plant native to Australia, introduced into Italy at the beginning of the 1900s, for reforestation. Its rapid growth has spread the eucalyptus as a windbreak in coastal areas, as an ornamental species, for the paper industry, and it is possible to extract from it an essential oil used in the

chemical-pharmaceutical industry. Some alien species help beekeepers to sustain economic benefits. Honeydew honey also benefits from an alien species: the insect *Metcalfa pruinosa* (Rhynchus Homoptera) which is native to North and Central America, incidentally introduced into Italy. Since 1979, when it was first reported, the spread of this pest has progressively extended southwards, thanks to the absence of antagonists and, above all, to its polyphagy which allows it to live on a great number of plant species, both spontaneous and cultivated.
The introduction of domesticated pollinating insects (e.g. *Apis mellfera* and *Bombus terrestris*) where they did not exist, such as in South America and other places on the Planet with unique and important natural resources, will generate effects that we can only partly imagine: the spread of diseases and parasites, competition for resources and reduction in biodiversity. [758]

ECOSYSTEMS THREATENED BY THE INTRODUCTION OF ALIEN SPECIES

ALIEN SPECIES

The voluntary or involuntary diffusion of species into new ecosystems is probably the second most important cause of mass extinction recorded on the Planet, after the destruction of natural ecosystems due to population growth and consumption. In the USA, of the 958 native species at risk of extinction, 400 are at risk of extinction due to competition by artificially introduced species. [698] The process of manipulation and mixing of the flora and fauna of the Planet, which began thousands of years ago along human migratory routes, has accelerated in recent decades to the point that in some areas of the World non-native plants outnumber the native ones. The displacements on wheels, by ship, and by plane, cancel millions of years of geographic separation. An experiment, unprecedented in the history of the Earth, which leads to the standardization of ecosystems and their simplification, is underway.

All nations have thousands of non-native species. Alien species compete with native species and can extinguish them through various mechanisms such as predation, hybridization, spread of diseases, and removal of food resources. The artificial spread of non-native species is an ecological problem but also for our health, so it is monitored. [806] Non-native species can promote the outbreak of several diseases that are dangerous to the human species, such as malaria.

A well-known example of ecological disaster following the introduction of a new species is the Nile perch (*Lates niloticus*) released in Lake Victoria in the 1960s. Within a few years at least 200 species of fish (cichlids) were extinct due to the introduction of this new species. [1147]

In the USA at least 138 billion dollars a year are spent on invasive species control with not always satisfactory results. Unfortunately, the problem of the invasion of new ecosystems by species considered aliens is not likely to diminish. [1148]

Determining what species is invasive and what is native is not always easy: there may be several obstacles. First, we do not have enough information for many species, such as some invertebrates, plants, microorganisms, many ocean and soil inhabitants. It is also difficult to determine how far back we need to go to know whether a species well-adapted to an ecosystem is native or invasive. Humans have been manipulating the environment, for their own benefit, for thousands of years. With agriculture and breeding, useful species for human needs have been favoured already over 5000 years ago. Because of the difficulty of knowing what happened millennia ago, in many cases it is appropriate to talk of probable native species or possible artificially introduced species. For example, as early as Roman times, a number of species were introduced into the UK, such as the sycamore maple (*Acer pseudoplatanus*) and an herbaceous perennial plant (*Aegopodium podagraria*). [698] The Romans introduced the European hare, the rat, and the fallow deer into the UK. The changes, however, began long before the Romans arrived. Wild oats (*Avena fatua*) were introduced into the UK as long as 3000 years ago and during the First World War another species of oat (*Avena sterilis*) which generates considerable costs. Some of the introduced species have adapted so well that they make up a large proportion of the species present. In Great Britain, the 22 artificially introduced mammal species make up 46% of the 48 existing terrestrial species. The 8 species of amphibians recorded as artificially introduced account for 57% of the 14 species present. [698] As these data confirm, alien species, in some cases, constitute an important fraction of the species present and are in competition with the native species. In the case of Britain, if you go back much further in

time, probably up to 7000 years ago, it was connected to Europe by ice (at least for some periods). From Europe some terrestrial species could have walked to Britain and vice versa. Some species became extinct already during this phase, when the isolation of Britain by the sea began (e.g. the wild horse or *Equs ferus*).
A major danger facing transplanted species is that they are usually few in number and, therefore, have a considerable genetic uniformity. In the 1970s, a fungal disease transmitted by an insect (the scolitid beetle) on elm trees decimated these trees throughout Europe. Some elms were more affected than others, confirming that genetic variation plays a role in disease susceptibility. In England, one species of elm (*Ulmus procera*) was almost completely decimated. Genetic analysis confirmed that the entire species was in fact a single clone (propagated by cuttings), imported two thousand years ago by the Romans to provide support for vine cultivation. [704] Following the introduction of a new pathogen, the genetic uniformity of this cultivated plant caused its near extinction.
The accidental introduction of non-native species may quickly bring entire production sectors to their knees. In the nineteenth century, a new vine parasite native to North America was accidentally introduced in France: the aphid called the grapevine phylloxera (*Daktulosphaira vitifoliae*). To control this parasite, European vines were grafted onto naturally resistant American rootstocks. In this regard, it is interesting to note that, in Italy, viticulture is very ancient: in Abruzzo traces of it have been found dating back to the third century b.C. [773]
Alien species also damage beekeeping which, as we have already written, is put to the test by many parasites that have travelled the World, such as the *Varroa destructor* mite or *Vespa velutina*. Another example is the frog native to Central America (*Bufo marinus*) which has been voluntarily transported to other countries (e.g. Hawaii and Australia) with the hope of controlling some insects considered troublesome: this frog is capable of damaging bee colonies. [974]

THE VULNERABILITY OF ECOSYSTEMS TO THE INTRODUCTION OF ALIEN SPECIES

The introduction of a species into ecosystems is followed by its spread and the establishment of new and unpredictable balances. Over time, new ecological relationships are created and it becomes easier to measure the effects, such as the reduction in the presence of native species. Introduced foreign species are defined as alien to that given ecosystem. The vulnerability of an ecosystem to artificially introduced species is influenced by several factors:

- Isolation encourages the recording of greater damage (e.g. on islands rather than continents).
- Simpler ecosystems, i.e. those consisting of a few species, are more fragile and less resilient to change. Complex ecosystems, on the other hand, are more flexible and able to withstand negative pressures such as those resulting from the introduction of alien species. If ecosystems are simple and isolated, the effects of the introduction of alien species will be even more devastating.
- The length of time the foreign species will be there. The longer the duration of close artificial cohabitation, the greater the damage to the native species. To give an example, in the United Kingdom, with the aim of contrasting the number of rabbits, in 1953 a disease was introduced, the myxomatosis virus. In the early years a high rabbit mortality rate of over 90% was recorded, but after twenty years of co-evolution the mortality rate was reduced to 50% and in the 1980s to 20%. [698] Grain losses due to rabbits are estimated at about 0.5% of the grain crop per hectare, per rabbit, per year. In Britain,

rabbits promoted the extinction of at least one species of butterfly in the 1950s, which subsequently reappeared (*Maculinea arion*).

- The presence of other disturbing factors such as climate change, habitat fragmentation generated by urbanization and agriculture, the use of poisons (e.g. insecticides) or fires.

The movement of species to ecosystems other than those in which they are normally found is one of the major causes of biodiversity loss together with pollution, destruction of natural ecosystems (e.g. reduction of soil fertility, agriculture, fires, deforestation), over-harvesting (hunting, fishing, trade) and climate change. The spread of alien species is caused by human activity but may also occur accidentally: preventing this type of environmental threat has become almost impossible.

THE INVASION BY NON-NATIVE SPECIES IN EUROPE

In order to better quantify the intensity of the phenomenon of invasions by artificially introduced species, the so-called alien species, it is interesting to report some data that are certainly underestimates. [698] Probably at least 12,000 alien species have been introduced into Europe, including 88 non-native species of mammals, 140 species of birds able to reproduce (since 1850 more than 197 species of birds have been introduced; in Europe as a whole less than 500 species of birds have been classified), 1,590 species of arthropods of which insects make up the majority (most insects have been accidentally introduced with ornamental plants). [424,699, 810] 20 of the 88 mammal species considered alien were introduced from other areas of Europe.

Species generating the greatest damage include the common rat (*Mus musculus*), the grey rat (*Rattus norvegicus*), the nutria (*Myocastor coypus*) and the mink (*Neovison vison*). Some species such as rats have followed humans everywhere except in the polar ice caps and probably in one region of Canada (Alberta). A rat (*Rattus norvegicus*) can swim without stopping for 72 hours, can fall 50 feet without dying, can climb a wall or climb a rope, can hold out without drinking longer than a camel, eats anything edible (but also many things that are not like wood), reaches sexual maturity at three months of age, and can mate as many as 20 times a day. A female in a year can mate with many males and give birth to 12 litters of several pups each. The female common mouse (*Mus musculus*) is also very prolific: she can produce 500 pups in a year. It is estimated that they consume an enormous amount of food for human consumption and are carriers of over 70 diseases, including plague (which over the centuries has killed at least a billion people), cholera, typhoid, tuberculosis, hepatitis E, salmonellosis and several species of parasitic worms.

At least 120,000 species have been classified among flies: some are capable of carrying thousands of different bacterial species (perhaps over a million). The house fly (*Musca domestica*) can be a vehicle for numerous diseases such as tuberculosis, typhus, cholera, dysentery, and various parasitic worms. Under suitable conditions the life cycle of the fly is very fast: from egg to adult in less than twelve days. *Musca domestica* is the most significant species in livestock farming. The concentration of animals in livestock farms and the large quantity of manure create favourable conditions for the multiplication of flies. The presence of these insects is a continuous source of disturbance for the animals, so as to cause economic losses (reduction in meat, milk, and egg production) and annoyance for workers and the population living in the vicinity.

At least 688 alien species of fungi have been introduced into Europe, including some that cause serious problems for crops such as vines and potatoes.

The Japanese deer (*Cervus nippon*) is able to reproduce with the red deer (*Cervus elaphus*) native to Europe generating fertile hybrids. The introduction of this deer has generated

a new hybrid animal and has favoured the spread of a disease (the nematode *Asworthius sidemi*) affecting deer and roe deer. Also some geese and ducks introduced into Europe have been able to breed with native species. This type of genetic contamination is very dangerous for some species.
Hundreds of examples could be given: one recalls the North American grey squirrel (*Sciurus carolinensis*) that has supplanted the native European red squirrel (*Sciurus vulgaris*), even in Italy. Despite the fact that for years its eradication has been decided and trade has been banned (Bern Convention of 1979; Decree of 24 December 2012) the North American grey squirrel can be easily purchased and it is present in urban gardens, even in Italy. [564, 565] This trivial example highlights how difficult it is to ensure eradication, even when countries, through regulations, agree on the plan of action. In these cases, the costs generated by inaction are much higher than those of prevention and eradication. This aspect, which is of vital importance for the protection of biodiversity, is dealt with superficially and with insufficient determination.
Returning to the squirrels, it should be remembered that they are active gardeners. Some species are able to recognize a diseased seed that will not be preserved and will therefore be eaten immediately. They deposit healthy seeds in the ground to store them during winter periods of food shortage. Their storehouse may be emptied by premature germination. Some squirrels have learned to gnaw off the embryo before storing the acorns, so that they cannot germinate. Some plants, such as some of the oaks, have evolved an anti-rodent gnawing counter measure: they have placed the embryo so that it cannot be damaged. This evolutionary adaptation is the result of the eternal battle between prey and predators.

Many of the pests of the European honeybee (*Apis mellifera*) are alien species such as mites (*Varroa destructor*) or the Asian yellow-legged hornet (*Vespa velutina*), which was probably introduced through the importation of bonsai plants. This hornet is a very clever predator of bees even in its home territory, but it is in Europe that this hymenopteron can seriously threaten the existence of farmed bees. Southeast Asian bee species have adopted valid behaviours to fight this predator, such as piling up on the intruder, overheating it to death; these behaviours are, so far, unknown to the European bees.

Other harmful arthropods introduced into Europe can be mentioned:

- The corn rootworm (*Diabrotica virgifera virgifera*) is an insect (a beetle) of American origin, belonging to the order Coleoptera, which can cause serious damage to maize if the crop is repeated for two or more years in a row on the same plot. In 1992, in Europe, it was found in Serbia, near the airport of Belgrade; in the European Community, the corn rootworm was reported for the first time in 1998 in a maize field in the Municipality of Venice. [807] It is an alien species that damages a completely artificial crop.
- The yellow cyst nematode (*Globodera rostochiensis*) parasitizes solanaceous plants such as potatoes and tomatoes. It forms cysts near the roots. It is native to South America and was discovered in Germany in 1913.
- The eastern peach moth (*Cydia molesta*) is native to eastern Asia, which has easily spread to America, Australia, and western Europe. The larvae are 10-15 mm long and cause damage on shoots and fruit. [811]
- The citrus moth (*Prays citri*) damages mainly the flowers (the larvae penetrate into the flowers, gathering them together with silky threads, gnawing away the inside, up to the ovary, and can attack the buds and the small fruits, on which erosions appear, emptying them and causing them to fall). [812]
- Grapevine downy mildew (*Plasmopara viticola*), which is a microorganism (belonging to the class of Oomycetes) native to America, was accidentally imported into France around 1878, from where it spread throughout Europe.

- Downy mildew (*Phytophthora infestans*), which is a microorganism (belonging to the class of Oomycetes), affects in particular the plants of the solanaceous family such as potatoes and tomatoes. It was the main cause of the great famine in Ireland between 1845 and 1849. In this case the alien species generated a catastrophe: it destroyed the entire 1846 harvest and caused the death of about one million people and the emigration of a further million (probably 8.5 million Irish people lived on the island at that time). [813, 814]
- The white fly (*Bemisia tabaci*) was already recorded in Europe at the end of the 19th century, in Greece, while its appearance in Italy was registered in 1939. [822] It belongs to a family of insects of the order Rhynchota Homoptera, i.e. those that produce honeydew. They can affect 900 different plant species and transmit at least 10 different types of viruses to plants. [698]
- The tiger mosquito (*Aedes albopictus*) is an insect belonging to the Diptera order. The first specimens living in Europe were found in northern Albania in August 1979. The Albanian monitoring group assumed that the mosquito had been present since at least 1975 and that the means of transport was a cargo ship from China docked in Durres. In Italy it appeared in Genoa, probably in 1990, in a warehouse for used tyres imported from the United States. The tiger mosquito can transmit nematodes such as dirofilariasis and viruses responsible for diseases such as the yellow fever, St. Louis encephalitis, dengue, chikungunya, and zika. [808] The first European chikungunya outbreak was recorded in Italy in 2007. [698]
- At least 102 species of mites (these are parasites of the arachnid class) have been introduced since 1500. Mites are microscopic eight-legged members (insects have six) of the spider group and are, in most cases, too small to be seen with the naked eye. After insects, they are the most diverse group of animals on the Planet: at least 48,000 species have been identified, and they probably make up a small fraction of those in existence. They are very adaptable: they can settle in the trachea of a bee and can be easily transported from one flower to another, for example, on the beak of a hummingbird. [848] Mites include ticks which can transmit scabies and typhus.
- At least 43 species of alien ants have been recorded.

In Europe it is estimated that there are at least 12,000 exotic species of which more than 10% are considered invasive. Also in Europe, over time, there has been an increase in the number of arthropods considered non-native. [810] Between 2000 and 2008, an average of more than 19 new alien species per year were recorded, mainly consisting of phytophagous species. At least 700 alien arthropod species have been recorded in Italy.

In the UK and Ireland, the number of invasive plants recorded exceeds the number of native species. In 1870 an alien plant species from America (*Spartina alterniflora*) was introduced into the UK where it was able to cross with a native species (*Spartina maritima*) resulting in sterile hybrids. Between 1970 and 2004, a UK inventory recorded the introduction of 234 plant pathogens: fungi, bacteria, and viruses (79% of the non-native organisms recorded were fungi).

In France, since 1800, it has been estimated that 227 species of fungi have been introduced, of which 65% are plant pathogens, mycorrhizal fungi account for 30% and saprophytic fungi for 4%. [698]

The spread of foreign plant species may generate many problems such as the reduction of biodiversity but also direct costs for human health: the plant *Ambrosia artemisiifolia* arrived in Europe and Italy in the middle of the last century from North America and its pollen is allergenic. [559] One gram of ragweed pollen contains about 30-35 million pollen grains; a well-developed plant can produce more than 45 grams of pollen in a year. [561] About 10 pollen grains per cubic metre of air are enough to cause allergic rhinitis in sensitive people; 50 pollen grains of grasses per cubic metre of air are enough to generate unpleasant reactions. [561] In Europe, *Ambrosia artemisiifolia* causes costs amounting to several hundred million euros. In Austria, where the plant is responsible for 30% of allergies, it has been calculated that *Ambrosia* pollen causes an additional cost of about 600 euros per year per allergic person. [560] In France and Italy, the cost generated by *Ambrosia* allergies has been estimated at 2 million euros per year. [561] It has become so widespread in Europe that eradication is impossible.

To get another idea of the scale of the problem, in Europe, at least 11,000 plant species have been introduced (e.g. ornamental or horticultural plants) of which about 10% generate ecological damage and 12% generate additional costs. [558] Among the non-native plants introduced into Europe, there is the water hyacinth *(Eichhornia crassipes*). [562] This is a floating aquatic plant that grows on the surface of rivers, canals, and lakes in tropical regions: it has a very rapid growth and is native to the Amazon basin. In the areas where it has been introduced, due to its high growth rate and the absence of herbivores, it has become invasive. It has been included in the list of the 100 most harmful alien species in the World. [563] Among the unsustainable actions to counter the spread of alien plants, there is the use of chemical control, such as herbicides that generate a further ecological disaster without solving the original problem.

VITICULTURE AND THE (ALIEN) VECTOR INSECT OF FLAVESCENCE DORÉE: UNSUSTAINABLE CHOICES

Phytoplasma is a plant parasite, the survival of which is only possible within the host plant and the vector insect (phytoplasmas are bacteria without a cell wall). Flavescence dorée is a phytoplasma (it causes phytoplasmosis) that affects the grapevine. The disease is endemic to European regions, but did not pose a problem until the arrival of the vector insect *Scaphoideus titanus*, native to North America, where the phytoplasma of flavescence of the grapevine is not present (in America, *Scaphoideus titanus* is a vector of other phytoplasmas). The American grapevine leafhopper (*Scaphoideus titanus*) is an insect (belonging to the Order Rhynchota Homoptera Auchenorincha) that lives only at the expense of grapevine. It has been present in Europe since the beginning of the Sixties, where it has affected most of the wine-growing areas. The greatest danger is represented not so much by the stings made in order to suck the sap, but by the fact that it is the vector of the phytoplasma of flavescence dorée. The vector arrived in France in 1955, and in Italy it was reported for the first time in 1963, in Liguria. *Scaphoideus titanus* lives only at the expense of the species of the genus *Vitis* spp.: in addition to the European grapevine (*Vitis vinifera*) it has also been reported on some species of American grapevine.

The culture of using chemicals in agriculture is so widespread and accepted that in many cases it is imposed. Wine-growers are obliged to use pesticides against this insect. The control of *Scaphoideus titanus* is compulsory since, in the event of failure to apply the provisions, non-compliant winegrowers are reported to the judicial authorities (under Article 500 of the Italian Penal Code). [817] It is up to the Regions to establish administrative sanctions for the non-compliant, i.e. those who do not apply insecticides and growth regulators if these insects are present. Despite the fact that grapevines are not pollinated by bees, they can still be visited for the sugary substances in the grapes. Insecticide treatments on grapevines have long since resulted in high increases in bee mortality. [35] Wine-growing involves a high level of chemical pest control and is therefore very dangerous for insects, but not only for insects. In this regard, it should be noted that a document on the requirements for the accreditation of bodies that certify organic products provides, among the high-risk factors due to pesticides, the cultivation of table grapes and of rice. [77] Organic crops in the vicinity of traditional crops may be damaged by both drift and extinction of insects and other beneficial organisms (e.g. plants with flowers). In Italy, the national legislation of 2000 decrees: [817]

> *"The control of flavescence dorée of the grapevine sensu stricto and its vector Scaphoideus titanus Ball. is obligatory in the territory of the Italian Republic in order to control its spread. Within the area declared to be a 'hotbed', an area in which the presence of flavescence dorée has been officially ascertained and the eradication of the disease may be considered technically possible, any plant with suspected symptoms of flavescence dorée must be immediately uprooted, without the need for confirmation analysis*."

The provisions of the government of the Piedmont Region in this regard are given below: [818]

> "*From the moment the American Grapevine Leafhopper acquires the phytoplasma by feeding on infected grapevines, it takes about a month for the multiplication of the microorganism within the insect before it can transmit the disease to healthy grapevines. Therefore:*
>
> - *the first insecticide treatment should be carried out approximately 30 days after the onset of egg hatching. Note: If slow-acting insecticides, such as growth regulators, are used, the time of treatment should be appropriately earlier;*
> - *the second treatment against adults must be done 20 days after the first one;*
> - *if adult reinfestations occur during the summer, a third treatment may be necessary.*
>
> *In order to make the treatments as effective as possible, it is indispensable to carry out an accurate wetting on the whole vegetation in order to hit neanids and nymphs sheltered on the inferior pages of the leaves, in particular when using products without an asphyxiating action. For the choice of the insecticidal products and the periods of intervention it is necessary to follow the indications of the technical assistance services connected to the Plant Protection Service*."

The government of the Piedmont Region in 2019 provided: [819]

> "*In vineyards where the percentage of presence of the disease is less than 2%, it is compulsory to uproot the infected grapevines. In vineyards where the conditions for effective vector control do not exist and in vineyards with more than 30% of infected plants, determined even if only by means of a sample identified according to a statistically suitable method to guarantee its representativeness with respect to the entire vineyard, the Plant Health and Technical-Scientific Services Sector may order the uprooting of the entire vineyard*."

The insecticide molecules indicated by the Piedmont regional regulations to be used against leafhoppers are the following: acetamiprid (a neonicotinoid), chlorpyrifos methyl (a phospho organic), etofenprox (phenoxybenzyl ethers), flupyradifuron (butenolides) and the pyrethroids such as acrinathrin, tau-fluvalinate, beta-cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, lambda-cyhalothrin, and zeta-cypermethrin. In addition, for organic wine production in Piedmont: [819]

> "*At least three insecticide treatments must compulsorily carry out in vineyards managed with organic farming, using pyrethrum or potassium salts of fatty acids, of which at least two with pyrethrum, on the juveniles every 7-10 days, in the period May-June; the timing of the treatments must be established taking into account the flowering of the vine and the cycle of the American Grapevine Leafhopper. Both active substances act only by contact and therefore they must be distributed in such a way to involve also the lower page of the leaves where the juvenile stages are generally found. There are no active substances authorized in organic cultivation against the American Grapevine Leafhopper's adults, so interventions against the juvenile stages are essential to reduce the population of the insect vector*."

Therefore, the use of insecticides is also mandatory for organic viticulture. The Piedmontese regional legislation also provides for the following sanctions: *anyone who fails to comply with the obligations relating to the execution of compulsory insecticide treatments is punished with the administrative sanction of the payment of a sum from 400 to 2,400 euros*. [820] In Piedmont, the following provisions are also applied: [821]

> " - *compulsory execution of the plant-health measures provided for, with the costs being borne by the offender;*
> *- the suspension of any form of economic contribution in the agricultural sphere under any title administered by the Piedmont Region.*"

This alien species, which causes enormous damage to vines, has led the health authorities to the worst possible solution: to force vine growers to use devastating chemical weapons. We should have the courage to adopt different, albeit unpopular, strategies, rather than bringing toxic molecules to the table and poisoning the environment. This affair is a representative manifestation of the denial of the existence of natural biological and chemical rules, which are not submitted to selfish and inconsiderate economic interests. Nature has limits within which we can move with a presumed safety that we refuse to see. What today seems to be indispensable technological achievements, allowing humanity to (temporarily) overwhelm nature, will lead future generations to question why we let this happen: it is not impossible that they will despise us. We continue dangerously to ignore the rules of the game for survival.

NON-NATIVE SPECIES IN AMERICA

At least 50,000 species of plants, animals, and microbes (50% are plants) have been introduced into the United States in 100 years. 25,000 artificially introduced plant species in the USA are numerically greater than the 17,500 plant species considered native to the same territories. [558] Of the more than 25,000 non-native plants introduced, at least 900 have adapted to the natural environment present in Florida, so they are out of control. [698] At least 500 alien plants have become weeds and are spreading into areas with natural characteristics at the rate of hundreds of thousands of acres per year (at least 700,000 ha/year). In pastures, 45% of weeds are alien. A plant imported to the USA from Europe, salicaria (*Lythrum salicaria*), is moving at the rate of over 115,000 ha/year. In natural areas, this invasive species has caused the reduction of the presence of at least 44 native plant species. 138 artificially introduced tree species are also spreading into natural environments threatening the wild species on which many animals depend, because a chain reaction has been generated. A plant poisonous to cattle and wild

ungulates (*Euphorbia esula*), imported from Europe in the early 1800s, has become an invasive species in agricultural and natural areas. At least 1,579 plant species are recorded in the USA as being invasive in natural areas; some alien plants are favoured by hurricanes and floods. [823]
Many non-native animals have adapted well too, as has happened with 18 species of rodents, 6 primates, 99 birds, 69 reptiles, 533 fishes. Examples include the rat (*Rattus norvegicus*) and other usually tolerated species such as the wild boar, the cat, the house sparrow (*Passer domesticus*), the pigeon (*Columba livia*), and the common starling (*Sturnus vulgaris*). The house sparrow was probably brought to the USA in 1853 to control an insect. Today there are at least 29 known diseases that sparrows can transmit to humans or wildlife; they also generate losses to farmers by feeding on grain. Pigeons are vectors for at least 50 diseases, so as new species are introduced, their diseases are also carried with them.
More than 2,000 arthropod species have also been introduced into the USA. As an example, it is estimated that in Florida there are at least 11,500 native species of insects and 949 of introduced species, of which 42 were imported to implement biological control. In California, 600 human-introduced insect species are responsible for 67% of the recorded crop losses. [698]
The United States, like most of the agricultural areas of the Planet, are the result of an irreversible anthropization process, so that alien species damage wheat, corn, rice or farms such as those for chickens, which are also alien species and often artificial, i.e. created by man; in the USA alien species probably guarantee over 98% of the food of Americans. At the same time, the vast majority of insects that damage crops are alien species: at least 40% of the species responsible for the 13% reduction in harvests are species introduced by human activities. In agricultural contexts that constitute an artificial system, distinguishing between benefits and disadvantages generated by artificially introduced species is only possible if the point of view is exclusively anthropocentric. This is the perspective used when estimating crop losses in agricultural environments that host intensive monocultures of plants selected by the human species. An eco-centric view in such artificial settings becomes difficult and sometimes impossible because the system of chemical and intensive agriculture is, in turn, alien to the biosphere.

THE INVASION OF NON-NATIVE SPECIES IN AUSTRALIA

The impact generated by the introduction of non-native species on the balance of ecosystems is devastating and also translates itself into a reduction in biodiversity. A dramatic story is that of Australia where rabbits, foxes, cats, camels, goats, and dogs have caused an irreversible damage to marsupials.
In the Australian continent we can talk about a real invasion by foreign species: the number of alien plants introduced is estimated to be at least equal to the number of native species, that is, equal to 25,000 each. Among the invasive species of vertebrates that have generated enormous damage we can remember the European rabbit, the wild boar, and the cat. In 1859, an English settler released 24 rabbits in Australia. Ten years later, all over the continent, they were so numerous that not even the slaughter of two million specimens was able to affect their population. When in Australia, in 1950, a disease (the myxomatosis virus) was introduced for the biological control of the rabbits, there were at least one billion rabbits. [848] The introduction of this disease, and subsequently others, reduced the number of rabbits but selected resistant populations. Probably, the rabbits contributed to the disappearance of at least one eighth of all Australian mammals and of many plant species. Even today, eradicating the rabbits from Australia is a mission impossible due to several reasons such as prolificacy: a female gives birth to 30 puppies per year that become sexually mature after six months.

Invertebrates introduced into Australia include mosquitoes (genera *Aedes* and *Culex*) which are vectors of several dangerous diseases (e.g. encephalitis), ticks which are vectors of diseases for cattle, honey bees, some wasps, and species of ants. Honeybees (*Apis mellifera*) were introduced into Australia around 1822 and, by 1998, at least 670,000 hives were recorded.
Among the harmful insects introduced into Australia we can mention a hymenopteran (*Sirex noctilio*) which, between 1987 and 1989, caused the death of more than five million trees (*Pinus radiata*), which had been introduced in 1870 (they are native to North America). [698] An alien species caused the extermination of another voluntarily introduced species.
In 2016 there was the first report of the presence of the *Varroa* mite in colonies of *Apis mellifera* which is, for this Continent, another alien species.

THE SPREAD OF CERTAIN INSECT-BORNE DISEASES

Climate change and globalization are promoting the spread of insects that are dangerous disease vectors for humans. [791] The diseases transmitted by mosquitoes to humans cause hundreds of thousands of deaths every year, all over the World. There are more than 3,000 known species of mosquitoes but 3 are the main ones responsible for spreading diseases to humans. The *Anopheles* mosquito is the only recognized species responsible for the transmission of malaria. *Culex* mosquitoes can transmit encephalitis, filariasis, and the Nile virus. The tiger mosquito (*Aedes albopictus*) is native to Southeast Asia and thanks to commercial transport has been able to spread to many areas of the World: in the middle of the twentieth century it spread to Africa and the Middle East, followed by South America, the United States of America, Oceania and, lastly, Europe. The first specimens reproduced in Europe were found in northern Albania in August 1979 and in Italy it appeared in 1990 in Genoa, where they arrived by ship from the USA. [787, 791] From Genoa it spread practically all over the peninsula and is currently present in many other European countries. This mosquito is diurnal and the eggs resist drought for more than 6 months, but to hatch and start the larval cycle they must necessarily be submerged in water. The tiger mosquito has a limited range (less than 200 metres), so the places where eggs are laid are close to where its presence is observed. As with other mosquito species, only female *Aedes albopictus* require a blood meal for egg production. They supplement their energy needs with nectar and other sweet plant juices, just as males do. The female tiger mosquito (*Aedes albopictus*), which is responsible for bites to humans, can take several blood meals, 3-5 days apart, and under optimal conditions lives about 4 weeks. [788] Starting from about 60 hours after having taken the blood, the females lay between 40 and 80 eggs, placing them singularly just over the water level. A humidity of 60-70% and temperatures of 25°C are sufficient for about a quarter of the eggs laid to survive for 4 months. In addition to humans, *Aedes albopictus* bites other mammals and birds too. This mosquito can transmit various diseases such as nematodes like dirofilarial, and viruses responsible for diseases such as the yellow fever, St. Louis encephalitis, dengue, chikungunya, and zika. The tiger mosquito is also important in the veterinary sector because, for example, it can be a vector of parasitic worms of the genus *Dirofilaria*, agents of cardiovascular dirofilariasis in dogs and cats.
The tiger mosquito was the vector of the chikungunya virus that caused the first recorded epidemic of this disease in Europe. The epidemic was recorded in Italy, in the province of Ravenna, in the summer of 2007: more than 200 people were infected. In 2017, an epidemic of chikungunya affected more than a hundred people in Latium. In 2010, this mosquito was responsible for the spread of the dengue virus in France, Croatia, and Germany. [786]
Chikungunya is a viral disease characterized by fever and severe pain. After an incubation period of 2-12 days, fever and joint pains occur suddenly, limiting the movement of patients,

who then tend to remain absolutely motionless and assume antalgic positions (the name chikungunya comes from these symptoms, which in *Swahili* means "that which bends"). In most cases, patients recover completely, however, in some cases, joint pains can persist for months or even years.
Of viral origin, dengue is caused by four very similar viruses and is transmitted to humans by the bites of mosquitoes that have, in turn, bitten an infected person. There is therefore no direct contagion between humans, although humans are the main host of the virus. The virus circulates in the blood of the infected person for 2-7 days and during this period the mosquito can ingest it and transmit it. [789] Normally, the disease gives rise to fever within 5-6 days from the mosquito bite, with very high temperatures too. The fever is accompanied by acute headaches, pain around and behind the eyes, severe muscle and joint pains, nausea and vomiting, and skin irritation.
The transmission of the Zika virus to humans generally occurs through the bite of a vector mosquito. If a person bitten by a carrier mosquito is subsequently bitten by a non-infected mosquito, this can trigger a chain capable of creating an endemic outbreak. Inter-human contagion is possible and may occur through biological fluids (sexual route, transfusions, maternal-fetal passage). It is estimated that in 80% of the cases the infection is asymptomatic. The symptoms, when present, are similar to those of a flu syndrome, lasting about 4-7 days, sometimes accompanied by other symptoms (rash, arthralgia, myalgia, headache, and conjunctivitis) that appear 3-13 days after the bite of the vector mosquito. Hospitalization is rarely necessary. [790]
So globalization and climate change favour the spread of alien species and disease-carrying pests. Because of the increased possibility of insect movement, thanks to human activities and favourable climatic conditions, health problems will increase; the spread of exotic diseases in Europe is favoured and it is a worldwide problem.

SOME FACTORS FAVOURING THE ARTIFICIAL SPREAD OF SPECIES

Throughout the Planet, the installation of Botanical Gardens and urban parks has encouraged the spread of non-native plant species and their hosts, such as parasitic arthropods. The establishment of parks and gardens with imported plants has encouraged the expansion of this problem. To give an example, most of the non-native European butterflies (Lepidoptera) have been brought in by the introduction of exotic plants in parks and gardens, and some of these insects have colonized cultivated areas, as they are phytophagous.
Other important factors in the artificial spread of some species have been fishing (e.g. trout and salmon) and sport hunting (e.g. pheasants, wild boar, deer, ducks), the pet trade and the ornamental plant trade. For example, wild boars were extinct and later introduced for hunting: the wild boar was extinct in Britain 700 years ago.
There are many factors that can turn a native species into an invasive and harmful one. For example, in natural environments the artificial help provided by beekeepers makes these insects fearsome competitors for wild species that feed on pollen and nectar.
The use of herbicides has artificially selected the evolution of grasses resistant to these poisons. The same has happened with insects because of the use of insecticides. So a species can be native and become invasive because it is resistant to insecticides, herbicides, or for other reasons.
Some species cannot move independently and naturally between ecosystems, but may be moved artificially. For example, organisms living in one lake may be artificially moved to another one, which would otherwise be unlikely. Thus even native species may become, under

artificial conditions, a problem both ecologically and for the economic interests of mankind (e.g. because of the generation of additional costs to food production).

Another aspect that may have significant effects is the possibility of distributing non-native seed species in the environment. The spread can be generated by the use of feed from other States or Continents. A survey conducted on horses used for recreational purposes within a California Park showed that feces distributed 32 plant species of which 4 were non-native. [898] Thus, the importation of feed may promote the distribution of species that are dangerous to agriculture or harmful to wild species.

The transport of animals that feed on seeds is another mechanism for indirect plant spread. Birds such as pigeons or jays are the planes on which many plants naturally travel. Thanks to birds they can move much farther and faster. Through the spread of certain bird species, the colonization by many plants that have adapted to take advantage of this type of transport is promoted.

Strategies to slow down the spread of alien species include the establishment of protected zones and the identification of areas where potentially dangerous species are present if they were to be accidentally exported. In protected areas, the entry of goods and living organisms should be monitored and controlled. From areas where dangerous species have been identified, very strict measures should be implemented on exports and trade of goods. [815] Controls are usually based on visual inspections (by veterinarians, botanists, entomologists) and laboratory analyses. In order to be marketed in Europe, plants must have an appropriate certification, identified by the term 'plant passport'.

There are official lists of particularly dangerous organisms. For example, 20 harmful organisms, included in the list published by the European Commission in October 2019, are quarantine organisms, ranked at the top of the priorities for the member states of the European Union, based on the severity of the economic, social, and environmental problems they can cause. Prominent among the 20 organisms is *Xylella fastidiosa*, a (Gram negative) bacterium native to the American Continent. Since the late 2000s, *Xylella fastidiosa* (pauca spp.) has also been reported in Italy (first report of a presence in Europe), with infestations starting in olive groves.

A *quarantine pest* shall be designated as a harmful organism in respect of a defined territory if it meets all of the following conditions: [816]

(a) its identity has been established;

(b) it is not present in the territory or, if present, it is not widely distributed within the territory;

(c) it is capable of entering, establishing itself, and spreading within the territory or, if already present in the territory but not widely distributed, it is capable of entering, establishing itself, and spreading within the parts of the territory where it is absent;

(d) its entry, establishment, and spread have an unacceptable economic, environmental or social impact on the territory concerned or, if present but not widely spread, on the parts of the territory where it is absent;

(e) there are feasible and effective measures available to prevent the entry, establishment or spread of that pest within that territory and to mitigate its risks and impact.

Commercially traded plants must have been kept under quarantine conditions and be free of harmful organisms. Unfortunately, current prevention and monitoring measures are unable to limit the movement of thousands of dangerous species, as they are alien to the target ecosystem. In conclusion, humans are arguably the most effective facilitator of invasive species in Earth's history.

THE VOLUNTARY INTRODUCTION OF ALIEN SPECIES FOR BIOLOGICAL CONTROL PURPOSES

The intentional introduction of alien species is also carried out to implement what is called biological control, which consists of trying to limit the growth of crop pests by using their natural enemies. The possible examples are countless. Many species of carnivorous insects (insectivores) have been artificially introduced with the intention of helping farmers. In Europe at least 180 species of insects (parasitoid hymenoptera) have been introduced with the intention of curing certain plant diseases. [698]

The harlequin ladybird (*Harmonia axyridis*) is a beetle of Asian origin that preys on aphids, mealybugs, neuropterans, hoverflies, and numerous other phytophagous insects: for this reason it has also been introduced into Europe. In the USA the contribution of the ladybird in fighting an Asian soybean aphid has been very important, because the native predators of aphids would not have been able to control it on their own. [809] The harlequin ladybird is particularly dangerous for grapevines. In the summer, but especially near the harvest, it tends to take refuge inside the big and compact bunches of grapes and release substances with an unpleasant and nauseating odour, which if present in the must strongly alter the taste and smell of the wine. Its danger for the ecosystem lies in the fact that this type of ladybird is able to eradicate the native aphidophagous species and replace them.

The introduction of a butterfly (*Cactoblastis cactorum*) native to Argentina was carried out because it is a parasite of different species of cactaceous plants of the genus *Opuntia* (the well-known prickly pear and other types of cactus belonging to this group). [558] This moth (a pyralid) owes its notoriety to the fact that it was successfully used as a biological control agent in Australia, in the 1920s, to control some species of *Opuntia* (*O. stricta*) which had spread beyond all expectations, invading some millions of hectares of pastures. After its success in Australia, it was spread to other parts of the World, including Madagascar in 1925, South Africa in 1933, the Hawaiian Islands in 1950, and the Caribbean in 1957. This insect has also been reported in Florida and Mexico, where however it is seen as a potential threat to the biodiversity of local cacti. [567]

Another example is the introduction of a toad *(Rhinella marina)* into sugar cane cultivations in Australia and Costa Rica to limit the development of certain pests of this crop. The introduction was repeated also in other places of the Planet, such as the United States of America and Japan. This amphibian has been included in the list of the hundred most harmful invasive species in the World. [558, 568]

The mosquitofish (*Gambusia affinis*) is a small freshwater fish native to the basins of the Gulf of Mexico (Mississippi); it lives in fresh and brackish marshy waters. During the twentieth century, some specimens were introduced into many swampy areas of the World, including Italy and all of southern Europe, to control mosquitoes. This species is also included in the list of the hundred most harmful invasive species. [578]

A successful example of the introduction of an insect species for biological control of a plant is recorded in the United Kingdom. This is the Japanese psyllid (*Aphalara itadori*) introduced to limit the spread of an invasive Japanese plant (*Fallopia japonica*). [558] In this case, the successful transplantation into new ecosystems was facilitated by planning and conducting a preliminary study over a number of years in which, for example, it was established that the insect could not reproduce on similar native species.

In conclusion, the voluntary spread of non-native species has favoured ecological disasters, in fact some species distributed on the Planet to implement biological control are classified among the hundred most dangerous invasive species in the World.

QUANTIFYING AND MONETIZING MAN'S DOMINION OVER NATURE

It is complex to try to give an economic value to a plant or a bird that becomes extinct due, for example, to the introduction of rodents or to hunting: the hunting of bisons in North America had brought this animal, in just 30 years (1830-1860), from a few million specimens to a few thousand. [703] A research that has documented the causes of 680 extinctions of animal species has identified, for 91 of these, the introduction of alien species among the determining causes of their disappearance. [558] On some islands, the introduction of cats led to the disappearance of 14% of birds, mammals and reptiles. [558]

Monitoring the presence or absence of native and introduced species in order to quantify the ecological disaster, the numerosity and the extension of the occupied territory is not easy. Measuring the benefits or disadvantages generated by introduced species, in some completely artificial contexts, such as urban or agricultural, is not easy and very often useless. Fallow land in agricultural areas may occupy less than 10% or 5% of the total area, which may extend to hundreds of thousands of hectares. Therefore, identifying and classifying the few species that have managed to survive ploughing, herbicides, fires, alterations to river courses, and concrete constructions as well as those that are well adapted locally is of relative ecological value. These ecosystems are completely artificial and dominated by human actions. The case of natural areas is different, where the damage caused by foreign species to native ones is much more evident and serious.

An economic estimation of the damage generated by the introduction of alien species is very difficult. At a global level, some attempts to estimate the costs generated by the invasion of more than 120,000 alien species estimate several hundred billion euros per year. The costs, in these estimates, include mainly two items: damages, such as those to crops, and expenses to counter the spread of non-native species. Several strategies may be used to estimate the economic value of damage generated by non-native species such as:

- The estimated costs generated for trapping (e.g. of mice, rabbits or wild boars) and/or sterilization (e.g. of cats).
- The value of the damage generated to the crops. In this case the reduction of the harvest and the correlated economic loss are estimated: if a parasite generates the loss of 4% or 30% of the harvest it is quite simple to have a quantitative measure of the economic damage for the farmer.
- The damage generated by non-native animals to farms. For example, the number of eggs and chicks caught by rodents (or birds eaten by the mongoose).
- The damage generated by alien animals to wild species. For example, it is estimated that domestic, subsequently abandoned, and feral cats, which may number more than 60 million in the USA, can each kill eight wild birds per year (that's 480 million birds exterminated every year in the USA by feral cats). [698, 848]
- The cost of controlling the pest or weed in the crop. In this case the additional costs incurred to distribute insecticides or herbicides are added. In some cases, the eradication of non-native invasive plants is done mechanically. Again, estimating direct costs to the farmer is straightforward, but indirect costs such as those generated by contamination of the biosphere with pesticides and damage to human health are not estimated.
- The damage generated to structures such as houses or monuments (e.g. termites and pigeons).
- Additional health costs (e.g. from insect bites or snake bites). The waves of the bubonic plague, which ravaged Asia and Europe, were transmitted by the Eastern rat flea (*Xenopsylla cheopis*). [848] Estimating exactly how much diseases such as those

transmitted by rats introduced into the UK (salmonellosis, leptospirosis, toxoplasmosis, yersiniosis or plague bacillus) cost the community is not easy.
- The costs generated by controls carried out during international trade, such as those at customs or the costs of supporting quarantines, return to sender or destruction.

Estimates of the damage caused by the control of artificially introduced species are very difficult and often underestimate the problem. To give an example, in the case of artificially introduced plant species, very often economic estimates are limited to adding up the costs of herbicides with those generated by crop reduction. Estimating the damage generated by the introduction of foreign species into a given ecosystem is very difficult and suffers from a major limitation, that of the anthropocentric vision. Estimates are made almost exclusively of direct costs incurred in the short term, such as those faced by farmers. We should try to create a new environmental culture and encourage a vision on the side of ecosystems.

In order to quantify the intensity of the presence of artificially introduced species in a territory, it could be useful to estimate the fraction of the biomass occupied by foreign species compared to the autochthonous ones. This measure gives a more realistic view of the severity of the problem. For example, in Britain, alien species make up almost half of the biomass of the mammals and birds present. However, as already written, in the UK and Ireland the number of invasive plants recorded exceeds that of native species. [698] Therefore, the number of alien species compared to native species is not sufficient to give a realistic measure of the impairment of an ecosystem.

One part of the pesticides and other poisons used (e.g. rodenticides) is intended to slow the spread of invasive non-native species. It is not easy to try to estimate the damage generated by the use of chemical control, such as insecticides and herbicides. It is arduous and unpopular to put an economic value on the health damage generated by pesticides to farmers and the community. It is even more complex to estimate the negative value of soil and water pollution caused, for example, by herbicide contamination.

It is unthinkable to assume that we will succeed in slowing down the introduction of non-native species throughout the Planet and, therefore, the most realistic destiny that lies ahead is that of ecological uniformity. Homogenization generates simplification and extinction, favouring the arrival of the Eremocene, the era in which we will remain with few species. The artificial agricultural ecosystem, with the few dozen species useful to man, becomes dominant or even the only one. The current perspective is that of the simplification of ecosystems and the extinction of most of the biodiversity.

The degradation of natural diversity will lead to the collapse of the capacity of ecosystems to provide services essential to the survival of the human species, such as food, air, soil, and water that are not contaminated. We do not know what level of degradation wrought by humanity that ecosystems can sustain and buffer before entering a zone of insecurity and instability. We may soon discover that we have long since passed the safe operating zone for the human species, while we are distracted by inadequate and unrealistic estimates. Much more effective action needs to be taken because it is no longer a question of reducing the additional economic costs generated to farmers, but of avoiding an irreversible and terrifying environmental collapse.

In Europe, the cost of introducing alien species is estimated to be at least 12.5 billion euros per year. [558] This is certainly an optimistic estimate, but it is a useful starting point on which to build prevention and management policies.

THE VILLAGE OF PROGRAMMED BIOLOGICAL DESTINY

OTHER PROBABLE FUTURE DISASTERS: GENETIC ENGINEERING

The design and creation of organisms with characteristics that do not exist in nature and are the result of human talent have become a reality. It is possible to build microorganisms in the laboratory with new characteristics, designed to meet human needs, more or less objectionable from the point of view of ethics and safety. Some animal creatures have been designed to amaze, they are whims of scientists such as luminescent animals (they possess genetic characteristics copied from bioluminescent insects or marine organisms such as jellyfish). It is also possible to generate organisms with new functions, unknown to nature. The precautionary principle should be applied very firmly here, especially in the case of the manipulation of viruses, bacteria, and other organisms that are difficult to contain in a laboratory or company. How much longer can we not care about treating biological systems in the manner of industrial production in an economic system that does not accept limits?

These are disruptive new technologies that allow us to get into the most intimate part of the process that generates the variability necessary for evolution to do its work. Evolution works thanks to the existence of immense libraries on the genetic characteristics that each species possesses. The tools for modifying these libraries (DNA) allow for a very dangerous process: artificial selection. Biotechnology offers potentially very interesting tools such as the cure and eradication of genetic diseases. At the same time, they open up distressing horizons such as that of eugenics, i.e. the artificial selection of human beings. Attempts to catalogue class and race hierarchies and, consequently, to try to exterminate inferior ones are a horror perpetuated many times by humanity. [1192] The idea of the perfect Aryan race led to the extermination of millions of Jews, but we must not forget that even in the USA, until the 1970s, some states proceeded to the compulsory sterilization of males considered unfit (e.g. homosexuals, psychiatric patients, criminals, blacks). Between 1907 and 1963, at least 64,000 individuals were sterilized in the United States in compliance with the eugenic legislation. In North Carolina, from 1929 to 1974, a disproportionate number of those targeted for forced sterilization were blacks and women: of the 7,600 women who were sterilized by the state, between 1933 and 1973, about 5,000 were African-American. [1049, 1050] These horrors should not be forgotten.

The artificial modification of the genes of living beings may be used for military purposes, to favour commercial enterprises and to undermine the food sovereignty of competitors. There are many unknowns and we are being presumptuous if we think we can predict all the possible negative effects of new technologies, even when they are used exclusively for potentially beneficial purposes. Innovation in the field of DNA engineering highlights once again the gap between technology and reality, the separation between economics and ecology, and the underestimation of the dangers generated by inequalities.

BIOTECHNOLOGY OPENS UP NEW FRONTIERS

The technologies opened up by innovation in the field of genetics are fascinating because they make it possible to enter the control mechanisms of natural selection. It is possible to control and modify the variability associated with heritable traits and also with non-heritable traits, such as those established by cells not used for reproduction. In artificial contexts, completely detached, separate and unaffected (temporarily) by the rules of natural selection, one is able to promote the differential reproductive success of traits designed by biomolecular engineers. A great acceleration is given to artificial selection of phenotypic characteristics chosen in genetics laboratories. The change of a population of organisms over time can be orchestrated and directed, generating a great innovation: the artificial evolution of populations, species and of entire ecosystems. The ability to modify the environment is amplified enormously and, above all, irreversibly. The tools and techniques that can be used to generate this great revolution are many and some have the great advantage of being very cheap; this aspect is a source of new and greater concern. We will mention some of them.

CRISPR/Cas9 (*clustered regularly interspaced short palindromic repeats*) is an acronym for one of the most recent and promising technologies. It is a defence system adopted by bacteria against viruses. Bacteria store pieces of viral DNA in their own DNA every time they overcome the infection. They store them by interspersing them in repeated genetic sequences (identical repeats of about 29 letters separated by unique sequences of 32 letters). [1051]
The viral DNA fragments, embedded in bacterial DNA, are used to build RNAs: small, single-helix strands of nucleic acids. The RNAs copies of the viral DNA are used by the bacterium to search for similar sequences, i.e. viral genetic material. Once the invader, the agent of potentially deadly infections, is found, the RNA recruits an enzyme system (proteins such as one called *Cas9*) that cuts through the viral genetic material rendering it harmless. The genetic material that carries sequences homologous to those of the virus, when attached to a special protein system acts as a sentinel. Once it recognizes the genetic material of the invading virus, it binds to it and renders it harmless (by cutting it thanks to the enzymatic activity of proteins called nucleases). This is the bacterial equivalent of the human immune system. Each time the bacterium survives a viral infection it archives its genetic sequence, alternating it with repeated sequences. When the virus arrives again, the bacterium will already be ready to defeat it: it is a system equivalent to vaccination. This system, called *CRISPR*, has been found in many bacterial species (40%) and in most of the archaebacteria (90%) studied. [989, 1051] Bacteria also perform important functions in bees through symbioses developed during evolution: defense against disease (e.g. fungi), and digestion. Symbiontic bacteria of bees (e.g. *Bombella apis* found in the intestine of bees) have also been found to possess the *CRISPR/Cas* system to defend themselves against viruses. [1123]
The natural defense system present in bacteria can be reprogrammed in the laboratory to perform useful functions. It has long been discovered that a considerable fraction of human DNA (perhaps as much as 8%) bears traces of infections that have occurred over millennia (e.g. by RNA-based retroviruses).
Viruses have long been used to try to cure human diseases and are among the leading causes of bacterial deaths (probably up to 40% of the bacteria living in the oceans die every day from viruses). Some viruses (bacteriophages) have been used successfully to cure bacterial diseases (billions of phages are inoculated into the patient to defeat antibiotic-resistant bacteria). In experimental trials viruses have also been used to transport the necessary enzyme into a human being (e.g. into the brain of a Tay-Sachs patient). The infusion of high doses of viruses has also led to serious failures, such as the death of patients. [1051]
Viruses have adopted counter-defense mechanisms from bacterial protective measures, such as systems against enzymes that deactivate the *CRISPR* system.

It was discovered that viruses were one of the main causes of the inactivation (killing) of microorganisms marketed to make yoghurt and cheese, generating considerable economic damage (e.g. bacteria of the genus *Streptococcus, Lactobacillus*, and *Lactococcus*). [1029, 1051] Bacteria resistant to these viruses contained *CRISPR* sequences, which represent a kind of vaccine memory. This antiviral defense system requires RNA-based sequences and proteins that cut nucleic acids (called *Cas;* there are some that are specialized in cutting DNA or RNA in different ways). The *CRISPR/Cas9* system, consisting of a protein complex linked to a nucleic acid (RNA) sequence, is nothing more than a biochemical apparatus specialized in cutting nucleic acids (nucleases) such as DNA, programmable by varying the RNA sequence (about 20 - 30 nucleotides); restriction enzymes have long been used to recognize and cut nucleotide sequences but, usually, they recognize shorter sequences (6 nucleotides) so they can make mistakes much more easily. Without the specific RNA sequence or without the *Cas9* protein the complex does not work and, therefore, is not able to save the bacteria from viral infection. However, viruses have defense systems such as proteins (*Cas* nuclease) that can disable the *CRISPR* system. Several types of *CRISPR* systems have been identified, each with different specificities, and others have been created in the laboratory with the aim of meeting specific needs.

By modifying the nucleic acid recognition sequence (i.e. the order of the nitrogenous bases in RNA) of the *CRISPR* system, it can be directed to specific sites: it can be reprogrammed at will with artificial guidance based on synthetic nucleic acids (RNA). All living beings use the same letters of the genetic alphabet (nucleotides). This technique is like possessing a text correction system that can find a word and modify it, for example by cutting or deleting it or replacing it with another. So the nature of bacteria has provided molecular engineers with a precision scalpel to recognize DNA sequences of a few bases (about 20) and make cuts (or modifications) in close proximity. The recognition technique is not infallible just as the cutting system can make mistakes too. Theoretically, cutting a gene can deactivate it: here is a new tool to deactivate genes that produce disease-causing proteins. You can also test what happens to a cell if a particular piece of DNA is cut off (deactivated). It has also been possible to use the system to recognize particular sequences to replace certain nucleotides and, therefore, repair or modify point mutations. The *CRISPR* system can be used to try to modify several genes simultaneously by making cuts, insertions or deletions of parts of the DNA, potentially for all living beings, both in the animal and plant kingdoms. [1029]

An interesting application could be to alter the characteristics of a protein found in white blood cells that allows the entry of the AIDS virus. In Europe it has been discovered that a fraction of the population can be naturally resistant to HIV, thanks to the presence, in duplicate (homozygosity), of a mutated protein found on the membranes of blood cells (called CCR5 and presenting a deletion of 32 bases). The unmutated form of the protein allows virus entry. The *CRISPR* system could be used to modify the gene that produces this protein in stem cells so that it does not allow the HIV to enter. Theoretically, one could hope to make HIV-positive patients resistant to the infection (by taking lymphocytes, the T-type stem cells, modifying them in the lab and putting them back into the patient). [1051] The problems are many, such as reaching a large number of the patient's lymphocyte cells and correctly modifying their DNA without errors or alterations in non-target sites. These hurdles are not insignificant, but if the idea would work, an injection of a concentrate of *CRISPR* systems, properly designed, would be enough to heal. In one fell swoop, one could hope to make a ill person resistant to the virus or a healthy person resistant to the infection.

Another possibility offered by the *CRISPR* molecular scalpel system could be to cure sickle cell anemia, which can be caused by a single letter mutation in the base sequence in the gene that directs the formation of the protein called hemoglobin. This disease (autosomal recessive) is generated by the substitution of one nucleotide for another (Thymine replaced by an Adenine), which causes the change of only one amino acid in hemoglobin. This qualitative change is hereditary and is responsible for the disease. There are between 2,500 and 4,000 Italian patients affected by sickle cell anemia (25,000 in Europe), which is the most widespread hemoglobinopathy in the World. [1032] The haemoglobin modifications, typical of sickle cell anaemia patients, do not allow the parasite responsible for malaria (*Plasmodium falciparum*) to exploit cellular resources to transport its proteins. By employing the *CRISPR* system in cultures of cells carrying the sickle cell mutation, it was possible to correct the error and change the cell phenotype from diseased to healthy. [1029] Similar successes have been achieved in cultures of cells with different diseases such as cystic fibrosis (lung cells) and Duchenne muscular dystrophy (muscle cells). The hope is soon to have gene therapies to treat these serious diseases: for one type of anemia, a beta-thalassemia, a gene therapy strategy has already been approved.

The technique enabled by the *CRISPR* system potentially allows the defective gene to be corrected with several advantages, for example, having to insert a new working copy of the gene. There is no risk of continuing to have the expression of a gene that is harmful and could be passed on to the offspring. Furthermore, the defective gene when corrected remains under the control of the natural regulators of gene expression.

The *CRISPR* system allows us to recognize small sequences within the more than 3.2 billion letters (these are the nucleotides that form a genetic alphabet based on 4 letters) that make up our DNA which contains over 20,000 genes. This information is contained in 23 pairs of chromosomes found in the billions of billions of cells (10^{18} or one trillion) that make up our bodies. If you wrote the entire human genetic code of 3.2 billion letters in 3,000 character pages, you would need to print over a million pages. In 2018, it was possible to know the exact sequence of an infant's entire genome (the order of all 3.2 billion letters made up of the 4 nucleotides of DNA) in less than 21 hours. [1056, 1057] So in one day it is theoretically possible to know, within this encyclopedia, the possible presence of incorrect words or phrases (mutations, alterations), capable of generating diseases.

In 1995, the first bacterial genome was sequenced and within a few years it was possible to sequence the genomes of numerous organisms including at least 150,000 microbes. In 2001 the entire human genome was sequenced, in 2002 that of mice, and in 2017 that of homing pigeons. For at least three species of bumblebees (*Bombus terrestris*, *Bombus impatiens*, and *Bombus terricola*) and for the honey bee (*Apis mellifera*) genome sequences of the entire DNA are now available. [1239]

A surprisingly positive aspect is the reduction of the costs necessary to carry out the sequencing of the entire human genome, down to under a thousand euros and there are already portable sequencers as big as a mobile phone. [1058] For at least one third of the 20,000 known human genes, errors (mutations) capable of generating diseases have been identified. [1051]

With the *CRISPR* system in laboratory animals it has been possible to deactivate individual genes (it is like finding and editing individual words within this encyclopedia) linked to certain types of cancer. It is hoped to be able to do the same in the case of some human cancers by deactivating them with targeted biotechnology systems. Thousands of errors in the words of this encyclopedia (the nucleotide sequence of the genome) are known to generate usually rare diseases. One day it will be possible to repair these errors by deleting or replacing them: the *CRISPR* system makes it possible to try this path.

The winning features of this genetic engineering strategy include simplicity, speed, and cost-effectiveness. This technology has been widespread since 2013 and in seven years it has been possible to register more than 5,600 scientific publications (those with the acronym *CRISPR* in the title or the abstract on the *PubMed* site). [990] The great advantage of the CRISPR system is that it is programmable and modifiable depending on the target. The target, the DNA sequence that one wants to modify, can be chosen easily. Also the operation to be done such as cut (deletions), or cut and replace, or cut and paste, or cut and insert (insertions), or even recognize the sequence and modify the regulators (genetic and epigenetic) that influence the activation of genes (e.g. histone proteins to which the DNA is wrapped or promoters) can be designed. It has become possible, in a rapid way, to alter the functioning of many genes at the same time, for example, to study tumours in laboratory guinea pigs. It is possible to do many things that were also possible with previous techniques but the great advantage is the rapidity together with the lower expense. [989] It is a molecular scalpel programmable to make the desired changes in predetermined places: cut, replacement, deletion, insertion. Theoretically, it is possible to program in the laboratory the guide sequences (of the RNA of the *CRISPR/Cas9* system) for each of the approximately 20,000 known genes in the human species or any other species whose genetic sequences are known. Thus it is possible to hope to reach and modify any sequence quickly and cheaply. [1044] This technological innovation has probably been followed by too much enthusiasm and many hopes are the result of overestimating the short-term benefits and underestimating the long-term negative effects. But it is certainly a useful tool for research into certain diseases such as tumours. In cell cultures, tissues or parts of organs (e.g. intestinal organoid), it is possible to switch off genes with a probable oncogenic or anti-oncogenic action in order to study their mechanisms of action. In cell lines, it is possible to discover which genes are essential for the development or prevention of cancer. [1151] In laboratory animals, genes can be deactivated to test their importance in the occurrence of many different diseases. It is possible to deactivate genes in the zygote (newly fertilized egg cell) with a single injection and obtain an animal in which that function is deactivated (e.g. in mice, rabbits, and monkeys). The study of animal models makes it possible to improve the understanding of human diseases and opens up new therapeutic and diagnostic horizons.

APPLICATIONS IN THE FIELD OF MEDICINE: GENE THERAPY

Some applications generated by biotechnology are very important and well-known. Until the 1980s the hormone insulin (used to treat diabetes) was extracted from the pancreas of pigs and cattle. For many years now, insulin has been produced by specially engineered bacteria (e.g. *Escherichia coli*, an inhabitant of our digestive tract). Likewise, bacteria produce many useful protein molecules such as the growth hormone or some vaccines. There are more than 7,000 known hereditary diseases generated by gene mutation, affecting at least 30 million Americans. [1023] For very few diseases there is an authorized genetic therapy, such as the one that employs adenoviruses, a group of viruses considered not dangerous for humans (they are very small and can carry a single helix of DNA of about 5,000 nucleotides). These viral vectors do not generate an integration of the DNA they carry into the genome and do not cause a strong immune response: these are two notable advantages. It must be pointed out that viruses have not evolved to provide us with tools for gene therapy so there are many risks. However, there are some approved gene therapy treatments that use these viruses to treat rare diseases. In many laboratories around the World, however, other viruses (e.g. lentivirus) are also used for clinical trials. So a dangerous experiment is underway.

There are also other strategies of genetic therapy which, in general, exploit two possible strategies to treat somatic cells, i.e. those which are not transmitted to descendants (e.g. skin, muscle or blood cells). The first consists of taking cells from the patient, treating them in the laboratory and bringing them back into the patient (e.g. stem cells). This makes it easy to do numerous checks before placing the modified cells back into the patient's body. This strategy is possible for blood cells. In part this methodology is already applied, for example, with bone marrow transplantation, but modern biotechnology allows the sick person to not need a donor (the patient's own cells are modified and healed).

A second strategy instead uses vectors (e.g. modified viruses) or molecular scalpels (e.g. the *CRISPR* system consisting of proteins and RNA) to modify (in vivo) the target cells in an organism. This possibility therefore presents much greater risks since the administration must take place in the patient and not on the cells extracted and manipulated in the laboratory. There are some obstacles to reaching the target without making mistakes or changing other functions. It is also possible to create viral vectors (adenoviruses) that carry the *CRISPR* system: they are designed to reach particular target cells and release the *CRISPR* system with precision (e.g. in cells of the liver, lungs, retina or central nervous system). In laboratory animals (mice), it has been possible to treat the equivalent of human Duchenne muscular dystrophy (a recessive genetic disease associated with the X chromosome) by injecting adenoviral vectors carrying a specially designed *CRISPR* system. [1029]

The following is a summary of some gene therapy strategies authorized by the Health Service to cure some rare diseases. These are scientific successes that contribute to maintaining a very rich industry, which supports billion-dollar legal disputes to appropriate the exclusivity of patents of biological systems such as those present in bacteria for millions of years (e.g. *CRISPR*).

GENE THERAPY IS NOW A REALITY: THE CASE OF THE *STRIMVELIS* TREATMENT

The following is an example of an authorized genetic treatment in Europe. A malfunctioning gene inherited from both parents (homozygous recessive) impairs lymphocyte production in the children. The disease can be ameliorated with a marrow donor, otherwise the children have to live in an environment isolated from microbes, which would kill them. This treatment (*Strimvelis*) is indicated for patients with severe immunodeficiency (adenosine deaminase deficiency or ADA and causes the severe combined immunodeficiency or SCID), for whom a suitable consanguineous HLA (human leukocyte antigen) stem cell donor is not available. [994] This immunodeficiency (ADA-SCID) is an extremely rare condition with a typically infantile onset (the incidence of the disease is estimated at 1-5 cases per 1,000,000 new births). [995] The *Strimvelis* gene therapy involves the use of the patient's own stem cells, which are collected and modified to produce the normal protein (the ADA enzyme). Once the cells have been treated (they are modified using viruses), they are stored in the patient and, after settling in the bone marrow, generate new cells that are active in the production of the enzyme (the cells taken from the patient are modified using a retroviral vector containing the cDNA sequence[I]). [994]

In Europe, it is estimated that every year 15 children are born with this disease, which, if left untreated, leads to inauspicious outcomes even before reaching school age; the cost of the therapy is 594,000 euros for each patient. This gene therapy has been authorized for several years also in Italy: the Italian Medicine Agency (Agenzia Italiana del Farmaco) approved the reimbursability of *Strimvelis*, an *ex vivo* gene therapy based on CD34 and autologous cells, in 2016. If Italian patients do not experience the expected benefits over time, the company producing the therapy will be forced to reimburse the full amount to the National Health Service. [993, 996]

The history of this treatment, which uses viruses manipulated in the laboratory to modify diseased stem cells taken from the patient, is astounding. Among the advantages of this strategy, two can be highlighted:

[I] In molecular biology, complementary DNA (abbreviated to cDNA) is synthesized from messenger RNA, from a single-helix nucleic acid we obtain a double-helix one.

- The manipulation of cells with viruses takes place in a controlled environment, in the laboratory. It follows that it is possible to make checks before putting the cells extracted from the patient back into the same.
- The virus engineered to generate the desired genetic changes does not circulate within the human body.

This disease is fortunately very rare, so the potential beneficiaries are very few. It must be stressed that this is a very expensive treatment, so without financial support, such as that provided by the Italian Health Service, it would not be accessible. How many of the estimated 15 possible European patients could afford to spend almost 600,000 euros to hope to be cured? Interestingly, the agreement between the manufacturer and the Health Service provides for reimbursement of costs if the patient does not improve. This guarantee should be extended to all these types of treatments regardless of cost.

A FAILURE: THE *GLYBERA* TREATMENT

The cost of gene therapy, which is usually intended to treat rare diseases, is a major limitation. These are very expensive solutions often aimed at a small number of patients. We report the case of the *Glybera* treatment which was the first example of a gene therapy authorized in Europe in 2012, and was subsequently withdrawn in 2017. The therapy was developed against the lipoprotein lipase deficiency (*Lpld)*, which generates a rare disease: it is a pancreatitis that affects one in about one million individuals, in which the patient lacks an enzyme that allows to digest fats. In patients, the gene that encodes the enzyme does not work and forces them to eat a diet with only 20% of the necessary fats. In the most severe cases, for which it has been approved, patients suffer from recurrent pancreatitis. The muscles are the site of synthesis of this type of protein lipase, an enzyme that is released into the blood, where it helps to keep the concentration of fats low (fat globules formed by triglycerides, bound to proteins, are dissolved): the lack of this enzyme causes the concentration of lipids in the blood to rise enormously, causing pancreatitis. This treatment became famous for being the most expensive in the World: over a million dollars. [997, 1091] It was withdrawn in 2017, yet it had been presented as the breakthrough towards the provision of effective therapies against very rare diseases for which there are still no specific treatments; in Europe, it was probably administered to only one patient and in the World to 31 patients (among them 27 were participating in preliminary studies), of which only one was paying and insured. [998, 1051, 1091]
The *Glybera* preparation consisted of a special viral vector (AAV1), into which the normal DNA sequence of the gene for human lipoprotein lipase (LPL gene) was inserted. The preparation was injected into the muscles of the legs. The limitations in the use of the medicine were the impossible cost (initially 1,600,000 euros, later reduced to 1 million euros) and some doubts about its real effectiveness. In this case it was possible to document the possible beneficial effects only on a few patients and in a short interval of time. [999]

ZOLGENSMA GENE THERAPY

Spinal muscular atrophy (SMA) is a rare genetic (recessive) neuromuscular disease caused by the lack of a functioning gene (SMN1), resulting in a rapid and irreversible loss of motor neurons, which compromises the muscle function, including breathing, swallowing, and basic movement. In Europe each year about 550-600 children are born with spinal muscular atrophy; in Italy an incidence of 1 case per 10,000 live births is estimated, so every year about 40/50 children are born with SMA, which is a genetic cause of infant death (75% of children with this disease die before the fourteenth month). [1013, 1017] If left untreated, SMA Type 1 causes death or the need for permanent ventilation within two years of age, in more than 90% of cases. [1014] The Italian Medicines Agency (Agenzia Italiana del Farmaco) has included *Zolgensma* (*onasemnogene abeparvovec*), the first gene therapy for spinal muscular atrophy (SMA), in the list of medicines available at full charge of the National Health Service for treatment within the first six months of life of the patients. The treatment is administered only once in a lifetime by intravenous injection: it consists of adenoviruses (AAV9) that are carriers of a new fully functional copy of the SMN1 gene. In the newborn, approximately 1.1×10^{14} viral vectors per kilogram of body weight are introduced into the venous circulation (the new gene is regulated by a chicken *beta-actin* promoter and forms an extra-chromosomal genetic element in motor neurons, with a reduced probability of insertion of the patient's DNA). [1016, 1017] At least 700 patients have been treated with this strategy (in the US this gene therapy was approved in 2019). Initially, the cost of this treatment was over $2 million. [1015]

LUXTURNA GENE THERAPY

Luxturna is indicated for the treatment of adult and pediatric patients with vision loss due to hereditary retinal dystrophy and with sufficient viable retinal cells (this disease is caused by confirmed biallelic mutations in the RPE65 gene, which produces a protein necessary for the retinal cell function). [1018] It is a medicine that with a single injection is brought under the retina of the eye of both adults and children (it contains 5×10^{12} viral vectors per millilitre). [1019, 1020] Through a single administration it allows to improve the visual ability of patients. This therapy is also based on the use of an adeno-associated virus (AAV) employed as a vector. Hereditary retinal diseases are multiple and linked to mutations in more than 250 genes: this specific biallelic mutation affects one in 200,000 people; in the USA, there are probably between 1,000 and 2,000 potential patients who could benefit from this therapy. [1091] An early diagnosis, at an early stage of the disease, and a sufficient number of viable retinal cells are essential for the success of the treatment. In the USA, the medicine costs $425,000 per eye and was approved in 2017. [1021, 1022]

ZYNTEGLO GENE THERAPY

The transfusion-dependent beta thalassemia is a chronic disease, better known as Mediterranean anaemia, that in Italy alone affects more than 6,500 people. [1028] The beta thalassemia is a rare genetic disease of the blood, even if rather common in the Mediterranean area and in particular in Italy, where it is estimated that there are around three million healthy carriers. It is caused by a mutation in the gene that encodes the haemoglobin protein, forcing patients, in its more severe forms, to receive frequent blood transfusions and to manage the disease not only in terms of its physical consequences, but also in terms of mental health and social inclusion. *Zynteglo is* indicated for the treatment of patients aged 12 years and over with transfusion-dependent beta thalassaemia (they must have certain specific characteristics). [1024] It is administered intravenously: it consists of haematopoietic stem cells taken from the patient and genetically modified in the laboratory, using a viral vector (lentivirus) that provides the working beta-globin gene. [1025, 1026] The genetically modified stem cells are implanted into the patient's bone marrow. This therapy is also very expensive: over €1.5 million in Europe. [1027]

By 2020, there were over 900 possible gene therapy strategies registered in the USA: in less than twenty years, gene therapy-based treatments have experienced a significant growth. [1051]

The problem of the costs of medicines and therapies for rare diseases is more crucial than ever and has not yet found reasonable, ethically acceptable solutions. Moreover, it is really difficult

to predict long-term effects, for example, generated by the injection of viral vectors (modified viruses) into the body. Biotechnological techniques are probably still too untested to hope to employ them systematically to prevent genetic diseases in the human species.
Another aspect is also underestimated: we do not know all the positive effects of some genotypes that we consider negative. To understand what this is about we can take the well-known example of resistance to malaria conferred by sickle cell anaemia. It has been estimated that the mutation, which is present in at least 300,000 new births per year, originated about 7,300 years ago in Africa. After 250 generations it has spread due to the fact that it confers resistance to malaria.
Even the presence of a single gene carrying the mutation for cystic fibrosis, perhaps, generates an advantage such as an increased ability to defend oneself against tuberculosis. Unfortunately, we do not have sufficient knowledge to predict the long-term effects of irreversible changes, such as those in the germ line of humans. Evolution, with an unremitting work of over several billion years, has produced the human genome. We are very presumptuous if we believe that groups of human beings capable of manipulating our genome can make irreversible changes without generating unwanted and, most likely, negative consequences. The same principle applies to the irreversible changes in the genetics of the species with which we share the Planet.

The gene therapies currently on the market are very expensive, present risks that are still largely unknown, and can be used to treat diseases that affect very few people: between one newborn in every 20,000 and one in every million. Therefore, the possible beneficiaries of these technologies are few and must be rich. Other cheaper strategies, such as prenatal genetic analysis, carried out in the parents or in the embryo during the assisted reproduction procedure (IVF) or during pregnancy, can avoid suffering in an effective and sustainable way. Prevention costs less and can be used to assess many different risks, as is already partly the case (e.g. for the early diagnosis of trisomies in embryos). It follows that potentially risky and expensive gene therapy technologies are probably not necessary. There is already a number of examples of companies that have had to withdraw their therapies from the market, partly because they have not been able to make the expected profits. Simpler and safer solutions are certainly better also because they are cheaper.

ARTIFICIAL HUMAN SELECTION: PROGRAMMING ONE'S OWN GENETIC DESTINY

New scientific applications, such as those offered by genetic engineering, are becoming cheaper and more accessible. The result is an easier and more widespread possibility of experimentation and, at the same time, a greater number of useful applications, such as those in the field of health. At the same time, however, it is getting more difficult to control what goes on in the laboratories all over the World. Genetic engineering makes it possible to correct diseased cells by modifying genes that are repaired; or it is possible to replace the wrong gene with a healthy one, or it is possible to add a functioning one to the sick gene. It is possible to turn off negative genes or turn on positive instructions.

Human beings have been born all over the World for several years now thanks to technologies that make it possible to overcome the limits imposed by nature. Through investigations carried out during pregnancy it is possible to diagnose illnesses and prevent enormous social costs and suffering (amniocentesis, villocentesis, cordonecentesis, ultrasounds, examination of DNA in the maternal blood, examination of biochemical markers in the maternal blood such as the dosage of alpha feto-protein). [1088, 1089, 1090] Thanks to these techniques it has been possible to reduce the incidence of diseases such as Down's syndrome. Innovation always offers new horizons and molecular scalpels allow us to enter and modify the codes that govern life. Therefore, human beings unfit for procreation can be put in the artificial condition of obtaining healthy children. Some examples are given.

ASSISTED REPRODUCTION

For the first time, in 1978, it was possible to achieve procreation through laboratory techniques: artificial insemination (known as *in vitro* fertilization). There are now many couples who benefit from assisted reproduction to solve problems of infertility or possible hereditary diseases. In the World, at least eight million children have been born thanks to assisted reproduction (in vitro fertilization) in less than 30 years. With assisted fertilization it is possible to choose the sex of the embryo, therefore it is possible to avoid diseases associated with sex chromosomes such as the Y chromosome (it will generate a male). Gametes provided by a donor or a donor can be used to overcome other obstacles. It is possible to have a womb on loan, to carry out the pregnancy in a mother other than the one who provided the egg. Finally, unused embryos from artificial insemination can be stored (cryopreservation) and used for research purposes or future procreation programmes, even for years.
Assisted reproduction has been used successfully since thirty years ago to avoid sex-linked diseases. With this procedure it is possible to program the gender and avoid diseases associated with sex chromosomes, such as Duchenne muscular dystrophy which mainly affects males. In 1992, assisted fertilization was probably used successfully for the first time to select an embryo that did not have the possibility of developing a serious disease of which the parents were healthy carriers (they had already conceived a sick child): cystic fibrosis. [1091] Today, preimplantation genetic analysis can be used to avoid many diseases such as haemophilia, thalassemia, and Huntington's syndrome. Thus, these techniques are used to select artificially the embryos obtained in the laboratory.

CHILDREN FROM THREE PARENTS

Mitochondria are organelles in our cells that are important for many functions such as producing energy through respiration. These organelles possess their own extra chromosomal genome. The mitochondrial DNA contains few but very important genes, if they do not work they generate serious diseases or alterations incompatible with life (muscular problems, blindness, cardiac dysfunctions). Mitochondria are not contained in the spermatozoon, therefore they are inherited only from the mother. To avoid a hereditary genetic disease transmitted by the mother's mitochondria, it is sufficient to replace them with those of a healthy woman donor. Therefore the child will have the genes from three parents: the chromosomal ones from the two parents and the mitochondrial ones from a donor. These children are therefore obtained with assisted fertilization thanks to the genetic collaboration of three individuals: two women and a man (the father, however, does not influence the mitochondrial inheritance). Mitochondrial transplantation challenges the traditional concept of a family with two parents; in this case two mothers and a father. In the UK, the practice of mitochondria replacement was licensed, to prevent diseases, in 2015 (it was probably first successfully applied in the laboratory in 1997). [1029, 1091] Embryos obtained by this technique can be frozen while waiting for the nation where they were produced to authorize their implantation and thus birth, or one can move to countries where it is allowed. [1091]

HUMANS GENETICALLY MODIFIED WITH THE *CRISPR* SYSTEM

Cloning and permanent germ line modification of humans have been technically possible for several years. The famous sheep (Dolly) was cloned in 1996 and the first monkeys were cloned at least 20 years ago. [1029] Biotechnology makes it possible to transgress natural laws (or divine laws) by allowing the artificial selection of genetic characteristics such as sex, but also many genotypes regulated by single genes (and the resulting phenotypes). The ways to achieve eugenics are becoming more and more numerous, cheap, accessible and, therefore, fast and uncontrollable. The artificial modification of the human germ line should be banned but this option is a utopia.

The unnatural selection of genetic characteristics can easily slip into dangerous deviations, but it will be impossible to prohibit the use of simple and cheap biotechnologies: they can still be used secretly. Just as tax havens and sex tourism exist, we will soon discover that eugenic clinics are widespread. Medical tourism for assisted reproduction is already a reality, as it is restrictively regulated in many states, so those who can afford it emigrate to more liberal shores.

One principle might be to favour the strategy of assisted fertilization associated with prenatal (pre-implantation) genetic diagnoses, aimed at discarding embryos carrying known diseases in the family. However, in many countries even pre-implantation genetic diagnoses have been banned (in some cases there are some exceptions: it is allowed if it is aimed at avoiding certain diseases such as those associated with the X chromosome). [1029] Assisted reproduction and pre-implantation genetic diagnoses do, however, allow eugenics to be practised: it is possible to discard, from among the available embryos, those with the worst genetic characteristics.

In 2018, a group of Chinese researchers published an astounding result that at the same time raised new ethical questions. A taboo has been broken: genetically modified twins have been born. These are two human embryos obtained through assisted fertilization and modified with the *CRISPR* technique.[I] With this publication we can claim to have entered the era of molecular eugenics. Permanent modification of the germ line should be banned. It is to be hoped that luck has assisted the two newborns (and the researchers who created them) since the biotechnological procedure is not without uncertainties and, therefore, possible errors (e.g. mosaicism, undesirable DNA damage). In Chinese laboratories, using the *CRISPR* system, two copies of a gene (CCR5) have been modified in such a way as to hope to make two female embryos resistant to HIV infection. The twins were obtained by the technique of in vitro fertilization, from an HIV positive father and an HIV negative mother. The zygote was artificially genetically modified, creating a condition that exists naturally in a fraction of the population and confers resistance to AIDS (the deletion of 32 nucleotides in the CCR5 gene occurs naturally in at least 100 million people). [1059, 1060] The twins are different and if things went as hoped they should have acquired the ability to resist HIV even in the germ cell line (one is homozygous and the other is heterozygous for the desired deletion in the CCR5 gene and both probably have mosaicisms; so in one of the twins a copy of the gene does not have the deletion). [1051] To confirm that the genetic engineering procedure has worked it would be necessary for the twins to meet the AIDS virus and it would be necessary to have a confirmation of the absence of undesirable problems (e.g. damage in the genome that can be ascertained by sequencing or over time). Observing the health conditions, it could be discovered that the genetic manipulation (the double artificial mutation of the CCR5 gene) has altered functions considered completely disconnected, such as cognitive functions or life

[I] The top Chinese researcher in this ethically highly questionable application was sentenced to three years in prison. [1052, 1061]

expectancy, both positively and negatively. Basically, they would have to continue to participate in a lifespan experiment.
If everything worked as hoped by the Chinese researchers, the two girls will one day be able to transfer this trait to their children. These are the first widely publicized genetically modified human beings. We must remember that AIDS has infected at least 37 million people in the World and that fortunately the cure has made enormous progress, so it is useless to run these risks.
Even if bans are promoted, it will be difficult to stop the application of genetic modifications in human reproductive cells, i.e. the generation of permanent alterations. In the future, human genetic modification experiments may hold many surprises in store.

WILL BIOTECHNOLOGY HELP US OR MAKE THINGS WORSE?

New biotechnology makes it possible to play with the lottery of natural reproduction, guiding it to achieve specific goals. Researchers have succeeded in cloning numerous animals, from sheep to bulls to monkeys. Theoretically, genetic engineering technologies, for the first time, are able to provide the hope of extinguishing hereditary genetic diseases from humanity (and beyond). It would be enough to intervene on all carriers of a genetic disease that can reproduce. As it has been for some infections eradicated by mass vaccination, genetic engineering, in a near future, could help us to reduce the incidence of some hereditary diseases but it is necessary to abandon, in this case, natural reproduction.
The diseases that are the first candidates for gene therapy or genetic engineering, with the aim of eliminating the disease or its heredity, are monogenic diseases. These are the diseases generated by a single gene whose effects are well studied (there are several thousands of them). Among the genes that will probably be corrected, there are those of cystic fibrosis and sickle cell anemia; many diseases (e.g. Alzheimer's disease, heart diseases) and characteristics (e.g. body height, intelligence) are the result of the interaction of several genes, so modifying these hereditary traits is difficult or, for now, impossible.
For the first time in the history of mankind, it seems that it will soon be possible to control our genetic destiny. This prospect is probably exaggerated and futuristic, but many applications have already entered clinics and laboratories around the World. However, the risk of self-directed genetic evolution of the human species is just around the corner, and that of domesticated living beings has been practiced for millennia. Modern biotechnology makes it possible to accelerate artificial selection with mechanisms that did not exist before, since it has become possible to transfer genes between living beings belonging to different species but also to different kingdoms, and it is possible to construct synthetic genes and genetically modified organisms, i.e. those that do not exist in nature. It becomes possible to think of designing superhumans in the laboratory, an *elite* of people who are healthier or even have enhanced characteristics. We can see these new possibilities as an opportunity but also as a deliberate attack on the heredity of humankind. The modification of the human germ line is already a reality in many applications and allows a leap forward in eugenics, that is, the artificial selection of human beings. The ethical, social, and ecological implications are immense. It is a question of reprogramming the evolutionary machine consciously and with risks that are largely unknown or very frightening.

Even scientific research, that which is destined to remain in laboratories, in some cases raises important questions. For example, in many countries of the World it is possible to manipulate human embryos up to the age of fourteen days. Two weeks is the moral dividing line between cells and human beings. The newly fertilized egg cell forms the zygote, which reproduces during the first few days, generating cells that can give rise to any other cell. At this stage it is possible, for example, to artificially unite cells between different embryos and obtain chimeras, i.e. individuals obtained from different zygotes. Even if the research is carried out on embryos rejected by the assisted reproduction procedure and will never be implanted into the uterus to originate an individual, we might not agree on when an embryo acquires the rights of being human, or until when it can be considered a set of cells to be freely manipulated in the laboratory.

The current economic and financial rules are liberal, if there is a potential buyer surely somewhere a seller will appear. The economics of capitalism is also that of liberalism, if something can be done it must be done. In this kind of context hoping to stop the manipulation of genetic characteristics, even of human beings, is really difficult. To avoid intervening artificially on heredity and, therefore, on the evolution of human beings becomes unrealistic. Trying to respect limits, restrictions between what can be allowed and what is absolutely to be avoided is not easy either. Defining a threshold of universal moral and social acceptability for genetic manipulation, whether of humans or of other living beings, is difficult; perceptions of risk and material benefit vary widely as they are influenced by many factors: culture, religion, social taboos, social status, personal interests, knowledge of the subject, state of health, etc.

With biotechnology, mankind has acquired an unprecedented ability to control the evolution of its own and other species. The scope of possible applications and the power are immense and unimaginable just a few decades ago. The design and creation of genetically modified human beings is becoming increasingly simple and inexpensive. It is likely that sooner than we imagine it will be possible to insert artificial characteristics into the human genome that no child can naturally inherit. Technology is progressing much faster than the society's ability to make decisions about it. History confirms that humanity as a whole has never excelled in generosity, altruism, cooperation, harmony, and respect for nature. Who can control and slow down the speed of scientific progress: society or science itself? What is the limit between life-saving therapeutic treatment and enhancement or aesthetic treatment? How to control and limit possible eugenic applications?

TECHNOLOGIES ARE NOT INFALLIBLE

The use of molecular tools to modify embryos can generate several problems, both ethical and medical. From the technological point of view, any innovation always has weak points. The *CRISPR* system, to give an example, could:

- It also acts at sites other than the target site. So, in addition to getting the desired result there will be side effects that can be very serious. The *CRISPR* system built to correct the mutation that causes beta-thalassemia, could generate mutations in other parts of the DNA causing new diseases or problems. Molecular scalpels could mistakenly deactivate some onco-suppressor genes, promoting the appearance of cancers.
- Make unwanted errors at the target site.
- Acting only on a fraction of the target cells, generating the phenomenon of mosaicism. [1046] The modification of somatic cells (e.g. with the *CRISPR* system) practiced in an adult change the DNA in a hundred million cells or more. In each of these cells there may be different undesirable events with the result that it is easier to obtain unacceptable errors (e.g. new tumours). In some cells the modification may work but in others it may not (in some cases it may be sufficient to modify 20% of the cells such as T lymphocytes). [1051] To reduce this risk we can operate on stem cells (e.g. blood cells in test tubes) or on gametes or on the zygote (newly fertilized egg). Unfortunately, among the failures recorded by the clinical applications of the gene therapy, such as the administration of modified viruses to cure tumours, there is the

onset of new tumours: some failures confirm the existence of considerable unpreventable risks. [1051]

To have a higher probability of success the *CRISPR* system should be used on monogenic diseases, trying to inactivate the diseased gene (inactivating is easier than correcting). It should also be sufficient to inactivate the diseased gene on a small number of target cells, so that the change in phenotype from diseased to healthy may be more easily recorded. The system should be very specific, so as to reduce the likelihood of changes being generated at sites other than the target site.

Any technique can fail, making unwanted mistakes whose effects may not be easily detectable and predictable. Some problems may manifest themselves years later or even in subsequent generations. To give an example, in the USA, at least 13 million cattle a year have their horns removed and it is possible to generate cattle genetically modified to be born without this anatomical part, but it has been discovered that the biotechnological operation has alarming consequences: in the DNA, the GMO cattle have retained undesirable bacterial genetic sequences. [1082, 1083] Someone could object that there is always a side effect that can be evaluated and tolerated, as in the case of the use of medicines or in the case of therapies and treatments such as surgery.

EDITING MICROORGANISMS

The use of microorganisms in the laboratory and their deliberate modification and manipulation presents many risks. One of the first official acknowledgements of the dangers, by the international scientific community, dates back to 1974: the Asilomar Convention (in California). Since then, scientific innovation has revolutionized the ability to manipulate and engineer living beings in a way that was by no means foreseeable. The concerns raised more than 40 years ago are now greatly enhanced by much more effective and cheaper tools.

The laboratory constitutes an artificial ecosystem where different microorganisms bred and modified by scientists can encounter each other and wild (natural) ones. They can also accidentally contaminate operators or other living beings in the laboratory. Within the bodies of animals or plants, these microbes can modify themselves and exchange genetic information. The laboratory is a potential source of sometimes unpredictable hazards, such as spontaneous reorganization of microbes into other forms that may be more dangerous. In addition, there is always the danger that these microscopic beings may escape from the laboratory. Therefore, laboratories are potentially very risky artificial ecosystems. The situation is even more serious when modified microbes are deliberately injected into animals (e.g. insects) and plants. In some cases, checking that nothing leaves the laboratory may be much more difficult. In some cases, microbes are experimented directly in humans (e.g. voluntary terminal patients). We are conducting a dangerous experiment: we are gambling with living beings such as microbes. We have entered into the management of the genetic lottery by invading the intimacy of nature, disrupting it out of apparent necessity (e.g. to cure currently incurable diseases), but also out of whims that violate the dignity of living beings and the sacredness of the very essence of life.

DESIGNING HUMAN EMBRYOS: WILL THE DREAM OF THE PERFECT CHILD COME TRUE?

Another important aspect is that of the legitimacy of carrying out experiments on human embryos obtained from assisted reproduction and not intended for procreation. These embryos are destroyed after a few days of development in test tubes. It is possible to modify human embryos and clone them for the purpose of scientific research. In many countries of the World the implantation of manipulated or cloned human embryos in utero is expressly forbidden (e.g. Italy). Other countries have open policies or few restrictions so these technologies, which are increasingly cheap and accessible, could easily be used: either in the open or secretly.

Humanity should adopt the principle of not modifying the human germ line. Respect for this principle would be difficult to ensure even if all the countries of the World were to adopt appropriate regulations and measures, since technologies are becoming increasingly cheap. It is like prohibiting the copying of music files in a society where practically anyone can easily dispose of the appropriate tools to do so. We should therefore not be asking if but when manipulations will be carried out on human embryos. We should probably ask ourselves a subtler question: for what purposes of modifying the human genetic make-up must we set limits? In the case of serious diseases, many people are in no doubt, first and foremost the patients and their families. For less serious ones, such as albinism and some types of deafness, what should we do? For aesthetic characteristics or those that could enhance natural gifts such as athletic, mathematical, musical or memory, are rules necessary? Probably one day we can also hope to act on genes that influence behaviour (e.g. altruism) and intelligence. It is possible to imagine that in the future it will be possible to enhance human capacities (intelligence, strength, life expectancy, reduced need for sleep, immunity and resistance to certain diseases) or it will be possible to implement the artificial selection of aesthetic characteristics (skin colour, eye colour). Certainly some applications are ethically less tolerable, such as the enhancement of certain behaviours, but they are also the most difficult because these are traits influenced by many genes and complex mechanisms that involve a great influence from the environment. It is difficult to artificially manipulate traits defined by multiple factors, partly hereditary and partly resulting from environmental factors: diet, education, lifestyles, experiences, trauma, disease, social relationships, climate, etc. Many traits are regulated by the interactions of many genes with environmental factors (e.g. intelligence, height), so at present it seems difficult to hope to be able to modify them at will with simple genetic engineering techniques.

One of the worst nightmares could be that of the intervention of Governments in the programming of procreations. Unfortunately, the history of mankind has already experienced such dark moments (e.g. compulsory sterilization in the USA until the '70s). We must be able to avoid a repetition. Abandoning the lottery of evolution for planned and intentional modifications will surely have disastrous consequences. Imagining the design of a desirable society by trying to prevent or eliminate undesirables is foolish and frightening. These strategies increase discrimination, marginalization, inequalities, and may reduce genetic diversity and freedom in reproductive choices. Paradoxically, the reduction in biodiversity, potentially caused by the design of ideal and perfect offspring, decreases the ability to resist change and may increase the risks of diseases including genetic ones. These aspects should not be underestimated.

The Peto paradox is the observation that at the species level the incidence of cancer does not appear to be related to the number of cells in an organism. [1086] For example, the incidence of cancer in humans is much higher than the incidence of cancer in whales or elephants, despite the fact that they have many more cells than a human (assuming it is easy to study the incidence of cancer in animals that, by the way, will soon be exterminated). If the probability of cellular carcinogenesis were constant one would expect whales to have a higher incidence of cancer than humans. Cancer risk and body size appear to be positively correlated within members of the same species, even when other risk factors are controlled for. Animals such as elephants have been found to contain many copies of oncosuppressor genes, similar to those found in humans. It is probably possible to hope to achieve a lower probability of having tumours by artificially enhancing the action of these genes that are naturally present in our bodies.

There are already clinics that carry out assisted reproduction and offer special services: parents can choose the colour of their eyes and other characteristics if they wish. [992] In the future, it may become feasible to enhance abilities such as athletic (muscular) or other skills. Perhaps these more ethically questionable applications, such as those used for aesthetic enhancement or the enhancement of natural gifts, will be the most sought after. The reason is simple. Today, assisted fertilization techniques accompanied by genetic analysis make it possible to avoid the birth of sick children and, at the same time, to select healthy embryos among other embryos. Therefore, engineering a human embryo to avoid a hereditary disease is much more dangerous and uncertain than applying assisted fertilization techniques and pre-implantation genetic analysis. Especially for monogenic diseases, preimplantation analysis is certainly a simpler and less risky way: genetic analysis is carried out in a few cells taken from the embryo a few days after fertilization (at the fifth day it may consist of less than 300 cells). This strategy is simpler than engineering human embryos and, for many applications, is proven by years of success. However, it has disadvantages: it is expensive, it is invasive (for the woman) and 100% success is not guaranteed.

The selection of aesthetic aspects or characteristics such as those of muscle tissue can be carried out by modifying embryos. For the first time, the means exist to ask the question: who has the right to choose who is fit to live and who is not? Who draws the line between artificial genetic improvement to avoid suffering and other choices?

We should probably give some thought to an absolute ban on the voluntary modification of human germ lines, but allow the use of biotechnology for health reasons in cell lines that cannot be inherited by the next generation.

There are companies that, after having sequenced the genetic characteristics of the potential parents (for example by taking saliva samples), are able to establish the probability of having genetic diseases or the predisposition to multifactorial diseases (in reality some predictions may be useful and feasible even after birth). [1087] It is also possible to choose between different donors (e.g. of sperm) the one most suitable to the expectations of a perfect child: healthy, beautiful, tall, intelligent and strong. [1084, 1085] Custom-made children are designed using special algorithms that apply mathematical statistical models. Theoretically, by choosing the donor or donors, it is possible to select many characteristics and try to avoid over 1,000 genetic diseases whose genotype is known: for some years now, eugenic drifts have been open. In this perspective, procreation is no longer natural because it is too dangerous and uncertain. Pre-fertilization tests allow us to select certain desired genetic characteristics and discard others.

Applications that favour eugenics and all the worst consequences we can imagine should be banned. In the past it has been possible to restrict applications of biotechnology with considerable ethical implications. Stem cells are a potential tool for curing many diseases, but some promising strategies involve sacrificing human embryos. Fortunately, this strategy, where it requires human embryos, has been banned. [1091]

This eugenic perspective widens the gap between different social classes. Not everyone can afford pre-implantation tests or will be able to afford assistance in modifying embryos. It will be difficult to make decisions that accommodate different religious and cultural sensibilities, but one thing is certain: these technologies will increase inequality. In a futuristic

perspective, one could imagine a society formed by genetically predetermined castes, a bit like what happens naturally among insects. Among ants, differences in body size and structure can be considerable, not least because they are destined to perform completely different functions. Modern biotechnology only exacerbates the burning issues that humanity has always faced without a solution.
We have desecrated the temple of life and we are entering the Age of Biocapitalism which will increase the differences. Cheapness and greater simplicity make it possible to accelerate scientific research: these are advantages. From another point of view, it is very alarming that technological innovation reduces costs and makes the tools for genetic engineering increasingly simple to use. You do not need super laboratories to permanently modify an animal or a plant. [1029] An example: for a few hundred euros it is possible to buy a *CRISPR/Cas9* system that can be used with simple equipment to modify the genome in living beings, including humans. [1012, 1044, 1045, 1051] In a way we can say that innovation in this field makes science more accessible and democratic. As a result, it is becoming increasingly difficult or, in some cases, impossible to prevent abuses or dangerous situations, such as the release into the environment of organisms created in the laboratory. Among the unnecessary and dangerous applications that could result, there is what is known as *gene-art* or genetic engineering in the service of something that is mistakenly called art (e.g. luminescent plants and animals, such as rabbits). A whim that could cost dearly.

ASTONISHING AND FRIGHTENING APPLICATIONS: IT IS NEITHER SUSTAINABLE NOR REASONABLE TO MAKE EVERYTHING WE CAN

Biotechnology makes it possible to achieve results that until only two decades ago belonged to science fiction. Some applications that are astounding are summarized below. [989]

- Some proteins are sensitive to light (at certain wavelengths; this is called optogenetics). This ability can be exploited to turn on or off nerve circuits at will, using light. With this technique it has been possible to artificially implant and activate negative memories in the minds of laboratory animals. When the light is switched on, the rodents behave as if they had perceived the negative experience, so they become alarmed following the activation, with the light, of an artificial memory implanted in their brain.

- It was possible to artificially create the minimal cell. Bacteria naturally have a few thousand genes. Starting from a bacterium of the genus *Mycoplasma*, all the genes considered non-essential to the life of a prokaryotic cell have been deactivated or eliminated, thus obtaining what has been called the minimal cell (less than 500 genes). A miniaturized cell was designed, capable of ensuring the minimum viability useful for scientific applications. The possible future evolution is to design artificial genomes, completely new. It has become possible to create new organisms by redesigning much of the information necessary for life: with microorganisms this has been achieved.

- Many products marketed to produce yoghurt and cheese, which are derived from cultures of microorganisms, have been modified by making them artificially resistant to viruses thanks to *CRISPR* bio-technology. [1051] In a few years, this technology has invaded the laboratories of the dairy industry and products fermented by microbes. Thus, through fermented products, the *CRISPR* system has also entered our food.

- Probably hundreds of thousands of people around the World are on waiting lists to receive an organ or tissue from donors that could change their lives for the better. There are clinics that transplant corneas or heart valves from pigs. [1051] The possibility of using animal tissue is becoming a reality.

- Salmon that have been genetically modified to produce the growth hormone are famous (they contain an extra gene controlled by a promoter from another fish species). The advantage for the breeders is the higher growth rate: almost the double; they eat more and grow faster. [1033] Breeding these finalized animals for human food has been allowed in North America. We must hope that the containment measures, to prevent these animals from swimming freely in the oceans (mating with wild Atlantic salmon), will work and that never, errors or accidents or unforeseen events will cause the distribution of the specimens in the seas. These containment measures are a utopia.

- By generating a change in a single nucleotide of DNA in the gene that controls muscle formation it has been possible to have a much more muscular dog (CRISPR). [1029] Using the same principle, cattle, pigs, sheep, rabbits, and goats were generated with a much stronger muscle tissue (to generate very obvious changes in the increase of muscle tissue of some animals it was sufficient to intervene on a single gene, that of myostatin, using the *CRISPR* system).

The athletic skills of a human being are regulated by many different genes, at least two hundred. The mutation that deactivates one gene (the myostatin gene) has been found to occur naturally (but exceptionally) in some humans who are characterized by having an uncommon strength. [1029] In addition, the myostatin gene is implicated in diseases such as dystrophy. Therefore, this gene may soon become the target of biotechnological therapeutic methods. Other genes that could be the target for enhancement strategies are the ones that generate stronger bones, the ones that reduce sleep requirements or the ones for erythropoietin[I] (illegally used by some athletes to increase red blood cells and thus oxygenation capacity). [1029] It might be useful to draw a line between life-saving modifications and others, such as those for aesthetic purposes or to enhance sporting ability. Unfortunately, it will not be possible to exclude health tourism to clinics located in paradises for geneticists that will allow freedom of action to eugenicists. Controlling one's genetic destiny may be an opportunity but also a terrifying reality. The World could in the future be divided into new categories of castes, those resulting from the enhancement and care offered by biotechnology and the others, generating not a few problems. [1050]

If one day we have a biotechnology system, with side effects acceptable to humans, how can we prevent someone from trying to enhance their children or even themselves?

- Biotechnology is making it possible to obtain goats with longer hair (wool) and more muscles (more meat), goats that produce spider silk in their milk, cows without horns (in the US alone at least 13 million cattle have their horns removed every year), plants that are resistant to herbicides, etc. A medicine has been approved (ATryn) that contains *antithrombin alpha*, an

---

[I] Erythropoietin (Epo) is a hormone synthesized by the kidney and, to a lesser extent, by the liver and the brain, whose main function is to regulate the production of red blood cells. Its discovery in the late 1970s was followed by the development, through biotechnology, of various types of recombinant human erythropoietin (rEpo), which are used to treat various diseases. Recombinant erythropoietin, because of its ability to improve tissue oxygenation (such as muscle tissue), is misused by athletes, especially in endurance sports. Athletes competing over long distances in athletics or endurance sports, such as cycling and cross-country skiing, may illegally use a variety of methods to increase tissue oxygenation in order to improve athletic performance. These include autotransfusion and the subcutaneous or intravenous administration of recombinant erythropoietin. [1047, 1048] anticoagulant naturally present in the human body. [1082] In cases where blood clots form spontaneously, the body eliminates them thanks to *antithrombin alpha* before they become dangerous. However, some people lack the gene responsible for creating *antithrombin alpha* and are therefore susceptible to thrombosis, which can pose serious risks during surgery or childbirth. The European Union and the United States of America have authorized the use of this medicine produced using transgenic technology: it is derived from transgenic goats that produce it in their milk and will be used to treat people who lack the gene needed to eliminate blood clots. [1034, 1035]

- A medicine is produced in the milk of genetically modified rabbits: it is an esterase inhibitor. [1036] It is used to treat angioedema, a rare autosomal dominant hereditary disease characterised by swelling (oedema) of the skin, mucous membranes, and internal organs, which can sometimes be fatal. [1037]

- Another medicine is extracted from the eggs of transgenic hens: they contain a protein that functions as an enzyme in humans. [1039] This protein (a medicine) is used to treat a rare hereditary disease that impairs the ability to metabolize fats (due to a deficiency of enzymes called lipases). [1038]

- A few dozen medicines (e.g. insulin and the human growth hormone) are produced by genetically modified organisms, mainly by microorganisms (bacteria).

- There are at least 30,000 strains of mice created with genetic characteristics such as to meet scientific objectives, such as the study of cancer or respiratory diseases. The possible applications are now numerous, and while some are for noble purposes, others raise many concerns.

- Even plants are modified to produce useful medicines for humans, for example by introducing foreign genes using bacteria (*Agrobacterium*), viruses (e.g. tobacco mosaic virus) or with mechanical systems (micro bullets of tungsten or gold coated with the genetic material to be transferred). From plants it is hoped to obtain vaccines (e.g. against hepatitis B from tobacco plants) or medicines against cancer (also from tobacco plants). [1040]

- Edible mushrooms have been created that maintain themselves longer by deactivating the gene responsible for blackening. This modification does not involve the insertion of foreign genetic characteristics but the deactivation of a naturally occurring gene.

- Another example is that of maize, which contains starch composed only of amylopectin: the gene that produces the other component of starch, amylose, has been deactivated. [989]

We should ask ourselves how far we can go. [1041, 1042, 1043] Intervening in the evolutionary journey, both of our own species and of many others, means gambling with nature. Is it permissible to create and breed an artificial zoo that is the result of tens of thousands of different experiments? All that can be done does not need to be done.

It is worth reflecting on the desirability of commercializing genetically modified pets. Micro-pigs, a little bigger than a cat, have been generated with the idea of marketing them as pets (using a biotechnology called TALENs or *transcription activator-like effector nucleases*, the growth hormone has been deactivated). [1030, 1031] Several luminescent fish have been created and are called *GloFish*: *Danio rerio*, *Gymnocorymbus ternetzi*, *Puntigrus tetrazona*, *Epalzeorhynchos frenatus, Brycinus longipinnis, Betta splendens, Gyrinocheilus aymonieri*. [1009, 1010, 1011] The fish appear bright under normal white aquarium light and fluorescent under blue light. These are genetically modified fish with the aim of being marketed for the aquarium market. They can be superficially referred to as "ornamental fish". The genes responsible for fluorescence may have different origins: from the jellyfish *Aequorea victoria*; from the colonial polyp *Renilla reniformis*; from the corals *Discosoma*, *Montipora efflorescens*, *Scleractinia* spp., *Pectiniidae* spp., *Lobophyllia hemprichii*, and *Dendronephthya* spp.; from the anemone *Anemonia sulcata.* In 2003 Taiwan became the first state in the World to allow the sale of a genetically modified organism as a pet. Over 100,000 *GloFish* would be sold, in less than a month, for a few dollars each. [1011] In this case, it is an unnecessary and dangerous experiment. No one can foresee the disasters generated by these whims that show superficiality and ecological illiteracy. Just as natural selection does not carry through all possible variations in the evolutionary process, we should learn to limit our ability to gamble with evolution by avoiding artificial selection, at least for those cases in which it does not meet the requirement of being useful and necessary to the entire collectivity, such as that of the *GloFish*.

We must hope that the thousands of artificial organisms, all fruit of the human talent, remain confined to farms forever. It is difficult for this to happen for animals (think bacteria or insects) and much more difficult in the case of plants: how to confine pollen? How to control the spread of transgenic animals marketed as pets or for aquariums? Even more unlikely is the certainty that the micro-organisms bred, kept and manipulated do not leave research centres. Bacteria can exchange information, even between those we have classified as different species, and those bred in the lab may encounter those in the bodies of researchers and other living beings. Viruses can also carry genetic information in prokaryotic cells, such as those of bacteria, and eukaryotic cells, such as those of animals and plants, or exchange it with each other. Therefore, confining and managing the invisible (viruses can be a hundred thousandths of a millimetre in size) is difficult if not impossible. The laboratory ecosystem is completely artificial and it is presumptuous and superficial to believe that we have the situation under control.

Biotechnology continually offers new opportunities that are cheaper and easier to use. [1045] At the same time, they make it possible to design strategies that are considered as dangerous as nuclear and chemical weapons, such as possible applications in agro-terrorism or those aimed at damaging ecosystems and biodiversity. Biotechnology therefore has two faces, the positive and the negative one. [1153] Unfortunately, it has become realistically possible to create microorganisms or other organisms that are harmful to humans and/or other living beings. Military agencies classify some of the methods offered by biotechnology as other weapons of mass destruction. [1029, 1051]

Over the centuries, the use of infectious diseases to defeat one's enemies was a widely used practice among many peoples. During the Middle Ages, for example, the corpses of plague victims were catapulted inside enemy walls, the Americans used blankets infected with smallpox to distribute them to the redskins, while in New Zealand prostitutes infected with syphilis were sent to harm the Maori. The Hittite people used as a "bacteriological" weapon sheep infected with tularemia, a serious infection caused by the bacterium *Francisella tularensis*, which if not treated in time can cause death, even today. This terrible disease was first recorded in the Middle East in 1335 b.C. in a city sacked by the Hittites. The Hittites also unknowingly took some of the animals infected with the disease and infected the rest of the livestock, spreading the infection and creating the “Hittite plague”. The first bioterrorists in history may have been (unknowingly) the Hittites, over 3,300 years ago. [1053, 1054, 1055]

In the past, when microbiology was not yet a science, arrows were used whose tips were contaminated by soiling them in rotting corpses (probably in this way they spread microorganisms such as *Clostridium perfringens* and *Clostridium tetani*). More recently some

uses of biological weapons in warfare have been recorded, against animals and humans: the Japanese used them between 1932 and 1945. [1053]
Terrible diseases categorized as potentially effective in warfare include the microorganisms of smallpox, anthrax, plague, and tularemia. Unfortunately, it is also possible to think of strategies for the destruction of plants or farmed animals. There are probably many countries that conduct research and programmes for the offence, as well as for the defence against possible attacks with biological weapons. The production of weapons that are of biological origin is much cheaper than other types and the enemy can easily remain untraceable. [1152]

Another interesting but worrying aspect is that the genetic characteristics of tens of thousands of microorganisms are known: for example, the genetic sequences of thousands of influenza viruses are known. The genetic sequences, i.e. the hereditary characteristics that confer the pathogenicity and infectivity of an infinite number of pathogens, are known and public. This knowledge makes it possible to design and create microbes with the desired characteristics in the laboratory (e.g. microbes created through genetic engineering with hybrid characteristics of different pathogens such as the HIV virus and the malaria agent). Scientific innovation enables potentially beneficial applications but, at the same time, it also opens the door to scary projects that become easily feasible because they are inexpensive and involve accessible technologies. The harmful applications, or military applications, of biotechnology are easily imaginable and within the reach of many laboratories, so they are uncontrollable and usable without an obvious declaration of hostility. [1152, 1053] These are strategies that can be designed to create much damage among living beings anonymously, even by small groups of people. Innovation has resulted in a significant acceleration in the simplification of the biotechnology's use and affordability and, therefore, a significant increase in potential users. As a result, security and peace concerns have increased.

A biological weapon to be effectively usable against humans should have several characteristics:

- Be easily and quickly transmitted.
- Generate a high mortality rate and weaken the health care system.
- Generate panic and, consequently, the weakening of social security.
- Require expensive and complicated prevention and control systems.
- Leave no easily identifiable trace of its origin.
- Possess damage limitation systems for the fraction of the population that is not part of the target. An effective offending system must at least safeguard those who produce it, and to achieve this goal there are several possibilities such as vaccination, the use of antibodies or medicines (unless they are projects that involve the suicide of those responsible). Genetic engineering makes it possible to engineer pathogens that specifically target particular cells (e.g. those that contain a surface protein that allows the entry). Thus it may be possible to create biological systems directed towards genetic traits that are more prevalent in a particular geographic area or age.

It is interesting to note that some microorganisms such as the agents of smallpox, tuberculosis, plague, Spanish flu, polio, Ebola, anthrax bacterium, and coronavirus (Covid 19 also known as *SARS-CoV-2* or *Severe acute respiratory syndrome coronaviruses*) possess, to varying degrees, some of the characteristics just listed. [1053] In 2001, in the USA, the anthrax bacterium was used in terrorist attacks that killed five people and poisoned 17: anthrax spores were delivered to newspaper editorial offices and two senators in a series of packages.

The manipulation of microorganisms in the laboratory, even if carried out exclusively for useful purposes, such as the understanding and treatment of disease, carries considerable risks. The Asilomar Conference on Recombinant DNA took place in California in 1975. The main objective of the Conference was to address the biological risks posed by genetic manipulation. For the first time, risks were recognized and precautionary measures were suggested. The artificial ecosystem in laboratories can encourage the contact between different pathogenic microorganisms, between microorganisms and humans, between laboratory-bred animals and wild animals in the surrounding environment. Different microbes may naturally exchange information and generate other microbes that may be more dangerous. Human activities favour processes that occur naturally, but which are accelerated and more harmful due to overcrowding and the impossibility of maintaining physical and biological barriers (different viruses easily recombine, i.e. they can exchange genetic information between themselves and with the host cell, when they are simultaneously in the same organism). These conditions often occur also in many urban environments where overcrowding, insufficient hygiene, the degradation of natural environments, and the simultaneous presence of wild and farmed species contribute to the triggering and promotion of negative processes for human health and for other living beings. These processes are natural and predictable, and are facilitated by artificial conditions. Epidemics often originate as consequences of complex interactions between humans and other animal species, including pathogenic micro-organisms. Altered ecosystems can facilitate the emergence of new pathogens and the spread of epidemics. More than 330 dangerous infectious diseases have been recorded since 1940 and the trend seems to be increasing, also due to deforestation and various anthropogenic pressures. [1190] It must also be considered that the colonization and degradation of remote areas increase the opportunities for contact with wild animals that are potential disease vectors. [1276] In addition, we voluntarily and involuntarily transport living beings and their diseases everywhere on the Planet, promoting a speed of spread unprecedented in human history.

The effects generated by the reduction of biodiversity will be devastating but largely unknown in detail and speed. Probably some types of epidemics will spread more easily in a degraded, simplified, and artificial environment. [1276] Some infections may become more dangerous (e.g. *Hantavirus*). [1177] The increase in biodiversity reduces the possibility of spreading diseases between animals belonging to the same species. Even institutions, such as those of the European Economic Community, recognize the importance of biodiversity in reducing infectious diseases: [1182]

> "*... Nature is important not only for our physical and mental well-being, but also for our society's ability to cope with global changes, health threats and disasters. Nature is indispensable to us. ...To be healthy and resilient, a society must give nature the space it needs. The recent Covid-19 pandemic teaches us how urgent it is to take action to protect and restore nature: it is making us aware of the links between our health and the health of ecosystems, as well as demonstrating the need to adopt sustainable supply chains and modes of consumption that do not force the limits of the Planet. All of these aspects highlight that the risk of infectious disease outbreaks and spread increases as nature is destroyed. Therefore, to strengthen our resilience and prevent the emergence and spread of future diseases, it is essential to protect and restore biodiversity and well-functioning ecosystems.* "

GENETICALLY MODIFIED HONEYBEES

In the honey bee it will probably soon be possible to artificially induce particular behaviours useful to the beekeeper. It could be hoped to enhance the behaviour that some bees in nature naturally possess, namely that of cleaning each other by removing parasites such as mites or that of removing diseased larvae. Genetically modified bees could prove more resistant to fearsome pests such as the *Varroa* mite.

Molecular scalpels designed by nature to defend bacteria from viruses, their natural enemies, have been found to be able to be used in the laboratory to modify the genetic expression of many living beings. In 2013, the *CRISPR* system was used to study the function of genes by observing what happens by deactivating them in the fruit fly (*Drosophila melanogaster*) and the silkworm (the *Bombyx mori* moth) (these are probably the first published applications for arthropods). [1112, 1126] The *CRISPR* molecular scalpel has subsequently been used to selectively deactivate certain genes in other insects such as mosquitoes, bees, and butterflies, but has been found to be less efficient than in mammals. Deactivation occurs by generating deletions or insertions in the target gene sequences. Injecting the *CRISPR/Cas 9* system into freshly laid eggs of the queen bee was able to artificially achieve the blockage of gene expression (eggs laid less than 2 hours before). [1112] Micro-injecting the molecular scalpel early into the newly laid egg reduces the risk of mosaicism and increases efficiency. By inserting the appropriate guide (a laboratory-designed RNA) into the *CRISPR* system, it was possible to generate the block of genetic activity in both target alleles in *Apis mellifera* embryos*;* the shutdown of the desired genes (*knockout mutant*) was achieved with high efficiency. A fraction presented the phenomenon of mosaicism, i.e. only some parts of the embryo's body had deactivated the gene as desired; a chimera was generated: in some cells the target genes were deactivated and in others not. [1112] The mosaicism may be due to the delayed entry of the *CRISPR* system injected into the eggs. These results confirm that the *CRISPR* system allows the selective deactivation of genes and the creation of homozygous transgenic bees.

In bees (*Apis mellifera*) a series of genes that code for the production of proteins present in royal jelly are important for differentiating larvae into queen bees (they are proteins produced by the hypopharyngeal gland and it has been discovered that they are also present in the brain, so they certainly have other functions). [1116, 1117] By using the *CRISPR/Cas9* system, it was possible to deactivate (*knockout*) one of these genes (*major royal jelly protein 1*) and obtain queen bees that transmitted this genotypic characteristic to the progeny formed by drones, in which the absence of the production of this protein does not seem to generate problems (at least in the early stages of development). [1115] Also in this experiment, the biotechnological kit consisting of protein and RNA (the *CRISPR/Cas9* system) was injected into eggs laid within three hours and the phenomenon of mosaicism was recorded. The queen bee, under artificial conditions, is induced to lay unfertilized eggs that will give rise to males. The examination of the genotype of males confirmed the hereditary transmission of the artificially induced genetic change: deactivation of the production of a protein secreted by worker bees to form royal jelly (deactivation was achieved by deletions and insertions thanks to the action of the *CRISPR/Cas9* system). The artificial insemination between laboratory-derived males and unmodified queen bees, a technique that has been widely used in beekeeping for many years, also made it possible to obtain genetically modified worker bees (in heterozygosity). By repeating a second time the cross between queen bees (heterozygous) obtained from the first cross (between mutant male and wild queen), homozygous worker and queen bees are obtained. This makes it possible to fix the artificially induced mutation in such a way that it is also transmitted to the insects that normally produce this protein (worker bees at a certain stage of their life). With this technique it is possible to study the effects on the health and behaviour of the colony resulting from the shutdown of a single gene.

Bees have already been produced, again using the *CRISPR/Cas9* system, in which a gene that produces a protein found in the central nervous system (*mKast* or *middle-type Kenyon cell-preferential arrestin-related protein*) has been deactivated. [1118] By deactivating single genes, whose alteration does not generate obvious problems for reproduction, it is possible to hope to understand the functions carried out by a protein in the behaviour or sensory capacities of insects.

Transposons are defined as certain genetic elements present in the genomes of prokaryotes and eukaryotes, capable of moving from one position to another in the genome. In particular, in prokaryotes they can move to new positions on the same chromosome (because it is unique, or on plasmids), while in eukaryotes they can move to different positions on the same chromosome or on different chromosomes. Transposition may result in the functional inactivation of a gene if the transposon is inserted into the gene, or in the modification of gene expression levels if the transposon is inserted into the gene promoter. [1114] Specific functions such as those of new genes may be added in the transposon sequences. These pieces of DNA can also be used to carry genes and, therefore, new functions. In bacteria, transposons may carry antibiotic resistance genes. Transposons in eukaryotes are very similar in structure to those in prokaryotes. They possess genes that code for proteins necessary for transposition (cutting and insertion) and that allow them to excrete and reintegrate at various places in the genome. [1113] Since too much transposition activity can destroy the genome, many organisms have developed various mechanisms to be able to reduce transposition to acceptable levels.

Transposons could insert themselves by inhibiting the gene expression on one allele (heterozygosity) or on both (homozygosity). In the latter case the inhibition, e.g. in protein synthesis, will be greater (by using transposons it is possible to hope to achieve the complete deactivation of a gene).

In 2014, using the transposon technique, it was possible to create transgenic bees (DNA molecules, i.e. plasmids and transposons, are injected into the eggs). [1113] It is technically feasible to integrate stable sequences, i.e. so that they are heritable, with regulatory functions (DNA sequences with functions as promoters or inhibitors of specific genes). Thus it is possible to artificially manipulate the expression of genes to study their effects (phenotypes). In this research 25% of the embryos that underwent micro-injection (after being placed in queen bee cells) were accepted by the colony. The progeny of queen bees manifested the genetic modification generated thanks to the use of the transposon technique. Thus, heritability and acceptability by the colony is confirmed, despite the manipulations. In each queen bee the transposons integrated themselves at different points of different chromosomes. Another relevant aspect is that the desired results are obtained with a low efficiency so it is necessary to micro-inject the DNA molecules synthesized in the laboratory in a high number of embryos. [1113]

One of the worst enemies of beekeepers and bees is a small mite: the *Varroa*. By applying genetic engineering to a bacterium that lives in the digestive tract of honey bees, it has been possible (in the laboratory) to control the mite and a virus that it can transmit. [1133] The bee bacterium (*Snodgrassella alvi*) has been modified in such a way that it can kill the parasite. [1134] Using a technology inspired by a natural mechanism of protection of eukaryotes against viruses, it was possible to induce in the bacterium a modification lethal for the mite. In particular, double RNA helices promote the degradation of homologous messenger RNA sequences (e.g. viral ones). These molecular tools (double helix RNAs) can be artificially supplied to the insect by injection or by feeding. By engineering the appropriate ribonucleic acid sequence it has been possible to alter the expression of genes present in bees and counteract the virus that causes wing deformities. Administering this ribonucleic acid to bees is not easy and may have side effects (e.g. on non-target sites). To overcome this obstacle, a bacterium - that normally lives in symbiosis with bees (*Apis mellifera*), which host it in their intestines - has been genetically modified. The bacterium (*Snodgrassella alvi* wkB2), introduced into the digestive tract, multiplies and remains inside the bees' bodies. [1134] It can then produce ribonucleic acids that immunize the bees and are potentially transmitted within the colony without an outside intervention. This strategy has the potential to generate long-lasting immunity and to spread autonomously among bees. The nucleic acids responsible for the protective action are found in different parts of the insect's body, such as the haemolymph where the bacterium does not reside, so they are effectively disseminated. The ribonucleic acids produced in the genetically modified bacteria have the ability to inhibit (silence) the production of proteins essential to the life of the parasites: the mite or the virus. With this strategy it is possible to hope to inhibit artificially the expression of one or more genes, either in bees or in their parasites, by modifying a symbiotic bacterium. By acting on the microbiome of a living being, including humans, it is possible to hope to cure or improve the ability to fight certain diseases. This approach is fascinating and amazing, but at the same time it makes possible applications that could be harmful or that could hold surprises.

Researchers have new techniques to improve the knowledge of gene functions in the honey bee as well: individual genes and functions can be deactivated at will. One problem could be generated by the social behaviour of bees, as embryos obtained by this artificial procedure could be eliminated from the colony because they have many unnatural chemical and physical traces.

This research presents results that are preliminary and promising but, in the future, especially for social animals such as bees, there may be many obstacles that are unforeseen today. Genetically modified bees must be kept under conditions that ensure they cannot leave the laboratory. How is it possible to study the interactions between genes, environment, and behaviour without leaving the laboratory? This type of experimentation shows many unknown risks, and if the modified insects were to leave the laboratory, an ecological problem of unknown dimensions could be generated, but it must be avoided.

MOSQUITOES HAVE INFLUENCED THE FATE OF MANKIND

The more than 3,500 species of mosquitoes over the millennia have influenced the fate of humans and other species: they have been existing for 190 million years; one of the oldest fossil mosquitoes was found in amber in Canada and is about 105 million years old. One of the earliest historical records of a mosquito-borne disease was identified in Sumerian tablets dating back to 3200 b.C. (between the Tigris and Euphrates rivers). Traces of malaria have been found in skeletons dating back 9000 years in Turkey and 5200 years in Egypt (Herodotus reports that in Egypt people tried to relieve the symptoms of malaria with a urine bath). [1283]

Confirming how the fate of mosquitoes has been linked to human habits and vice versa, in 400 b.C. Japan imported the rice cultivation with malaria from China, and this disease helped to defend Rome from Hannibal around 200 b.C. The name malaria derives from "bad air", i.e. an infection from stagnant water. Galen, in the II century a.C., reported that in Rome to cure malaria (and not only) bloodletting was practiced and objects (e.g. amulets) with the magic inscription "abracadabra" were worn.

The female mosquitoes, through their stinging and sucking mouthparts, take a few milligrams of blood, injecting an anticoagulant: they prefer to sting individuals of blood group 0 and pregnant women; they are able to sting many times animals belonging to different species but there is no blood exchange, so they cannot transmit HIV. The males can feed on nectar, they mate several times and form swarms (up to 300 metres high) to attract females. The females always lay their eggs in the water, live a few weeks and, exceptionally, up to five months, and rarely move more than 400 m from the place where they were born (some mosquitoes, such as those of the genus *Aedes*, can lay their eggs in the mud and are able to resist desiccation for years).

Different species of mosquitoes are able to spread diseases such as malaria (5 different types caused by protozoa), Nile fever, Zika virus (it can also be transmitted sexually), yellow fever, filariasis (a worm that causes a disease called elephantiasis), and dengue (these insects spread diseases also in other animals, for example at least 29 forms of malaria are known in reptiles).

Most human diseases caused by organisms are zoonotic, i.e. they also affect animals: the common cold comes from horses; bird flu, herpes, and chicken pox come from chickens; cattle have transmitted smallpox, tuberculosis, and measles. Mosquitoes, because of the serious diseases they can transmit, may have been responsible for the deaths of nearly half of the humans who have lived on the Planet: over 52 billion people in the last 200,000 years. Even today, the 15 most important diseases transmitted by these insects to humans are responsible for over 800,000 deaths per year. [1283]
The yellow fever (caused by a virus) is the only disease to have had an effective vaccine for over 70 years, although it continues to cause up to 50,000 deaths a year, mainly in Africa. There are effective treatments for filariasis but, despite this, it causes suffering to at least 120 million people a year, mainly in Africa and South-East Asia. [1283] Dengue probably affects 400 million people every year.
At least 300 million people contract malaria every year through the bite of *Anopheles* mosquitoes. The severity of the disease depends on many factors such as the strain of malaria contracted; it is possible to be infected by several strains at the same time.
The five types of human malaria are transmitted by about 70 of the 480 known species of *Anopheles* (the protozoa nest in the human liver and attack red blood cells by feeding on hemoglobin; fever may exceed 41°C). *Plasmodium falciparum* is the most deadly parasite; *Plasmodium vivax* has a very low mortality rate, less than 5% in Africa and 2% in the rest of the World. Some parasites such as *Plamodium vivax* remain in the liver and can cause relapses after many years.
The *Plasmodium* mates in the mosquito, from the digestive tract it moves to the salivary glands and once it has entered our body it is able to induce the production of an odour that will attract female mosquitoes, so as to favour the continuation of the life cycle. When it is in the salivary glands of the mosquito the parasite induces it to bite more often by inhibiting the production of the anticoagulant: the quantity of blood collected with each bite will be less and, consequently, it will bite more times to obtain the quantity necessary for the development of the eggs (up to three times the body weight). *Plasmodium falciparum* can generate a mortality rate between 25% and 50%, and more than 75% of deaths are African children under 5 years of age. This disease interacts with others that are particularly widespread in Africa; for example, malaria increases the ease of developing HIV and the latter, by weakening the immune defences, makes the disease generated by *Plasmodium* more serious. These two diseases favour each other and are widespread in some of the poorest areas of the World, where the population earns less than one dollar a day. In Africa and Asia, the majority of ill people cannot afford to buy medicines.

A defense devised by natural selection against malaria was sickle cell anemia: children who inherit a single altered hemoglobin gene are 90% immune to *Plasmodium falciparum* (they are the heterozygotes). It is estimated that there are at least 60 million people who carry this genetic trait, which appeared as a counter to the long struggle for survival against mosquitoes in Africa. At least 10% of the World's population possesses genetic traits that confer resistance to the two most important types of malaria: *Plasmodium falciparum* and *Plasmodium vivax*. More than 95% of the inhabitants of some African areas are "Duffy negative", i.e. they possess proteins external to the red blood cells that do not allow the parasite to enter, so they do not contract the disease. 3% of the World population is carrier of the genetic characteristics of thalassemia that significantly reduce the probability to contract *Plasmodium vivax*.

One of the oldest natural remedies used in the fight against mosquitoes was the chrysanthemum plant: the dried flowers were used as insecticides, as they contain natural pyrethroids, already around 1000 b.C. in China and still today chimpanzees chew the leaves of a plant (*Vernonia amygdalina*), used in the traditional cooking of some African areas to treat malaria. [1283] Perhaps also for this reason, in countries where mosquito-borne diseases have historically been important, chrysanthemums are associated with funeral ceremonies. Another plant with notable anti-malarial properties, described in a 2200 year old Chinese medical text (entitled: *52 recipes*), is sweet wormwood (*Artemisia annua*). An important anti-malarial medicine was extracted from the cinchona tree (*Cinchona*) and used by the indigenous peoples in Peru.

The Agro Pontino, in Latium (Italy), has been infested with mosquitoes and malaria for millennia, and Julius Caesar and Napoleon Bonaparte had already imagined plans for land reclamation. Much later Benito Mussolini managed to implement the ambitious project, with considerable sacrifice, before the Second World War. In the 1920s, throughout the World, at least eight hundred million people were affected by malaria with a mortality rate of around 4 million per year. In the same years (1918-1919) the Spanish flu infected at least 500 million people, killing between 75 and 100 million, that is many more than those who died for the First World War: between 1914 and 1918, 65 million people fought, 10 million were killed and 25 million were wounded. [1283]

In 1923, malaria affected 18 million people in the former Soviet Union and killed 600,000, while flea-borne typhus affected 30 million people and caused 3 million deaths between 1920 and 1922.

In 1923 in Germany, an insecticide based on hydrocyanic acid was designed to be used against mosquitoes (Zyklon, which means cyclone in German, was synthesized by the Jewish chemist Haber who won the Nobel Prize but was forced to emigrate: unfortunately Zyklon is sadly known to have been used in the gas chambers of extermination camps such as Auschwitz). DDT was synthesized in 1874 but its properties as an insecticide were discovered in 1939 (Müller, the discoverer, won the Nobel Prize in 1948).

An interesting but crazy story involves the use of not particularly lethal strains of malaria to treat a severe form of syphilis in 1917. It actually worked because the increase in body temperature generated by the malaria killed the syphilis bacterium. In 1922 the treatment was carried out on several patients, for example in the USA.; in 1927 the creator of the therapy (Jauregg) won the Nobel Prize but soon after, following the discovery of antibiotics, the treatment strategy was no longer used (penicillin was identified by Alexander Fleming and this discovery earned him the Nobel Prize in 1945; in 1940 the antibiotic was produced in great quantities).

Returning to the reclamation of the Agro Pontino, carried out by order of Benito Mussolini, we must remember that it was successful in that the incidence of malaria was drastically reduced (it was the '30s). In 1944 this result was cancelled by a biological attack carried out by the Nazis who flooded the reclaimed fields and spread the mosquitoes infected by malaria. In a few weeks a job that had cost years of sacrifices was destroyed and the incidence of malaria increased rapidly: it was a real biological war directed against the Italians and the Allies. Mosquitoes were released in the Agro Pontino and at the same time the Germans confiscated most of the stocks of medicines (it was quinine) and confiscated the mosquito nets owned by the population in order to reduce the protection. The Germans flooded the countryside and installed mines, sabotaging Mussolini's reclamation work. Terrible suffering was recorded for at least the next three years. Malaria was used as a biological weapon with success but, predictably, the illness and death were also recorded among the Germans. During those years the Germans, in the concentration camps, conducted inhuman experiments on the prisoners to try to have more knowledge about malaria: in 1946 a German responsible of these terrible atrocities was condemned for *crimes against humanity* and was executed. The Americans also conducted cruel experiments on prisoners, using various microorganisms including malaria, and these atrocities continued until the 1960s. In Asia the Japanese experimented biological warfare on the Chinese: they killed at least 580,000 people by distributing flies carrying the agents of cholera and plague by air (in 2002 the Japanese confirmed their responsibility for this biological warfare). [1283]

Between 1947 and 1980 it was possible to fight malaria thanks to the massive use of DDT: its production increased almost 1,000 times and its use was widespread in agriculture. The systematic distribution of this insecticide in Italy, in 1948, contributed to zero deaths from malaria and Europe was considered free from this parasite in 1975. Similar results were recorded all over the World thanks to the use of DDT. In the long fight against mosquitoes, however, strains of insects resistant to both antimalarial medicines (e.g. artemisinin, quinine, chloroquine, mefloquine) and insecticides were rapidly recorded: the first mosquitoes resistant to DDT were identified in 1947 and confirmed in 1956 (mosquitoes can develop resistance on average after seven years of exposure to the insecticide but in some cases immune insects have been recorded after two years; also resistance to medicines, in some cases, has been recorded after just a few years of use). In the 1960s DDT-resistant strains were detected in most areas where these disease vectors were present. Still today DDT is used in some places on the Planet even though the mosquitoes carrying malaria have often become resistant (DDT is produced in some countries such as India and North Korea). It must be underlined that the mosquito has returned to Europe, for example in 1995 there was an epidemic of malaria that affected at least 90,000 people.

During the writing of these pages the news of the approval of the first vaccine against malaria to be used in children was spread. The World Health Organization has authorized a medicine that promises to save hundreds of thousands of children, mainly Africans (remember that vaccination eradicated smallpox, which during the twentieth century caused the death of at least 300 million people). [1284] The antimalarial vaccine is recommended to prevent the disease caused by *Plasmodium falciparum* and is administered in 4 doses starting at 5 months of age. This vaccine will probably not succeed in eradicating the disease but could reduce mortality by 30%. [1285, 1286] The vaccine has been tested in seven African countries but the results obtained were not as expected, in fact it was possible to prevent malaria in 56% of children between 7 and 17 months and only 31% of children between 6 and 12 weeks. Moreover, the effectiveness of the vaccine decreased after one year. On the basis of the trial results, however, it was concluded that, despite its limited effectiveness, the benefits outweighed the risks. [1286]

Unfortunately, many factors such as climate change, declining biodiversity, and the intensity of travel (over 4.3 billion people, in 2018, travelled by airplane) suggest that mosquito-borne diseases are set to spread. Today, technologies provide new opportunities, in some cases wonderful but at the same time frightening. Biotechnology (e.g. CRISPR) makes it possible to engineer in the laboratory and implement the extinction of species, including malaria-transmitting mosquitoes. This is a new potential weapon of systematic destruction, which among other things is cheap and within the reach of small laboratories, so it is difficult to control. The possibility offered by biotechnology to manipulate entire ecosystems raises distressing moral questions, it is biologically fallible and is certainly disturbing: the genie is out of the bottle and suffering from a delirium of omnipotence.

UNNATURAL SELECTION: THE GENETIC CONTROL OF INVASIVE SPECIES OR SPECIES CLASSIFIED AS HARMFUL

No one knows exactly how many species are alien to a given ecosystem because they were introduced accidentally: probably at least 18,000; for some invasive species the damage is very evident. At least 2,500 plant species and 3,200 insect species have been classified as invasive. [1133] Little information is available for some categories of organisms, such as the smaller ones (microorganisms).

Unfortunately, invasive species are not only a problem for agriculture or human health, but also for natural areas of particular importance and marine protected areas, which cover about 13% of the land surface and 7.7% of the sea surface. Invasive species diminish biodiversity. Some plants alter soil characteristics and encourage fires. Other organisms facilitate the spread of diseases or are pathogens or predators of native species. The effects may be rapid and devastating. For example, the introduction of cats (*Felis catus*) into new environments led to the extinction of at least 14 species of vertebrates, including mammals, birds, and reptiles, and the introduction of a parasitic fungus (*Batrachochytrium dendrobatidis*) caused the extinction of 90 species of amphibians. [1133] Defence against invasive species is a biosecurity issue of global importance, recognized as a priority by many nations (e.g. Australia and New Zealand). To counter the spread of alien species, different control strategies have been employed, such as chemical (e.g. pesticides, pheromones), physical (e.g. capture and/or killing) and biological ones (e.g. sterilization). Biological methodologies include those offered by biotechnology.

Biotechnology is making it possible to modify the genetic variability of living beings in an increasingly invasive and less controllable way. With biotechnology, the level of control over biodiversity is reaching unprecedented levels. Genetic engineering offers tools that can be used to engineer the extinction of a species. In this case, human ingenuity inspired by nature has the means at its disposal to modify the biosphere very quickly and irreversibly. Technological innovation reduces costs and simplifies laboratory procedures, making them accessible to ever larger groups. Thus controlling abuses and ethically questionable behaviour becomes difficult if not impossible. It becomes easier to irreversibly modify plants and animals, and some biotechnological innovations also raise legal issues, as innovation proceeds faster than the apparatus that sets the rules. For example, the inactivation of a gene naturally present in an organism, employing the *CRISPR* system, does not add extraneous information: these are living beings that have been genetically modified but are different from regulated ones (in 2015 at least 94% of soybeans and cotton, and 92% of all corn grown in the USA were classified as Genetically Modified Organisms). The distinction between natural and unnatural is becoming increasingly difficult. Artificial DNA modifications can generate significant changes because they are irreversible but sometimes invisible, i.e. not easily identifiable in the laboratory (no foreign genetic material is added).

Two examples of invasive species control are given as follows.

- The Mediterranean fruit fly (*Ceratitis capitata*) is a phytophagous insect. Its larvae develop inside the pulp of many fruits. It is one of the most economically important pests affecting summer fruit produced in the Mediterranean environment (it affects more than 250 species of agricultural interest). [1004] In Australia, the use of sterile males to eradicate this insect dates back to the 1980s: in 1984 it was successfully eradicated. [1006] This strategy may not always lead to the desired results. Despite the release of hundreds of millions of sterile males, the fly was not eradicated in California. [1005] The artificially bred insects carried a mutation capable of eliminating only female eggs, as they were not resistant to the incubation at high temperatures (a character linked to the Y chromosome).
- In Africa, the *tsetse* fly (*Glossina morsitans*) causes sleeping sickness and claims about 10,000 victims a year (it is a vector of the African trypanosomiasis). The use of the technique of releasing sterile males (obtained by exposing them to gamma rays) has successfully generated the eradication of this insect, in 1997, in the island of Unguja, in Zanzibar, an archipelago of Tanzania off the coast of East Africa. [1007] The ratio of sterile males released by aircraft to wild males was (in 1995) greater than 100:1 (more than 8.5 million sterile males were released). The time it took to allow six generations since the last release of sterile males was sufficient to reach the eradication of this harmful insect from the island. [1008] 18 years later the island was still free of this dangerous insect, confirming that in a confined environment this technique works. The interesting fact is that to be successful, the number of sterile insects to be freed must be enormous: 100 sterile insects for every wild animal. Thinking of extinguishing insects

distributed in larger areas and with much greater populations is impractical: it would be necessary to rear an excessive quantity of animals.

Science has made progress in the meantime, so that more efficient strategies can be adopted. The genome of some insects, such as the *Anopheles* mosquito, has been sequenced: in this case it has been possible to identify genes that are essential for the transmission of the malaria agent. It has been possible to create mosquitoes in the laboratory that are antimalarial, i.e. not capable of carrying the parasite. By distributing these mosquitoes in the environment (males because they do not sting), equipped with new genetic mechanisms conferred artificially, one can hope to propagate these characteristics useful to humanity to the entire population of wild insects. Up to 100% of the offspring of the males modified in the laboratory will carry the desired change (with sexual reproduction, genes usually have a 50% chance of being passed on in the offspring). It is just a matter of having to wait, and you do not have to release huge numbers of insects (you could encourage malaria resistance or genetic sterility, as was done with radiation). This is a new great and frightening power, not least because many new biotechnologies are cheap and therefore accessible to non-specialist laboratories with fewer economic resources. The strategy of transmitting a characteristic to all the progeny, as if it were a homozygous dominant character, allows to obtain the diffusion of the desired characteristic very quickly. The mechanism is called *gene drive* and the *CRISPR* system can potentially be used. Normally with sexual reproduction each individual acquires a copy of each chromosome from both parents. Therefore, each variant of a gene has a 50% chance of being inherited. Using biotechnology (*CRISPR*) in the laboratory it has been possible to prove that it can effectively transmit a hereditary characteristic (e.g. the colour of the eyes of the fruit fly and other phenotypes) in 97% of the offspring. Thus, one could think of facilitating the spread of negative traits, such as sterility in malaria vector mosquito females which is a recessive trait, in most offspring. In this way, it is not necessary to release more laboratory-modified individuals into the environment than those that are present in the wild. In practice one can realistically hope to be able to extinguish a wild species by releasing into the environment a reduced number of individuals carrying negative traits that are not inherited by the mechanisms of Mendelian genetics. The first experiments in nature that try to extinguish some mosquitoes by exploiting this new genetic strategy have already begun a few years ago: biotechnology (e.g. *gene drive*) is considered less dangerous than the use of pesticides to control mosquitoes that carry diseases such as malaria, the Zika virus, yellow fever, and many others that together generate more than one million deaths every year. [1029]

Mosquitoes are vectors of many diseases such as malaria, which kills over 400,000 people and makes at least another 2 million ill every year, due to the bite by the female of the genus *Anopheles* (it needs blood to feed its eggs). In the World, it has been possible to fight this disease by killing the mosquito vectors of the parasite using enormous quantities of insecticides such as DDT (*dichloro-diphenyl-trichloroethane*) and destroying the swampy environments. An example is the history of the malaria eradication programme in Sardinia, implemented after the Second World War (the vector mosquitoes on the island were *Anopheles abranchiae*, *A. algeriensis*, and *A. claviger*). Sardinia, in Italy, was one of the regions most affected by the disease: about 2,000-2,200 deaths a year in a population of almost 800,000 inhabitants. [1069] To try to extinguish the mosquitoes, which probably arrived on the island in 502 BC, at least 267 tons of DDT were distributed in the 1940s, also using airplanes and helicopters. In the month of June 1947 at least 85% of the inhabited centres of Sardinia were treated with this insecticide (including the interiors of the houses). The disease was defeated but the mosquitoes were not eradicated as completely as hoped (they were probably reduced by 99%). In 1950, for the first time, not even a single case of malaria was recorded on the island but the success was due to the synergy of several factors, including the use of insecticides, land reclamation and monitoring.

Genetic strategies to eliminate pests such as mosquitoes that transmit the malaria agent (*Plasmodium*) could be varied. It is possible to create mosquitoes that cannot transmit the malaria vector by appropriately modifying their genetic characteristics. Mosquitoes created in the laboratory should be distributed in the wild with the hope that over time they will replace natural populations. Another possibility involves the extinction of the vector mosquito by releasing into the environment large numbers of animals rendered sterile or with lethal genetic defects. By mating with natural populations, they could cause their extinction. This strategy envisages, for example, the distribution of sterile males and is not a new idea, since, as previously written, it has already been used several times with success. With this methodology it has been possible to eradicate the *Cochliomyia hominivorax* fly, whose larvae (in the tropical zones of the New World) are able to feed on the living tissues of various animals, including man. In this case, the distribution of sterile males (millions of insects), obtained by exposing them to radiation, allowed the eradication of these dangerous insects from many territories: in 1982 they were eradicated in the USA and in 1991 eradication was obtained from North Africa, where the insect was an alien species. [1002, 1003]

It has become possible to design cheaper and more durable strategies such as releasing genetically modified insects (*CRISPR*) into the wild that can spread self-destructive traits. [1075] In this way, in theory, it is sufficient to release a number of insects equal to a fraction of the natural population and wait. With the most advanced biotechnologies (*gene drive*) it is possible to hope to overcome the obstacle constituted by the fact that to extinguish wild insects it is necessary to spread an enormous number of animals selected in the laboratory. With appropriate genetic modifications, the probability of transmitting desired genetic characteristics is increased. Therefore, it could be possible to introduce into the environment a number of genetically modified insects equal to 1% of the natural population in order to hope that the genetic characteristics introduced will spread to the majority of individuals in a few generations. [1065] In the laboratory, by using the *CRISPR/Cas9* system in mosquitoes (*Anopheles gambiae*), it is possible to promote the spread of sterility in females, causing the collapse of the population in less than 11 generations (females homozygous for the artificially mutated character are sterile). [1072] It becomes possible to hope to extinguish mosquitoes, and therefore malaria, from the most affected areas of the Planet (e.g. Nigeria or the Democratic Republic of Congo) in four years. [1137]

Various heritable genetic alterations can be introduced in the laboratory that can generate the weakening and collapse of mosquito populations such as *Anopheles gambiae* and *Aedes aegypti*, for example by inactivating a gene important for egg production (the vitellogenin gene) or by inhibiting an important enzymatic activity in the blood. In these experiments, the *CRISPR* system was injected into the thorax of the mosquitoes. [1138]

Another type of genes identified by the researchers as a possible target to obtain in nature the numerical reduction of mosquitoes is that of flight muscles. In mosquitoes (e.g. *Aedes aegypti* and *Culex quinquefasciatus*) it has been possible to identify a gene that produces a protein essential for flight only in females. [1141, 1143] Therefore it is possible to hope to reduce the wild population by favouring characteristics that make females unsuitable for flight (males, even when homozygous for the mutation have normal wings, are able to fly and reproduce; in females even the condition of heterozygosity, that is those that still have a copy of the gene that is not mutated, can compromise flight). [1143]

It must be remembered that in these insects it is the female that stings and transmits diseases. Therefore, another strategy exploits the alteration of the numerical ratio of males and females. By acting on appropriate genes it is possible to hope to obtain (starting from an allelic frequency of the mutation of 2.5%) the collapse of the population (of *Anopheles gambiae*) in less than 14 generations, due to the reduced number of females compared to males. [1144]

Disrupting the expression of certain genes - in insect vectors of many diseases such as mosquitoes - is a new strategy to counteract serious health problems. To ensure that the genetic modification is stable, it must be passed on in germ cells. Working in mosquito embryos or zygotes to obtain a modification that is definitely heritable requires more experience as it is more difficult. In order to overcome this technical obstacle, molecular vectors have already been designed that are injected into adults but modify germ cells (in *Anopheles stephensi*). [1139]

These studies suggest that new tools may be available to control serious diseases. However, there may be unforeseen and underestimated events, such as the development of systems capable of counteracting and nullifying the effects desired by the researchers. For example, mosquitoes could have mutations that would override the effects of the *gene drive* (of the *CRISPR* system). [1140] In order to improve the safety of these technologies, systems should be designed that are reversible (it should be possible to restore the previously deactivated gene) and that can be confined only to certain populations (e.g. by choosing target genes that are present almost exclusively or predominantly in the target population and not in all or the majority of individuals of the species).

Another objective could be to avoid exterminating mosquitoes but rather to disseminate genetic traits capable of making them incapable of being disease vectors, for example by inactivating the expression of a protein present in the salivary glands of the mosquito (*Anopheles gambiae*) that plays a key role in the development of the malaria agent (*Plasmodium berghei* and *Plasmodium falciparum*). [1142] Mutant mosquitoes, in the laboratory, actually show a reduced ability to sustain the spread of the malaria agent. This type of approach aims to create insects that are immune or less able to host and, therefore, spread the parasite. It will be necessary to understand if the absence of the production of this protein (salivary in this case) makes the insect less able to survive in natural conditions.

Another research group has followed the same strategy by obtaining transgenic mosquitoes (*Aedes aegypti*) with a reduced ability to transmit a serious disease caused by a virus (Zika). The female mosquitoes, obtained in the laboratory, contain far less viruses in their salivary glands. [1145] By exploiting the natural ability of some mosquitoes to be incapable of transmitting the virus, it is possible to try to spread this characteristic in the wild mosquito population.

One technique is to shut down the synthesis of a protein by inactivating the function of messenger ribonucleic acids within the cell cytoplasm. The technique of interfering with the activity of messenger RNAs is based on the artificial transfer into the adult animal (e.g. the mosquito) of a vector of an RNA double helix, which contains the information necessary to reach the sequence of the target RNA and generate a cascade that destroys it. [1127, 1154] The final effect is the absence of the production of a protein determined and chosen in the laboratory. In this way it is possible to study the effects of gene shutdown, under controlled conditions. The genomes of many mosquito species have been sequenced so it is possible to design in the laboratory the sequences needed to turn off one target rather than another. In the case of mosquitoes, different techniques can be used to transfer the nucleic acid that will inhibit the synthesis of the desired protein: immersion of the larvae in a solution containing the interfering RNA, injection into the body of the insect, ingestion of nanoparticles that carry the RNA in the digestive tract protecting it from digestive enzymes, use of vector microbes (bacteria, yeasts, and adenoviruses) and algae. [1127] Bacteria and algae, genetically modified to carry nucleic acids, can be administered (dead or alive) in food. Microbes are easy to manipulate in the laboratory and protect the genetic material to be transported inside the insect. The use of microbes and nanoparticles could allow applications to control some plant pests as well.

In order to obtain mosquito males with disadvantageous genetic characteristics it is necessary, in breeding, to eliminate the females (also because they sting). One technique involves the genetical transformation of the insects so that when they are bred in the presence of a substance (e.g. the antibiotic tetracycline) they deactivate fundamental functions: for example they are born without wings and are therefore unable to live in the wild. [1135]

The common wasp (*Vespula vulgaris*) is a typical species of the Northern Hemisphere, although widespread and acclimatized in Australia and New Zealand where it is considered an invasive species. It is included in the list of the 100 most harmful exotic invasive species in the World. [1125] The queen wasp, which is the founder of the colony, is the only one to lay eggs (thousands) which the workers take care of. The nests can host more than 10.000 specimens. By modifying the activity of genes responsible for spermatogenesis in males with the *CRISPR/Cas9* system, it is hoped to manage to control the numerosity of the populations in the places where it has been introduced. [1124] Altering the ability to produce fertile sperm can generate infertile males. By spreading the genetically modified individuals in the wild population it is possible to hope to decrease the wild population in 10 generations, i.e. in 10 years (under the conditions present in New Zealand, distributing 100 modified insects per 100 hectares, where there are on average 13.5 wild nests per hectare). To achieve this eradication, the mutation that causes sterility in males should be spread in at least 96% of queen wasps. With the *CRISPR* system it is possible to think of spreading mutations to eradicate this species or simply reduce it numerically. Thus, at least theoretically, it is possible to design biotechnological experiments to reduce the population without extinguishing it completely (partial sterility). [1124] It will probably soon be possible to extend this *gene drive* practice to other wasp species such as the Asian hornet (*Vespa velutina*), also known as the yellow-legged hornet, which is a hymenopteran native to Southeast Asia. Since 2005 it has been introduced into Europe and worries beekeepers as it is a skilled predator of bees.

Inexpensive and fairly simple biotechnological techniques open up new opportunities that may, however, have unforeseen and negative side effects: mistakenly or accidentally the biotechnological system designed to control a species considered harmful could spread to very useful insects such as honeybees, bumblebees or other wasp species. In addition, the lethal genetic feature could accidentally be spread to places where the insect is not invasive, even long after the last artificial release in the wild. It may not be worth taking these risks, even in the laboratory. Common sense suggests that we should not design experiments at all in laboratories located in geographic areas where insects are wild and, therefore, naturally occurring. We cannot foresee the cascade of all possible effects and events, especially at a distance of time. Genetically modified animals, once released into the environment where they are not indigenous, could over time arrive in areas where wild individuals naturally reside. That is, the mutated animals may become invasive and lethal in areas where their wild ancestors not modified in the laboratory reside. In this case, one can talk of post genetic modification back invasion. If the biotechnological system for clockwork self-destruction were to work well, the extinction of the wild species in the natural environment could also be a matter of time. A true ecological disaster. The only way to be sure that this does not happen is not to insist on this path. Perhaps we sin in presumption as nature is likely to somehow provide against this lethal genetic pollution, the result of human ingenuity.

Also in the agricultural field various tests are underway for probable future applications. The example of the nocturnal moth known as the yellow tomato moth (*Heliothis armigera*) is given here. This moth can be found in Europe, Africa, Asia, Australia, and New Zealand. Extremely polyphagous, in subtropical regions, it infests mainly cotton and corn. In Italy, it damages tomato crops, but also many others: pepper, eggplant, potato, tobacco, broad bean, pea, bean, artichoke, cucurbits, salads, and ornamental crops. [1129] In southern Italy, the *Heliothis armigera* moth accomplishes up to 4 generations per year, with the most numerous occurrences from July to September. Females can lay from 300 to 2,700 eggs, depending on the environmental conditions. These are laid in isolation or in groups on the vegetation of the host plants. The larvae eat leaves, flowers, and fruits. The insects cannot synthesize certain fats

(sterols such as cholesterol), which must therefore be taken from the diet. The *CRISPR/Cas9* system has been used to interrupt the production of a protein (a membrane protein called *NPC1b*) necessary for the absorption of fats essential for their survival. [1128] Larvae in which this gene has been deactivated die. In genetically modified insects, the protein is not found in the intestine, where it is normally present. This gene could be a target for hoping to artificially spread a genotype with an insecticidal effect.
In nature, the larvae of *Heliothis armigera,* like those of other moths, are very susceptible to diseases caused by the nuclear polyhedrosis virus. Isolated mainly from Lepidoptera, nuclear polyhedrosis viruses are obligate endocellular pathogens, which require a viable cell for reproduction. Belonging to the Baculovirus group, they are present in many environments across a narrow host spectrum, which includes neither plants nor vertebrates. [1130] The virus must be ingested by the larvae and the effects are not immediate (farmers are used to the effects of insecticides being evident after a few minutes or hours). There are commercial products based on this virus (Baculovirus) to be used in agriculture against different insects. [1131] The distribution in agricultural fields of solutions containing viruses selected in the laboratory (on the same moth against which they are used in the field) to control insects harmful to crops could generate undesirable and unexpected effects (e.g. damage non-target species). Again, this is a dangerous experiment whose long-term effects are unknown.

These possibilities are astonishing and terrifying in that the use of cheap biotechnological techniques could easily allow a small group of researchers to plan the extinction of a species without any control, acting covertly. The release of animals with these modifications into the wild should be banned: military applications, such as biological weapons, or those aimed at gaining advantages may be numerous. How can dangerous experimentations be avoided when the technologies are relatively simple and cheap?

Unfortunately, experiments in releasing genetically modified insects (e.g. sterile male mosquitoes) into the wild have been implemented for over 10 years. Genetically modified insects have been released in countries such as Brazil and in the Grand Cayman Island of the United Kingdom. In Florida (in the Keys) a massive deployment of transgenic insects (they contain a dominant lethal gene) was planned for 2021 to control mosquitoes (*Aedes aegypti*) that have become resistant to insecticides. [1069, 1070] In Africa, releases of mosquitoes (*Anopheles gambiae*) modified using various biotechnologies are underway. In a first phase, sterile males are released, which when mated with wild females are unable to produce offspring. In a second phase, fertile males are released, but they produce mainly other males. [1136]

In Brazil, in the city of Jacobina, which is considered to be surrounded by sufficient geographical barriers to prevent the natural movement of mosquitoes, 450,000 genetically modified mosquito males were released every week for at least 27 weeks (between 2013 and 2015). [1071] The subsequent monitoring (27-30 months after the massive release of transgenic mosquito males) showed that individuals had appeared that were able to reproduce and possessed traits of the transgenic insects in their genome. This result confirms that losing control of the situation is easier than one might think. Transgenic "hybrids" capable of reproducing in the wild were unintentionally created; moreover, the transgenic mosquitoes were created from a Mexican insect population, thus creating a new strain resulting from the artificial mixing of three populations, one of which was designed in the laboratory and one of which was foreign. Prior research, which allowed the authorities to approve releases into the wild, had not foreseen this ecological disaster. Unintended consequences following the release of genetically modified animals into the wild can be unpredictable and cannot be determined in the laboratory.

One possible target for trying to counteract insect pests is egg-laying related genes. The functions of several categories of genes involved in egg production and laying have been discovered. These genes are potential useful targets for trying to obtain animals capable of transmitting the inability to complete egg production. [1119] An example in the *honey bee*: the

gene is that for vitellogenin which is involved in many functions such as ovary function and is present in many other insects. The hymenopterous insect *Nasonia vitripennis* is a parasitoid of flies infesting farms. This useful insect is used in biological fly control and genes have been identified that affect both egg and sperm production. [1119, 1120, 1121] The insect *Rhodnius prolixus* is a hemipteran that can transmit a parasite fearsome to some plants. [1122] By inhibiting the production of a protein (*Autophagy-related protein 8* or *ATG8*) the ability to oviposit is altered (inactivation achieved by the RNA interference technique). [1119]
In insects, more than 1,000 genes have been identified that could be a useful genetic target for the diffusion of characteristics unfavourable to survival: those for the production of the yolk, those that regulate the development of the ovaries, those of moulting and embryogenesis, those that produce receptors for particular odours or those of colour sensitivity (favouring the localization of the host plant). Among the important proteins, there are some that influence reproductive behaviour, such as the choice of the place where to lay eggs (*pherormone binding protein*, present in many species of insects) or the identification of the odour of the host plant to be parasitized by oviposition. Theoretically, by deactivating a single gene it is possible to obtain a lethal reproductive disadvantage to be spread in wild insects. As you can guess the surprises could easily disappoint us and the risk of spreading modified animals in the environment should be avoided.

Another possibility is to produce disease-transmitting mice in the laboratory: the white-footed mouse (*Peromyscus leucopus*) may be a vector for Lyme disease, generated by a bacterium (*Borrelia burgdorferi*). This microorganism is carried by a tick (*Ixodes scapularis*) of deers and affects at least 300,000 Americans every year. It is possible to engineer the release of genetically modified animals into the wild to spread the inability to transmit the disease. [1074] With this strategy, it is possible to hope to interrupt disease transmission throughout the ecosystem by acting on an important host: the mouse. Genetic biocontrol can be designed to reduce the numerical presence of a sex, so as to promote imbalances that can lead to the collapse of the rodent's population. [1150]
Mice and rats transported by human activities to other places on the Planet are the main cause of predation on bird and reptile species (they eat eggs), as it happens in New Zealand. On some islands in this country, it has been possible to eradicate an invasive rat (*Mus musculus*) by using poisonous baits (spread by helicopter at doses of 8 kg/ha). [1150] Hundreds of rodent eradication projects from small islands around the World have had good results: at least 637 projects up to 2017, most of which have used the anticoagulant rodenticide brodifacoum delivered in wax or cereal seeds. This practice has actually experienced failures too and, in general, has only worked on small islands.
Rodent eradication was successfully achieved by employing poison baits also in Italy, where in 14 islands it was possible to eradicate the rat (*Rattus rattus*), with the management of a plan that employed rodenticides (using between 4 and 10 rodenticide distributors/hectare containing bromadiolone and/or brodifacoum, in the years 1999-2017). [1150] In the Mediterranean islands, the rat was the cause of the reduction of seabirds (such as *Calonectris diomedea* and *Puffinus yelkouan*). Rat eradication has resulted in a significant increase in breeding bird colonies on the islands. However, the eradication is not necessarily permanent as the rats could be transported to the islands again, either accidentally or voluntarily, as it happened in the eradication attempt on the island of La Scola in Tuscany. In this case at least three new invasions were recorded in the 15 years following the eradication, probably partly as a result of deliberate introductions by saboteurs of the project. [1150]

The use of poisons (insecticides such as fipronil) has made it possible to eradicate the Argentine ant (*Linepithema humile*) from a small island in New Zealand (220 ha). [1150] The systematic distribution of poisoned baits, even using helicopters to extinguish rodents, or of insecticides certainly generates undesirable effects on non-target organisms. For example, the rodenticide *warfarin* has led to the emergence of rat populations resistant to this molecule, and poisoned grain or wax baits have also been eaten by other animals: birds, snails, and insects. Rodents such as mice (*Mus musculus*) and rats (*Rattus rattus* and *Rattus norvegicus*) are vectors of several diseases and are very dangerous invasive species. Rodents pose a threat to many wildlife species and therefore efforts are being made to develop molecular strategies to reduce this risk. One possibility could be to encourage the spread of genetic traits that render sperm non-viable. [1146] By exploiting the behaviour that requires the female to mate with more than one male, it is hoped to amplify the spread of genetic traits that can promote the numerical reduction of these animals. Using the technique of *gene drive* it is possible to hope to overcome the frequency of transmission of hereditary characters provided by Mendel's laws. By altering genes present in the male chromosome (Y) it is possible to inactivate spermatozoa. Heterozygous males transmit the negative trait to 100% of the progeny (instead of 50%): females (XX) will have the phenotype of males, therefore fertile females will tend to disappear in the population. [1146] Therefore, by releasing the modified males it is possible to hope to obtain a drastic numerical reduction of wild animals in a few generations.

Biotechnology also makes it possible to design animals with genetic characteristics that do not allow breeding between modified and wild individuals. These compatibility factors can manifest their maximum expression in the individuals obtained from the mating between laboratory-modified animals and those present in nature. Theoretically, by modifying wild animals (e.g. insects) with this artificial genetic feature, one can favour the creation of new evolutionary paths that could favour speciation. From a population of animals two could be created, one of which modified in the laboratory and which is not able to generate fertile offspring if it mates with wild animals (modified animals, when they mate among themselves, are able to generate fertile offspring). [1132] Possible applications are diverse such as reducing the possibility of genetic mixing between laboratory-created and wild animals.

These are only some of the possibilities of eradication of alien species because, in reality, there are many applications and they exploit completely different strategies depending on the category of living beings to be controlled. [1150]

Alien species that are genetically modified with the intent of artificially promoting self-destruction may return to the places from which they started. Species, which due to emigration favoured by human activities have colonized new environments, may accidentally return to their original locations. They carry the genetic modification of the invasive species as they are able to damage their wild conspecifics, in the environment where they naturally live. We could call it a return invasion of genetically modified emigrants. The effects of the biological invasion and the effects of the return invasion in the natural habitats of the genetically modified animals could generate disastrous ecological effects. The greatest problems may only become apparent after a long time. In countries that are rich in wild environments and unique species, the protection of living beings is a national priority (e.g. Australia, New Zealand). [1133] The release of modified organisms into the environment, whether authorized or unauthorized, seems increasingly inevitable. Technologies improve, experimentation increases, and species considered harmful continue to generate many problems. The release of living organisms designed to facilitate the numerical reduction of species classified as harmful presents many risks, largely unknown and unmanageable. The release of individuals modified for genetic biocontrol (e.g. through *gene drive*) is not compatible with the application of the precautionary principle. [1149] In situations where there is little evidence of potential serious and irreversible damage, it is far-sighted to prevent it. The precautionary principle should be applied whenever scientific information is insufficient to rule out risks, so as to avoid irreversible damage.

Humankind is imperfect, it invests enormous amounts of energy to prepare for conflicts or conduct wars: in the World, in 2019, at least 38 violent armed conflicts classified as wars were recorded. [1066, 1067, 1068] We are the result of an incomplete and constantly evolving

evolutionary process: how can we hope to prevent biotechnology from being used for military purposes or by terrorists or madmen? This is a dark, frightening side of technological innovation, which clearly manifests that it is proceeding much faster than human society's ability to evolve social and genetic mechanisms that inhibit its own destructive capacity.
Biotechnology is fascinating but, at the same time, very worrying. These experiments should probably be avoided and, in any case, it should be ensured that there are insurmountable physical and geographical barriers at the sites where the laboratories are located, so as to prevent the accidental release of modified animals into the wild. The potential offered by these technologies, which are, moreover, cheap and accessible, is alarming. Unfortunately, military applications or those aimed at undermining the food security and food sovereignty of a territory are also greatly enhanced.

Environmental degradation and mass extinction are greatly reducing the chance of many species to survive. Biotechnology may offer the means to save the eroded genetic diversity of many animal and plant species that are so numerically reduced that they have little chance of survival. The numerical reduction, together with many other factors, decreases the genetic variability and, therefore, the possibility to overcome the natural selection (but not even the artificial one imposed by the degradation operated by the human species) for lack of the necessary diversity. To mitigate this human disaster, biotechnology offers a way out: artificially modifying plants and animals to make them capable of surviving certain adversities such as pathogens. For example, America's chestnut tree is ravaged by a fungus from Japan. It is possible to generate genetically modified plants in the laboratory that are artificially given the ability to resist the fungus by inserting genetic traits that are naturally present in wheat. Are we going to let the chestnut trees be destroyed by the fungus or is it better to allow reforestation with genetically modified plants? The human species, in order to defend itself from the degradation caused by itself, adds other variables making the environment more and more artificial and controlled by technological innovation, rather than by nature. The prospect that presents itself is that of contrasting the extinction of some species, consequent to the disaster operated by humanity, by manipulating nature at a more intimate and irreversible level. The level of intrusion and hazard on selection, which becomes increasingly driven by human ingenuity, is unprecedented.
Another impressive and gruesome example is the attempt to revive extinct species through cloning into similar animals, using cells from tissues stored in freezers. Scientists have tried this with the Pyrenean ibex (*Capra pyrenaica pyrenaica*). In the late 1980s and early 1990s, about 10 animals survived. [1062] In 2000, the last living Pyrenean ibex, a female, was found dead and the species became extinct. It was decided to try to resurrect the species by cloning as there were no other possibilities, but the animal obtained through biotechnology died soon after birth. [1063] Despite the efforts of biotechnological de-extinction, it has not yet been possible to produce pure and viable offspring of extinct specimens, although such an attempt has already been made several times. [1064]

THE FARM WITH THE HUMANIZED GENETIC PROGRAMME

Humankind for the first time is able to intervene in the genetic codes created by nature (or according to religious people by the Creator). It is not easy to predict what the practical consequences will be (for example we know little about the interactions between different genes, and between genes and the environment). Certainly it will be difficult to hope to stop the experimentation of these new biotechnologies also in humans. Unfortunately, biotechnology allows dangerous or deliberately harmful applications. The modification of the human germ line should be banned, but unfortunately a few years after the first experiments on humans, it is already considered a morally acceptable technology, if it is to prevent diseases. The management of heredity in the laboratory to prevent disability and health problems is now a reality. Even if we succeed in promoting and sharing a planetary ban, work will probably continue invisibly or in the few places where there are no restrictions. We could call them *havens for eugenics* that in fact already exist: think of in vitro fertilization procedures, three-parent children and other procedures that are not authorized in all countries.

Many scientists have called for a halt, at least temporarily, to experimentation on human germ lines, but clinics offering countless services have existed for some time in many countries around the World. The possibility of redesigning the genetic characteristics of human beings is becoming a reality. Like many applications that the human species would have had to abandon because they were dangerous or immoral, such as nuclear weapons and drugs, biotechnology will not be held back. There are multiple applications that are about to invade the biosphere to fill our tables, to satisfy the medicine and various industries.

Biotechnology greatly increases mankind's ability to alter the biosphere and modify his genetic destiny. The speed of possible changes is enormously amplified, both those that in the anthropocentric view are considered positive, and the nefarious applications, such as biological weapons. Whether biotechnology, like many other technologies, will help us to improve life (e.g. to eradicate hereditary monofactorial genetic diseases) or instead accelerate the speed of degradation of the Planet (impoverishment of biodiversity) is still an open question. Certainly, if one looks at some of the most cultivated biotechnological applications in the World, such as plants genetically modified to be resistant to herbicides, those who have benefited have mainly been the managers of this technology, the patent holders and the vendors of technologies. Innovation proceeds much faster than the ability to evolve social (and genetic) mechanisms of self-defense. We are not prepared to handle such a capacity to intervene in the most intimate mechanisms of evolution and the frightening possibility of altering ecological balances that have emerged as winners over millions of years of evolution. We are optimistic if we believe that we can predict the effects of such a powerful disruption of the biosphere. It is very likely that even the applications we consider most useful, necessary, and least risky will reserve nasty surprises, such as the laboratory design of human embryos free of certain diseases.

The massive release of genetically modified organisms into nature surely increases the speed of destruction of nature and the delicate ecological balances necessary for our existence. We have incredibly increased the speed at which the biosphere is being transformed into a completely artificial environment, anarchically managed by commercial interests. We are systematically and at an unprecedented rate replacing nature with farms with biological features programmed to meet selfish needs, we could call it the *humanized gardens* or *humanized farms*. The chances that things will improve for mankind are very low, but at the present time it is difficult to imagine being able to stop this race towards an artificial World, deprived of naturalness and therefore less resilient. We are violating the basic rules of evolution with superficiality and presumption, simplifying nature and irreversibly contaminating the biodiversity of the Planet. Moreover, inequalities are destined to increase.

The replacement of natural environments with *humanized farms*, in which even human beings are the result of a self-managed evolutionary project, is a crazy and frightening idea. In this perspective, the reduction of biodiversity undergoes an impressive acceleration with consequent irreversible changes: less capacity to resist changes and greater susceptibility to diseases, including genetic ones. The replacement of procreation and natural selection with biotechnologies governed by economic rules and impossible dreams, such as that of desirable (or perfect) individuals, is a frightening project.

HABITS THAT ARE DETRIMENTAL TO BEEKEEPING AND AGRICULTURE

INDUSTRIAL AGRICULTURE LEADS TO A LACK OF NUTRIENTS FOR POLLINATORS

The current agricultural landscape, especially in plains such as the Po Valley (in Italy), is a real desert for bees, but also for other animals such as birds as it is lacking in nutrients. In France, between 1960 and 1990, 60% of hedges, spontaneous trees and uncultivated areas between fields were replaced by crops of little interest to bees such as grasses (maize is visited for its pollen). [243] It has been known for more than 50 years that semi-natural areas allow the survival of at least seven times as many wild pollinators as cultivated fields.

Among the effects generated by the reduction of biodiversity is food monotony, i.e. the reduction of the nutritional value accessible to pollinating insects (pollen and nectar). Reducing the quantity and quality of available pollen weakens the colony and wild insects; no scientific demonstration is needed: if bees suffer from starvation or malnutrition they are doomed to die. [163] Forced deprivation of pollen (using mechanical traps) causes several negative effects to the colony such as decreased weight of drones, delayed development of drones, reduced sperm production and reproductive problems. [1179] On the contrary, supplementary nutrition improves the reproductive qualities of males. [991]

In order to promote honey production, it will soon be essential to cultivate plants specifically for this purpose, which will provide the nectar and pollen necessary for the survival of honey bees: the use of supplementary feed will not be able to compensate for the lack of flowers. The degradation of agricultural and semi-natural ecosystems will ensure that beekeepers will cultivate the flowers necessary for honey production, otherwise this delicious food cannot be produced in the desired quantities.

The negative effects of pesticides are amplified if bees cannot feed properly. Lack of important nutrients, due to decreased pollen availability, increases the negative effects of pesticides such as insecticides (e.g. clothianidin). [1236] As one would expect, malnutrition, even in the case of bees, makes the organism weaker and more susceptible to the effects of other negative factors such as the exposure to pesticides or parasites. In order to ensure a better health for bees and a greater ability to withstand damage from pesticides as well, an adequate variety of plants must be available to feed them in a sufficient and balanced manner. Access to abundant and varied quantities of pollen is a prerequisite for the health of honey bees (mortality is reduced). [1236] Encouraging an increase in biodiversity in our countryside improves the health of pollinators and helps the farmers.

Monoculture may mean large quantities of pollen but only of one quality and for a short period. Pollen is important for its protein content, which varies from species to species, and it is important for feeding the juvenile stages of bees. Bee colonies that have access to five types of pollen, instead of just one, have a better nutritional intake and, therefore, will be more resistant to diseases (e.g. to the *Nosema ceranea* fungus). [243] As you might expect, dietary diversity is very important for maintaining the bee colony health, just as it is for the human species.

Artificial selection of plant varieties with characteristics that are more useful to humans often does not take pollen and nectar production into account, so the attractiveness to bees can vary considerably. The intensity of bees' frequentation of the flowers of different sunflower varieties, between the most attractive and the least attractive, may vary by a factor of four. [243]

Mowing before flowering (e.g. of alfalfa and clover to produce silage) is one of the agronomic practices that reduce food availability for pollinators. Mowing or weeding of flowers near orchards also decreases food availability for insects. Chemical and industrial agriculture has gone so far as to devise unsustainable preventive measures such as preventive chemical or mechanical weeding of uncultivated areas near cultivated fields to avoid attracting bees to flowers contaminated by pesticides distributed in orchards.

Flowers should be constantly present within a few kilometres, for about 7-8 months per year. In beekeeping, the lack of environments in which different blooms alternate constantly throughout the year is compensated for by nomadism and the use of feed.

A livestock practice that reduces the food supply of bees and pollinating insects is grazing. Farmed animals not only reduce the food supply to wild animals, but also damage underground nests such as bumble bee nests through trampling. [361] The movement of herbivorous species (cattle, sheep, goats), which are alien in certain ecosystems, may favour the extinction of some pollinators through the destruction of plants with which they have developed their symbioses. [481]

Due to several factors, bees are forced into an artificial diet managed by beekeepers. At an extreme, we may consider industrial beekeeping as an enterprise that transforms feed based on water, sugars, proteins, vitamins, and mineral salts (with the support of insecticides, fungicides, acaricides, hormones and, sometimes, antibiotics) into honey. Rice pollen, brewer's yeast, soy flour, sunflower flour, sorghum flour, milk powder, casein powder, egg yolk powder, and chestnut flour may be used as protein supplements. [35, 974] More than 20 kg of sugar per beehive is also needed in difficult seasons, such as 2017, which was marked by intense drought in Italy.

Honey is the natural nourishment of the bees, but it is taken away for economic purposes and must therefore be replaced by feed, which must necessarily have a lower economic value, in order to ensure profitability for beekeepers. Therefore, feeds may be necessary even when there is an abundant supply of pollen and nectar: it depends on the intensity of honey withdrawal.

Industrial agriculture forces bees into a simplified diet consisting of single pollen varieties that alternate through nomadism by beekeepers. There are many artificial alternatives to flowers. We could imagine the future of honey bee farms in greenhouses where the main purpose of the plants is to create a less alien environment and most of the nutrients will be provided by artificial flowers, which deliver feed enriched with vitamins, amino acids and minerals. We would not be surprised if economic interests came to induce the recycling of bees, their eggs, and dead larvae, in the form of meal, as is done in animal husbandry and aquaculture.

Feed (e.g. syrups with 50% sugar in water) fed to bees should be recorded. Knowledge of the quantities of feed used in the different periods of the year could also provide very useful information for understanding the health problems of the colony. In Italy, the legislation required this registration, but beekeepers were exempted from the obligation in 2004. [222, 223] Beekeepers can also recycle unsold honey by diluting it with water and sugar. This practice could encourage the spread of certain diseases. It would be useful to register not only sugar substances, but all types of feed used, such as protein sources.

Bee diseases, besides being spread by nomadism, are also favoured by the importation of feeds such as pollen, which may contain bacteria and fungi. Therefore, the movement of this raw material, should be carefully supervised.

MIGRATORY BEEKEEPERS: BLIND OPTIMISM AND SELECTIVE DEAFNESS

Historical precedents of the practice of nomadic beekeeping can be found in Egypt of the Hellenistic period and in Greece. [972] Bees are moved to increase the profitability of the breeding, but also because of the considerable reduction in floral biodiversity. Movements are often carried out weekly and for hundreds of kilometres, whereas honey bees would hardly be able to cover more than 5-8 km per year. Nomadism and trade in queen bees promote several problems:

- The spread of diseases among farmed bees (e.g. mites and viruses). Pests may be moved to geographical areas where they do not exist.
- The spread of diseases from farmed to wild bees. The movement of farmed bees promotes the spread of pathogens into new territories because, in some cases, parasites can easily colonize them. This problem is more pronounced when bred bees are moved outside their native territories. [486]
- The spread of diseases among plants.
- The extinction of local phenotypes. Nomadism, together with the possibility of buying these insects from anywhere on the Planet, favours the phenomenon of genetic homologation that extinguishes local ecotypes. Characteristics that make bees unique and adapted to particular environments are lost forever. Genotypic and phenotypic characteristics that are important and useful for beekeepers themselves (e.g. resistance to diseases) are systematically lost.
- Competition for resources such as pollen, nectar, and nesting sites between farmed and wild bees increases. The reduction of local wild biodiversity is favoured. Food resources are taken away from wild species and some crop pests may be favoured. Bees subtract pollen from many arthropods such as wasps, hoverflies, diptera, and coccinellids, which perform the natural function of feeding on crop enemies (they have both carnivorous and herbivorous stages, as it happens with some wasps whose adults are insectivorous but whose larvae feed on plants). In an already poor and fragile ecosystem, the sudden arrival of platoons of foragers, artificially supported by beekeepers, reduces the natural possibility of containing crop pests. Some insects that parasitize others also need pollen and nectar for survival. Therefore, competition for plant food resources between bees and wild carnivorous insects may help to reduce an important limiting factor for agriculture.
- The modification of the qualitative and quantitative composition of plant species such as the extinction of some native plants. The reduction of local biodiversity may promote the destruction of the network of balances such as symbioses between plants and wild pollinators. Farmed bees, because they are able to pollinate different species, are more resistant to changes in the composition of wild flora. Thus, the nomadism of honey bees may negatively affect local plant biodiversity. [486]
- The increase in the frequency with which medicines will be used to treat diseases will be more likely to occur, due to nomadism itself. A side effect is the selection of parasite strains that are resistant to the medicines used (insecticides, acaricides, and antibiotics).

Unfortunately, insufficient information is available for most of the critical points listed above, such as competition for food resources and qualitative and quantitative variation of plants, in natural areas and in the long term. Even less information is available on the effects generated by the breeding of pollinating insects other than the honey bee (*Apis mellifera*) and in some territories such as Africa and Australia. [486] Most of the publications analysed examine European and North American contexts (also in North America the domesticated bee is an alien species).

In general, problems increase when domesticated bees are moved outside their native territories (e.g. from Europe to North America).

The movement of pollinating insect herds should be restricted or prohibited in areas of biodiversity conservation concern. In the case of honey bee nomadism, a safety zone of at least 15 km of distance from the boundary of the area and/or the species to be protected could be recommended. Safety zones and preventive measures (e.g. for trade in queen bees and bee colonies) should also be regulated in order to limit the spread of diseases in farmed bees and from them to plants.

THE NOMADISM OF BUMBLEBEES: A DANGEROUS EXPERIMENT

Like the honey bee, bumblebees are bred and sold all over the World to pollinate crops such as tomatoes and raspberries grown in greenhouses and strawberries, blueberries, alfalfa, cucumbers, eggplants, beans, blackcurrants, peppers, and apples. These are nests to lose i.e. disposable. This international trade in insects is harmful to the environment because it generates the same problems caused by the nomadism of the honey bee. In the case of bumblebees, the hybridization between the bumblebee and related but different species is well known. Hybrid animals are the offspring of a donkey and a mare which is called a mule, or that between a donkey and a stallion which is called a hinny; zebras crossed with donkeys are almost always sterile. [848] Male bumblebees (*Bombus terrestris*) may mate with female *Bombus hypocrita*, which is indigenous to Japan, but the mating is sterile. Hybridizations between *Bombus terrestris* and native species have also been observed to produce offspring. [688] In this case the environmental disaster is even greater.

87.5% of wild plants need pollinators to varying degrees and 20% benefit from the presence of bees. [1239] Bees include at least 260 species of bumblebees which, like all insects, are in decline. In Europe at least 21% of the 63 bumblebee species and in North America 26% of the 47 classified species are in decline or at risk of extinction. [1239] The causes of bumblebee decline are many and, as with honeybees, pesticides and the destruction of natural ecosystems are the main cause. Habitat destruction, habitat fragmentation and degradation due to agricultural expansion are important causes too. For some species, particular causes are well documented. A bumblebee native to Argentina (*Bombus dahlbomii*) is also in decline as a result of the arrival of *Bombus terrestris*, which is an invasive species in this area. [1239] The trade in farmed bumblebees has also generated problems for native populations in North America, China, and Mexico. From Chile in 1990, *Bombus terrestris* spread rapidly (200 km per year) across the Andes to Argentina, where it arrived in 2006. The expansion of *Bombus terrestris* was followed by the disappearance of Argentina's native bumblebee (*Bombus dahlbomii* or giant Patagonian bumblebee). [481]

Species that would never have met without the human insect trade are mating, endangering the survival of native species. This is a dangerous experiment that leads to the reduction of genetic biodiversity. The nomadism of bumblebees has encouraged the spread of diseases among wild species and the extinction of wild bumblebees. Some species of bumblebees are cold-hardy and climate change shrinks their habitats. For example, the area of the Alps suitable for *Bombus alpinus* has risen by more than 450 metres since 1984 due to global warming: the minimum altitude at which these insects can live is rising, and over time they will disappear. In Switzerland, between 50% and 85% of bumblebees are threatened with extinction due to climate change. Vulnerability to such rapid changes is a crucial aspect. Climate change with drought and heavy rainfall alters the floral availability by reducing food sources.

The erosion of natural habitats due to various factors (climate, agriculture) also generates fragmentation and isolation of populations that are forced into lethal geographical and genetic isolation (e.g. for *Bombus veteranus* and *Bombus distinguendus*). [1239]

Another negative factor is the spread of pathogens favoured by the trade in both bumblebees and bees used for pollination in agriculture. The artificial nomadism generated by the symbiosis between farmers and beekeepers favours the spread of many diseases: *Bombus terrestris* in South America has favoured the spread of an intestinal parasite (*Crithidia bombi*). [1239] The parasite *Nosema ceranae* affects honeybees and bumblebees, so artificial migration greatly amplifies its spread. Trade in reared pollinators has facilitated the spread of viral diseases from honeybees to bumblebees.

Bumblebees are raised to aid pollination in greenhouse crops such as tomatoes and may transfer diseases to wild ones. The spread of diseases due to nomadism has been recorded in North America for several bumblebee pests *Nosema bombi* (a fungus), *Crithidia bombi* (a protozoan), *Locustacarus buchneri* (a mite) and the deformed wing virus. [361]

Some bumblebee parasites are also potentially very dangerous for honeybees, such as the small *Apocephalus borealis* fly, which in North America can transmit viruses such as the deformed wing virus and the parasite *Nosema ceranae*. [1155] The fly lays its eggs in the bees' bodies, from which the larvae will emerge. Genetic analysis has shown that the flies that parasitize bumblebees (*Bombus vosnesenskii* and *Bombus melanopygus*) are the same as those that affect the honey bee (*Apis mellifera*). [1155]

There is no longer any doubt: the nomadism of insects bred for the service of pollination also promotes the spread of diseases to wild species. The information on this is inadequate and should invoke the precautionary principle. We are therefore conducting an uncontrolled experiment. If we wanted to distribute bumblebee diseases (e.g. mites) across the Planet quickly and reduce genetic diversity, we could not do better.

Another alarming aspect is that, in Europe, a few hundred tons of pollen are needed every year to breed one million bumblebee colonies. Bumblebee breeders can obtain this huge amount of pollen by subtracting it from honeybees. The pollen can be collected by beekeepers by placing special mechanical tools in the hives. The sale of pollen can be another source of income for beekeepers. Thus, the artificial condition is created in which the honey bee is bred to feed another species, the bumblebee. This trade of pollen taken from *Apis mellifera* may favour the transmission of diseases to *Bombus terrestris* (e.g. the deformed wing virus) and they spread them all over the Planet: through pollen and insects, diseases may be transmitted to other insects but also to plants. This industrial practice generates another environmental damage as nourishment (pollen and nectar) is taken away from wild pollinators. In some cases, pollen is marketed as packaged feed to feed colonies in difficulty (not only honey bees but also bumble bees). In 509 pollen samples from 14 countries, specimens of 71 insect families and 27 mite families were detected. [670]

In conclusion, nomadism, along with the insect and feed trade, threatens the health of farmed and wild insects and even plants.

BEEKEEPING DEPLETES THE FOOD RESOURCES AVAILABLE TO OTHER POLLINATORS

The introduction of honeybees wherever there are humans and a crop to pollinate has generated competition for resources such as pollen and nectar. To give an example, European bees were introduced into North America in 1620; competition between honey bees and native wild insects, such as bumblebees, has favoured a reduction in the reproductive success of the latter. [361] It must be remembered that an apiary, i.e. the hives placed on a site by an industrial beekeeper, may consist of more than 50 colonies of bees. Each hive will consume, in one year, between 9 and 59 kilos of pollen and between 54 and 408 kilos of nectar. [388] A colony with a maximum population of 50,000 bees can consume more than 120 kg of honey in a year, which is necessary only for the survival of the adult bees. Overall, the entire colony can consume over 200 kg of nectar and between 10 and 30 kg of pollen per year. [972] A colony of this size can yield between 20 and 40 kg of honey per year to the beekeeper (at least part of this production comes from sugar-based feeds provided by the beekeeper rather than nectar; some beekeepers interviewed in Piedmont in 2020 claim to provide their hives with at least 30 kg of feed per year, composed of 50% sugar and 50% water, before the onset of winter).

Natural *Apis mellifera* colonies in Europe probably no longer exist, but the natural density of these can be assumed to be between less than one and a few colonies per 100 hectares (this density is that of some wild bees with a similar biology). [972] Artificial densities managed by beekeepers can be two or three orders of magnitude higher: they can reach over 500 colonies per 100 hectares instead of less than 10.

The displacement of numerous colonies in a natural area or where wild species reside generates a considerable competition with available food resources. An apiary of 40 colonies will probably consume the pollen needed by 4 million wild bees (in three months). In flowers, pollen, unlike nectar, is not continuously replenished, so the number of insects per number of flowers is very decisive for the possibility of survival. Unfortunately, there is not much information on the long-term effects generated by this artificial competition supported by beekeepers, but it is easy to predict the reduction of biodiversity.

The competition between wild pollinators and bees is an aspect that could be interesting to deal with in depth. On this theme and on the problems generated by nomadism such as the spread of pathogens, competition for resources, inbreeding that reduces biodiversity and the qualitative and quantitative variation of the flora (e.g. extinction of native species) there is little and fragmentary information. [388, 486]

Solitary bees do not live in groups, they feed mainly on pollen and may have different diets depending on age and sex. The nomadism of honey bees generates a sudden and massive presence of hundreds of hives, which are supplied with resources that are limited and useful to other species. Undoubtedly, bees, thanks to human help, become formidable competitors capable of damaging wild pollinators, as they will deprive them of the necessary food resources.

It is possible to estimate the maximum number of individuals that may be fed (i.e. larvae and adults), based on the number of flowers per unit area; the number of flowers may be less than 10 flowers/m^2 for sunflower, may be more than 50 flowers/m^2 for clover and more than 11,000 flowers/m^2 for rape (*Brassica napus*). [13] Even more direct may be the correlation between the maximum number of insects that can survive per quantity of pollen produced per unit area. Plants, with the intention of promoting greater dissemination, release pollen gradually so that they have to be visited several times before they have released all the pollen grains to the insects. It is also important that nutrients are available during the different months of the year, and not only for the 2-3 weeks of the monoculture flowering. A critical aspect is food monotony to which pollinating insects are exposed, which for honey bees is partly compensated by artificial feeding by beekeepers and nomadism (an environmentally unsustainable energy investment).

Wild pollinators, such as some solitary bees, build their nests in the wood or the ground, so the absence of trees and tillage prevents the completion of their life cycle. A considerable negative impact for wild species is the fragmentation of agricultural land, which means having to travel greater distances to find hedgerows and areas with flowers. In addition, barriers to movement increase. Insects, like all living beings, have to reckon with energy efficiency: once certain distances have been overcome, which in many cases may be of the order of hundreds of metres or a few kilometres, the probability of reproductive success decreases in a decisive and irreversible way: it is possible to estimate the distance beyond which the probability of returning to feed the larvae is reduced by 50% or 90%, or to estimate the maximum flight time, which may vary between 3 minutes and 170 minutes for insects between 7 and 12 mm in size. Once passed a certain distance or a certain time of flight, if the necessary pollen and/or nectar have not been collected, the death by starvation becomes inevitable. Therefore all these factors could be explored in depth to help the agricultural system regenerate a new, more lasting and sustainable balance.

SOME IMPORTANT ENEMIES OF BEES

The spread of a bee disease is promoted by factors such as nomadism, pesticide use, artificial selection, and other beekeeping activities, and is an increasing problem. Beekeeping practices that promote the spread of a disease include trading in queen bees (they are usually shipped with a small group of worker bees) and joining bee families to reinforce weak colonies. Other activities that promote the spread of a disease are the collection of swarms, the transfer between hives of materials such as combs and wax, and equipment such as the smoker or the honey extractor. The first cause of the spread of bacteria and their spores is precisely beekeeping. According to some researchers, the pollinator trade may be the main cause of the spread of particular diseases in wild bumblebees. The importation of bumblebees for greenhouse pollination in Canada has encouraged the spread of a pathogen in wild species (*Crithidia bombi*). [442]

In order to know the distribution of bee pathologies (and in general of animals) in the World, it is possible to consult a special site of the World Organization for Animal Health. [671] This Organization mainly monitors the spread of six bee pathogens: *Acarapisosis*, American foulbrood (*Paenibacillus larvae*), European foulbrood (*Melissococcus plutonius*), *Aethina tumida*, *Tropilaelaps*, and *Varroa*. Some of these pests can be very resistant. For example, the spores of the bacterium that causes American foulbrood (*Paenibacillus larvae*) can remain in the environment, resisting heat and cold, for up to 35 years, while the spores of the *Acarapisosis* fungus can remain active for 15 years (e.g. in wax, honey, and pollen) and the parasitic fungus (*Nosema ceranea*) can remain active in wax for up to one year. [670, 744] A bee larva may be infected by the ingestion of less than 10 spores of *Paenibacillus larvae,* but a dead pupa can release 2.5 million spores, generating the rapid spread of the bacterial disease in the hive. [974]

Beekeeping practices are one of the main causes of the spread of some diseases across the Planet. The fungus that affects the respiratory tract and feeds on the hemolymph of bees (*Acarapisosis*) can be transported by queen bees that are traded between different continents, using fast postal services (they can travel by aircraft). [670] The trade of colonies or queen bees involves a well-established rule of veterinary prevention which requires the verification of the guarantee of the absence of disease reports, at least in the previous six months, in the area from which the insects originate and within a radius of at least 100 km (this is a safety measure to prevent the spread of parasites). As an example, the parasitic beetle *Aethina tumida* can fly and actively move up to 13 km from infected bee colonies and can also parasitize bumblebees (*Bombus terrestris*). [670] Adult insects can live several months and the pupal stage is spent in the ground, within a few tens of metres from the hive.

Honey is primarily used for human food, but it can be used to feed bees in harsh conditions. Honey may also be a vector for diseases, such as those generated by the American and European plague bacteria. 600,000 spores of the American plague agent bacterium *Paenibacillus larvae* can be found in 5 grams of honey. [670] Bee larvae, which are traded by beekeepers to help weak colonies, can transmit diseases such as some viruses or the European foulbrood (through spores).

A very important sanitary practice is the destruction of infected colonies to reduce the probability of disease spread. However, prevention should be adopted within a radius of at least a few kilometres, not less than three: all hives that are within 3 km of the colonies that are definitely diseased should be destroyed. This preventive health action is not accomplished by beekeepers, who may refuse to report the presence of infected colonies to the health authorities, as required by the veterinary regulations. To encourage this preventive strategy, financial aid could be provided to compensate for at least some of the losses (e.g. compensation for the destruction of the hives due to the presence of the plague, i.e. *Melissococcus plutonius* bacteria).

Artificial practices carried out by farmers include the collection and marketing of the drones' semen for the purpose of assisted fertilization. It has been shown that the liquid containing the sperm may carry certain viruses. In the wild, a queen bee may mate with up to 28 males during her nuptial flight, thus receiving dangerous viruses from different colonies.

During trade a preventive measure is the so-called quarantine, i.e. for a period of time the material that risks spreading pathogens is subjected to isolation, observation, and analyses to determine the probable onset of the pathology. In some cases it is necessary to carry out disinfestations with special substances (e.g. sodium hypochlorite at 1% for 30 minutes for equipment at risk) or it is sufficient to subject the material to low temperatures: for example in the combs the temperature of -12°C, maintained for at least 24 hours, is able to reduce the risk of propagation of the beehive beetle (*Aethina tumida*). [670] The small beetle (*Aethina tumida*) is native to South Africa and can cause damage to the combs, loss of honey and pollen, up to the extreme consequence of the loss of the colony. [672] If the infestation is particularly serious, it may cause swarming. In addition, it may cause damage to stored combs and unprocessed honey. The larvae dig tunnels in the combs where they eat and defecate, causing the honey to ferment.

The beetle *Aehtina tumida* reached the USA after 1996, in 1998 it was recorded in Florida, in 2002 it arrived in Canada and Australia, in 2004 in Portugal, and in 2014 in Italy. [243] The speed of the spread of this parasitic insect has been favoured by the trade of bees and their products (wax, pollen), although it is able to fly about 5 km. Wild bees are able to fight it by covering it in propolis or by adopting behaviours such as abandoning the infested colony. [243] Attempts have been made to control it not only with the usual chemical weaponry (coumaphos, fipronil, permethrin), but also with entomopathogenic nematodes (biological control). [218]

Diseases spread by nomadism and other practices such as the queen bee trade include the intestinal parasitic fungus *Nosema ceranea*, which appeared in Europe in 2005. [230] In the USA it was probably recorded for the first time in 1987, in Florida. In a few years it has colonized the bees of the whole Planet. [307]

A monitoring conducted in Italy on more than 1,300 hives, in 2009 and 2010, recorded parasites dangerous to bees with worrying frequencies: *Nosema ceranae* in 49.5% (between 30% and 69% of colonies in the various regions of Italy) and viruses in 75% of the colonies. [163] In other countries, the frequencies of *Nosema ceranae* were: Belgium 92.6%, Spain 65.6%, Germany between 5.2% and 35.4%.

The first official report of the presence of the *Varroa destructor* mite on European bees (*Apis mellifera*) was probably made in China in 1958 and in Italy in 1981. [35] The *Varroa mite* probably moved from the Asian bee to the European bee exported to Asia in the 1960s and arrived in Europe in the 1980s. [230] The *Varroa* mite promotes the spread of viruses in bees (at least 18 types of viruses are known to damage bees). Viruses may also be transmitted by feeding such as through the administration of royal jelly that the glands of young workers produce to feed the queen bee.

Various chemical molecules are used to control pests such as *Varroa* and other mites. Some parasites, such as *Varroa*, have in a few years seen the emergence of strains resistant to the pesticides used by beekeepers.
The adult female mite is an ecto-parasite, it sucks the hemolymph of the adults by placing itself on the thorax and is able to damage also the brood. Curiously, these mites are blind, the males live a few days and do not take food, as the mouth serves to transfer the spermatozoa into the female genital tract. Some bees (e.g. *Apis cerana*) have developed defensive behaviours such as locating and removing the contents of parasitized cells, self-cleaning or letting other bees get rid of the parasites (grooming). These activities are probably induced by chemicals produced by the mites. Beekeepers have learned to sprinkle the bees' bodies with powders such as powdered sugar, which induces greater self-cleaning and grooming behaviour. Through the distribution of powders on the body, micro-encapsulated pesticides can be dosed. [865]
It is possible to measure the density of mites present in a certain number of bees in order to quantify the severity of the infestation. In this way it is possible to assess economic thresholds below which the infestation is not so serious and intervention can be avoided (e.g. 2 mites per 100 bees, among those examined in spring). [35]

Vespa velutina or Asian hornet (also called yellow-legged hornet) is a hymenopteran native to Southeast Asia. This species probably arrived in Europe before 2004 in France and from there it spread to other states such as Spain (in 2010) and Italy (in 2012). In Europe it has been moving at the rate of 100 km per year (this wasp can fly 30 km in a day and a female can produce another 550 future queens in the course of a year). [13] It is equipped with a sting that can also be used against humans, and honey bees are one of its foods. This wasp forms colonies that may contain more than 6,000 individuals. Southeast Asian bee species (e.g. *Apis cerrana*) have adopted effective behaviours to combat this predator, such as piling on the intruder, overheating it to death by thermal shock. This behaviour is so far unknown to European bees. In fact, European bees bred for 50 years in Asia have adopted a similar behaviour, but less effective, as it is carried out by a smaller number of bees. [35, 150]
The Asian hornet can form annual colonies consisting of a few thousand individuals and forms nests with a diameter of 40-60 cm. It can prey on other insects in flight and feeds preferably on the flight muscles in the thorax. The adults of this wasp feed the juvenile forms with other insects but they are also pollinators. In an anthropized environment, that is, where sources of food are scarce, honeybees can make up 70% of their prey. The wasps stay near the hives, preying on the returning bees. Honey bees in the presence of this predator can interrupt their foraging activity, thus weakening the colony. It only takes five of these hornets to condemn a hive. *Vespa velutina* also poses a danger to other insects such as some wild pollinators, resulting in a likely reduced survival of flowering plants.

MODERN BEEKEEPING PROMOTES THE SPREAD OF DISEASES

The high density of apiaries generates problems also among the bees themselves because diseases will spread more easily (e.g. *Varroa destructor* and viruses transmitted by these mites) and they will compete with each other. It is well known that increasing the density of apiaries decreases their strength, increases winter mortality, and reduces honey production. [771] A density of hives that could be considered natural is between one and six colonies per square kilometre, i.e. every 100 hectares. Industrial beekeeping can achieve a density of more than 4-5 colonies per hectare, i.e. more than 500 colonies per square kilometre (an apiary can also contain more than 50 hives, which are then placed a few centimetres apart): in beekeeping, dozens of hives can be placed in a row next to each other. Artificial assemblage can only exist thanks to support by beekeepers: feed, medicine, nomadism (they travel on tyres), rapid replacement of entire colonies or the queen bee (she can travel by plane). The density exploited by entrepreneurs is hundreds of times higher than what could be considered natural. This generates overcrowding and competition, which damages the beekeeper himself.

Among bees it is a well-known phenomenon that both a forager and a drone can be accepted in a colony different from the one they come from: worker bees are more easily accepted if they are loaded with pollen or nectar.

The way the hives are placed on the same site may affect bee health. By arranging the hives in a circle, with the entrances facing outwards and 10 m apart, one can hope to get more honey, record less diseases (*Varroa destructor)*, and the bees will enter the wrong hive less frequently, compared to the same number of colonies placed in a row one metre apart. [771] However, this cannot be the solution.

Bee drift is another phenomenon that is more likely to occur between neighbouring hives, as it happens during apiary nomadism. Bees move from one colony to another, thus being able to spread pathogens such as bacteria (the plague) and other diseases. The economic exploitation of beekeeping implies having to manage in limited areas even hundreds of hives that will be in close contact with one other. Factors that may promote the spread of diseases (e.g. mites) include the looting of weaker colonies, carried out by the bees themselves, and the movement of drones (male bees), which during the mating season are also accepted by other colonies. The plundering may be latent, a small number of bees, a little at a time, takes away the honey from the weakest colony. In some cases the plundering may be very devastating and bloody, as the bees kill each other.

Artificial beekeeping conditions reduce aggressive behaviour among colonies for several reasons:

- foragers visit the same flowers and therefore the smells in the colonies will be similar;
- odours have a genetic component and bred bees are very similar to each other so chemical differences are also very small.

The presence of large food supplies, facilitated by artificial feeding (water and sugar provided by beekeepers), makes the colonies more docile and decreases imbalances in colony strength. A practice in beekeeping is to balance the strength of the colonies by taking frames from a bigger hive and inserting them into a weaker colony. Thus, intensive breeding increases the possibility of tolerance of intruders and between neighbours. This condition promotes physical contact between different colonies and, consequently, the spread of diseases.

An exemplary case is the migration that occurs in the almond orchards of California. To ensure the production of almonds in one of the most important regions in the World for this crop, between 1.3 and 2.5 million hives are needed: they represent more than 50% of the colonies present in the USA. [13] Practically the American bees meet in these almond groves. It is a very profitable mass migration, as beekeepers can receive as much as 160 euros per hive (for a service lasting a few weeks, the time of flowering). [196] The proximity of millions of bees, which are continuously moved, definitely has predictable negative effects. Besides spreading diseases and pests (e.g. *Aethina tumida*) among the colonies, bees can do so to other species of insects such as bumblebees, which in some countries are autochthonous species. [361] For example, in North America, where bees were introduced after 1620, at least 3,600 native bee species have been recorded: how many of these native species have been damaged by the introduction of foreign bees and to what extent is difficult to determine. [388] There is little information on the possibility of spreading diseases from bees to other insects, but problems have already been reported for other pollinators due to honeybees: for example, as a result of the spread of viruses such as deformed wings. [481] Parasites adapt themselves, they evolve, and the passage of the parasite *Nosema ceranae* from honeybees to bumblebees has already been recorded in South America. [388] The opposite may happen too, i.e. honey bees receive a parasite present in a certain habitat and then spread it in another place, as has already happened. Thanks to nomadism and to the trade of queen bees some parasites, in few years, have gone around the World with catastrophic consequences (e.g. mites). Nomadism has favoured the worldwide spread of the *Varroa destructor* mite, which was originally present in Asia and parasitized *Apis ceranea*. [483] The displacement of this parasite on *Apis mellifera* has also favoured the transmission of some diseases such as the deformed wing virus. Another important parasite, the intestinal fungus *Nosema ceranea*, was also spread from the Asian bee (*Apis ceranea*) to the European bee (*Apis mellifera*) through international trade and nomadism. The parasitic fungus (*Nosema ceranea*) was recorded in the USA in 1995 and in Europe in 1998 (spores are transmitted by ingestion). [483, 670] This fungus affects wild bumblebees too. [483] *Nosema ceranea* can weaken the immune system in honey bees, shorten life expectancy and alter the workers' behaviour. The presence of these parasites in bee colonies increases the susceptibility to pesticides. The doses needed to manifest lethal or sub-lethal effects (e.g. fipronil or neonicotinoids such as thiacloprid) are reduced in the presence of parasites such as *Nosema ceranea*. [483] Some pesticides (e.g. imidacloprid or fipronil) reduce the immune defence capacity of bees by favouring parasites. Therefore, new synergies are established between some pests and pesticides, which generate negative effects by reinforcing each other.

Nomadism can promote the spread of diseases among plants. [483] *Erwinia amylovora* is a bacterium (Gram-negative of the Enterobacteriacea family) agent of the plant disease known as fire blight of pome fruit. This disease affects more than 200 species belonging to 40 genera of the Rosaceae family, including many of great importance for human economy (apple, pear, loquat, quince, rowan), ornamental species (hawthorn) and other species such as the black locust. [17, 18] Among the vectors of *Erwinia amylovora* more than 70 genera of insects have been described, among which the most important ones are bees and wasps.

Another aspect concerns the use of wax, which is regularly recycled. Wax may contain amounts of pesticides that are 100 to 1,000 times higher than those recorded in honey. [230] With the goal of getting more honey, beekeepers recycle the wax so that the insects spend less energy building up this resource. Unfortunately, wax accumulates and stores dangerous molecules, such as pesticides used by both beekeepers and farmers. Synergistic and additive effects are amplified. This effect can also be seen in the wax purchased by beekeepers who adopt the criteria of certified organic farming and amplifies the phenomenon of bioaccumulation. [240] In order to reduce this problem, the companies that process wax and reconstruct the honeycombs with the cells to be inserted in the hives adopt procedures such as filtering with active carbon and acid washing. These processes are expensive, add other potential risk factors, and do not solve the problem of chemical contamination. [230] Other substances (e.g. paraffin) are also used during recycling and may have negative effects. Finally, wax recovery may facilitate the spread of diseases. Sustainable beekeeping should not recycle wax which, among other things, may come from hives living thousands of kilometres away.

A crucial aspect of sustainable beekeeping should be nomadism. Journeys that last even overnight or several days on trucks certainly weaken the bees and promote the effects already mentioned.

LIMITING NOMADISM

The system of rules on the possibility of movement of both reared pollinating insects (such as bees and bumblebees) and plants, which was designed according to anthropocentric principles, does not give an adequate protection from the spread of diseases. Regulations and customs accept placing groups of 50 hives (defined as apiaries) at a distance of 200 m from each other. [222] This distance is not sufficient to prevent the spread of diseases between colonies. No minimum distances are regulated between apiaries and natural areas, where species of particular naturalistic interest (e.g. wild insects) are present, and between the apiaries themselves. Some guidelines propose placing apiaries more than 6 km apart, but to ensure that they do not encounter bees from other apiaries or forage on the same flowers, this distance should be more than doubled. [388]

For example, the female mite (*Varroa*) is transported by adult bees to the larvae, where it reproduces (*Varroa* can live for two months). In addition to harming bees by sucking their hemolymph, the mite spreads diseases such as viruses. Drones are one of the best spreaders of *Varroa*, which during the breeding season are accepted and fed even in different colonies from their own. There are strains of bees that are naturally resistant to this mite and there are mites that have become tolerant to the insecticides used by beekeepers. In France, probably the first *Varroa* mite population to become resistant to fluvalinate (pyrethroid insecticide) was recorded in 1995. [243]

In conclusion, it is important to remember that 85% of all flowering plants depend on pollination by animals, so we must ensure the protection of wild pollinators to safeguard the community of plants that are essential to our survival. [388] At least 15,000-20,000 flowering plants need pollinators other than bees. We can say that the movement of armies of bees, everywhere and without limits, harms wild pollinators and the plants with which they have evolved. We are not in a position to quantify precisely the effects generated by nomadism and artificial competition, but the application of the precautionary principle can limit the damage: do not move bees near areas with wild species of interest, at least until the neutrality of this operation on natural balances is demonstrated. In addition, the number of hives per unit area should be limited according to criteria such as the availability of resources and the presence of wild competitors. Some limits that should be shared, in order to reduce the problems generated by nomadism to reared insects, are the following:

- The establishment of safety zones in which it should not be possible breed insects. Beekeeping and the use of commercial pollinators should be prohibited in these areas. These safety areas could be established to protect the biodiversity importance or to prevent the spread of diseases from regions where they are known to exist. Safety zones could be established around all geographic areas that are homogeneous in terms of ecosystem type, so as to avoid dangerous exchanges and contamination. Safety zones could help safeguard unique bee ecotypes.
- Insect trade should be national or regional. Movement between regions and even more so between countries should be avoided. Insects needed to meet crop requirements should be bred locally.
- Insect breeding for crop pollination should preferably be carried out using local species and native breeds.
- Trade and nomadism of bred insects should be avoided in all cases where they are known to generate hybridization problems with wild species.
- Wild pollinators compete for resources with bred pollinators so the number of colonies per unit area should be limited and managed by ensuring the availability of food for wild animals: in this case pollen and nectar for wild insects.

- Sustainable beekeeping, with a view to safeguarding the biodiversity of bees, should limit the trade in swarms and queen bees, especially in areas where there is evidence of strains adapted to particular conditions. The importation of bees reproduced and selected by humans generates a very dangerous reduction in biodiversity.

These containment and preventive measures are necessary to ensure the sustainability and health of insect farming as well. If people continue to trade and move insects without a serious consideration, beekeepers and breeders of beneficial insects will also suffer serious economic losses. A self-amplifying and self-destructive mechanism has been set in motion: honeybees are bred and their pollen is taken from their colonies and traded to breed bumblebees, which in turn are moved to pollinate crops and die within weeks. Honey is also taken from the honey bees. Honey bee colonies need to be provided with feed to compensate for the lack of pollen and honey. This trade takes place between continents with all the problems that come with it, not least the enormous costs. Considering all the costs, in comparison, dedicating between 10% and 20% of the agricultural surface to semi-natural areas and adopting the principles of ecological agriculture to ensure the survival of wild insects is certainly more economic. Continuing to entrust pollination to market and financial rules means that the number of wild pollinators will decrease and it is not certain that farmed insects will be able to compensate for this shortage. Already today the demand for pollination exceeds the supply. If we believe that we can replace the services freely provided by nature by market rules, i.e. by trade, we risk bankrupting agricultural production that needs pollinating insects. We need to invest in more environmental-friendly and cheaper alternatives before that it becomes too late. The species hitherto used to pollinate crops are few and the biodiversity of these farms is insufficient to provide guarantees of a good capacity for adaptation and survival over time (resilience is constantly diminishing). Insects and wildflowers are the insurance policy for agriculture in the coming years.

Trying to reason about the problems generated by the insect trade, artificial selection, and nomadism with professional beekeepers is difficult and confrontational. The use of pesticides in beekeeping is another unpopular or rather taboo subject. Blind optimism is often expressed, for example about nature's spontaneous resilience, but selective deafness is also evident. Despite the fact that beekeepers are themselves damaged by the unsustainable practices they have adopted, it is a difficult mission to try to convince them to self-limit, to make this activity more resilient. Yet without pollinators we must all imagine a diet less rich in cherries, apples, beans, almonds and many other delicacies. Clover, alfalfa, and other forage crops would be compromised and consequently also the meat production.

THE ANTHROPOCENTRIC GENETIC SELECTION REDUCES BIODIVERSITY

There are many ecotypes of honey bees adapted to different ecosystems and climates: at least twenty subspecies or geographical races of domestic bees (*Apis mellifera*) are known, which differ in their physiological, morphological, and behavioural characteristics. For example, *Apis mellifera ligustica* (widespread in Italy), *Apis mellifera mellifera* or black bee, and Apis *mellifera caucasica* or grey bee are native to Europe and Africa and have been exported to the whole World, where they have come into contact with species such as the Asian ones.

Among the causes of the weakening of bees, there is the disturbing erosion of the genetic heritage of the species *Apis mellifera* and the devastating reshuffling of different subspecies (and local ecotypes). Man-made selection is carried out by choosing colonies deemed best, according to commercial criteria, using artificial insemination and through the sale of queen bees. Among the most artificial practices in beekeeping, there is the collection of sperm for

artificial insemination and the production of royal jelly. Normally this food is secreted by some young worker bees and is not stored. Royal jelly is produced and used immediately to feed the queen bee. [670] Production for commercial purposes implies that bees must be artificially reproduced and forced to store royal jelly in special cells built by the breeders.
Breeding for the production of queen bees for sale began in 1861 in North America, but the technique was perfected in the early 1900s. [972] Therefore, it is a long timr that queen bees are artificially reproduced and commercialized, travelling around the World.
Among the traits chosen by genetic selection by beekeepers over the decades were: [490]

- the higher productivity of honey;
- a lower aggressivity;
- the lesser ability to produce drones;
- the lower propensity to swarm.

Other characteristics that might be desired are resistances to different diseases, the ability to fly at lower temperatures, a greater ability to accumulate wax or propolis, better hygienic instincts, i.e. behaviours such as grooming each other or keeping sick animals away.

Swarming, i.e. the old queen flying off in search of a new home with a big number of worker bees, has always been the reproduction system of colonies. Before swarming, the bees fill their digestive tracts with honey so that they can survive as long as possible away from the colony, i.e. a maximum of ten days. Industrial beekeeping selects bees that are not inclined to natural swarming, preferring artificial swarming, i.e. a reproduction managed by beekeepers. Artificial swarming can produce two or three swarms. The problems, which could be generated by nuclei of bees that are too few in number and without a queen, are compensated for by beekeeping techniques that allow reproduction (artificial insemination is also used), the sale of queen bees, and artificial feeding. The lack of queen bees can be temporarily compensated for by administering special dispensers of chemical substances (hormones) that mimic those produced by the queen bee.
Characteristics are selected that domesticate the bees by satisfying economic interests but make them less able to survive in the wild. Colonies of more aggressive bees, with an adequate number of drones that will spread their genes to other territories and with a marked tendency to swarm, are rewarded by natural selection, but not by the artificial selection operated by beekeepers. Beekeepers influence natural selection by the regular use of medicines against parasites (e.g. acaricides), protecting the bees from unfavourable weather conditions, feeding them artificially, building wax combs, and moving them continuously. As a result, losing characteristics are favoured as they weaken them and make them dependent on technological and energy-intensive beekeepers. As an example, in France, artificial selection with the help of assisted reproduction has replaced the local black bees, which are more suitable and resistant, with strains that have doubled the amount of honey produced. These hybrids are less adapted to the local ecosystem and are less resistant to diseases. [243] Local ecotypes are being extinguished and replaced by commercial strains, often selected in the laboratories located on other continents. Artificially selected strains cross with wild or native strains, extinguishing unique genetic characteristics. Genetic pollution leads to the extinction of phenotypic traits adapted to particular ecosystems.
Queen bee breeders, starting with a few bees and therefore a reduced gene pool, supply thousands of beekeepers all over the World. It is estimated that most of the colonies bred in the USA are derived from a few hundred queen bees. [484] The result is a genetic impoverishment, an amplification of artificial traits, and the pollution of unique traits that generate the erosion of biodiversity, throughout the Planet, with unprecedented rapidity. A general weakening and loss of local characteristics are achieved permanently. The trade in queen and colony bees (which in some cases is also smuggled) also promotes the spread of diseases. [484]

The possibility of buying and moving bees everywhere, together with artificial selection by man, have as side effects:

1) the reduction in the genetic diversity of bees, which also causes a greater sensitivity to pests, pollution and, in general, any change;
2) the extinction of bees adapted to living in certain environments, as natural mating cannot easily be prevented (between farmed and wild bees).

In the World it is estimated that there are at least 31 subspecies adapted to unique environments, which can mate with *Apis mellifera* generating a reduction in biodiversity among the bees themselves. Nomadism therefore allows hybridization with similar but wild species that are better adapted to particular environments: biodiversity is therefore reduced. Natural genetic and behavioural factors that lead to the resistance to parasites such as the *Varroa* mite become extinct. The reduction in the number of small beekeepers and the increase in the number of industrial beekeepers favour this phenomenon: in Europe, more than three quarters of the honey is produced by industrial beekeepers, i.e. those with thousands of hives. As the number of hives per beekeeper increases, the number of beekeepers moving around increases, as does the likelihood of using insects selected and reproduced in the laboratory according to profitability criteria.

Farmed bees have entered an era of anarchic hybridization on a planetary scale: they can be moved and bought at will, without any limits. The most rustic and wild subspecies mate with those selected by beekeepers, destroying important genetic resources forever. It has already happened that the crossing of wild species with bees selected in the laboratory has given rise to new hybrids of insects that have spread in the environment, creating many risks: in 1950, in Brazil, the mating of the African bee with the European one. [230] Hyper-selected bees with reduced genetic variability have spread into the wild with catastrophic consequences. Fragility is promoted, as has happened to many domestic animals (think of the wolf and the dog, or bovine animals), causing an increased susceptibility to certain diseases and to variations in environmental conditions.

The genetic diversity of honey bees is also at risk in Europe. [1244] In order to reverse this trend, action should be taken on several fronts, such as reducing the use of chemicals in agriculture and managing bee reproduction, which should favour the maintenance of local ecotypes. Local adaptations, through regional selection programmes, are a possible way to maintain and improve biodiversity. Beekeepers should be more regional and less global in order to favour strategies for sustaining biodiversity. [1244] Native bees adapted to specific and unique local conditions should be protected and encouraged.

Nomadism has brought several Asian pests to Europe (e.g. the *Varroa destructor* mite) which have participated in the extinction of native subspecies. [490] In order to protect the biodiversity of the different local ecotypes of bees, which are able to mate with bees kept by beekeepers, it is necessary to accept limits to nomadism, trade, and some beekeeping practices. In Italy, a call for the protection of the biodiversity of native subspecies of *Apis mellifera* was released in 2018. [490] This document points out that there are 31 subspecies of *Apis mellifera* native to Europe, Africa, the Middle East, and Central Asia. In Europe and the Caucasus area, 15 subspecies are classified, in Africa 11 subspecies and in the Middle East and Central Asia 5 subspecies. The honey bee has been transported by beekeepers to the rest of the World, such as America and Australia. Nomadism and the bee trade have genetically impoverished local populations, generating an irreversible loss of biodiversity. In order to protect the surviving biodiversity in Italy, consisting of 4 subspecies, it is asked to take targeted actions (*Apis mellifera ligustica, Apis mellifera siciliana, Apis mellifera mellifera* called black bee or German bee, *Apis mellifera carnica*; these last two are probably present only as hybridized populations with *Apis mellifera ligustica*). Unfortunately, this appeal, which is a legitimate and sensible request for greater protection of ecosystems and local biodiversity, was followed by an official communiqué from the Presidents of three Italian National Beekeeping Associations (signed on 31 May 2018). In this communication, some authoritative spokes persons of Italian beekeepers wrote that they do not share the good intentions and spirit of the initiative. Short-term commercial interests prevail: the possibility of setting limits to nomadism and the bee trade is lost.

In summary, actions that could be taken to reduce a further loss of biodiversity (and more) include:

- A ban on the introduction of different subspecies in areas with local ecotypes.
- The establishment of safety zones at least 20 km away from the sites to be protected (e.g. breeding sites). The protection of ecosystems where wild plant and animal species (e.g. pollinators) of particular naturalistic interest reside. Here too, safety buffer zones could be established where insect breeding could be prohibited.
- The ban on the importation of queen bees.
- Tracking all animal movements, including queen bees and small nuclei.
- The registration of wax recycling and trade.
- A ban on trade and breeding of other subspecies of bees in the territory to be safeguarded.
- The establishment of safeguard areas in areas of possible natural reproduction or from queen bee breeding (of at least 200 square kilometres).
- The prohibition of nomadism allowing only sedentary beekeeping.
- The protection of habitats where local ecotypes reside.
- The definition of maximum densities of hives per unit area, based on the type of ecosystem.
- The encouragement of the local reproduction of bee colonies, so as to favour the selection and maintenance of ecotypes with characteristics suited to the habitats of particular geographical areas.
- A ban on the use of medicines to treat bees.
- The registration of all diseases and health problems.
- The registration of all beekeeping practices, including artificial feeding and swarm blocking.

Beekeepers should be encouraged to keep all useful records which could be managed electronically, by using the internet and mobile phone applications. Very useful information could be obtained in real time, enabling rapid action to be taken. This would ensure a more effective prevention of epidemics.

It would be a good idea to set up working tables where we can begin to evaluate the implementation of these measures, which are necessary to save beekeeping.

Among the solutions researchers have put in place to preserve biodiversity, there is the cryopreservation of bee gametes for use in assisted fertilization. This technology may provide some help but it is not a solution.

Safeguarding biodiversity is an indispensable priority. The genome of the honeybee (*Apis mellifera*), which consists of 32 chromosomes (46 in humans), was fully sequenced in 2006. It is realistic to envisage the generation in the laboratory of genetically modified bees that will one day be sold to beekeepers. [167] Already in 2014, the first transgenic bees were generated, capable of transmitting the designed genetic modification to their offspring. [168, 169, 170, 171] It is not absurd to imagine the creation of bees resistant to insecticides-acaricides (used in the field and by beekeepers) and unable to generate productively usable queen bees. As happened with some genetically modified plants, unscrupulous entrepreneurs could hope to privatize and monopolize the pollination service and, more generally, undermine the food sovereignty of the community in order to make huge profits. Artificial selection has rewarded sterile plants with a resistance to herbicides. [175] Something similar could happen with bred pollinating insects. We

must remember that every seed in the heart of a fruit encloses an invisible orchard. The artificial selection of plants that produce sterile seeds or plants unable to reproduce sexually is a suicidal strategy and, for this reason, should be banned and opposed in every way possible. The fossil record indicates that plants with seeds date back three hundred and sixty million years. [704] The artificial selection by man destroys an evolutionary pathway that nature has selected as successful for hundreds of millions of years. Live seeds are the basis of a sustainable agriculture dedicated to biodiversity, in practice the opposite of monoculture.
Artificial selection carried out by man also favours plants that are less attractive to insects or even breaks the symbiosis between insect and flower. Plants that do not need pollination, fruits without seeds or flowers that do not produce nectar. This is the case with new varieties of sunflower that produce much less nectar and are therefore less attractive (the ancestors of today's sunflower plants originated in North America). [230]
Refuge zones, a kind of nature reserve, should be set up to prevent bees with unique genetic characteristics from becoming extinct through hybridization with bees super-selected by geneticists and beekeepers. The establishment of protected areas is the only way to preserve the different ecotypes of endemic bees, avoiding an uncontrolled genetic erosion.
Human intrusion has dramatically altered the necessary balance between pollinators and nature. Pre-printed wax honeycombs and artificial nutrition alter reproduction. The selection of commercial hybrids that travel around the World has impoverished the genetic patrimony, reducing it irreparably. The invasion of man in the life of bees has detached them from the rules of the game of natural selection. As a result, colonies have become hopelessly weak. Probably the wisest decision regarding the selection of bees would be to stop selecting them artificially and let nature do the work. Deciding, conscientiously or by gambling, which traits (genes) to kill off forever or to reward, according to anthropocentric and selfish criteria (submissive to economic rules) is very risky. Not playing the director of evolution in this case could be the winning strategy to save the honey bees from the dead end of the erosion of genetic diversity (and not only). Genetic variability must be kept high so that a chance may allow the survival of traits that can withstand the inevitable changes.

UNNATURAL PRACTICES

There are many activities carried out by beekeepers that interfere with the biology and health of the hive superorganism, some of which are highlighted below.

- In nature, a colony of bees generates several queen bees that will have to fight one with the other to win the loyalty of the entire colony. The bloody selection that takes place between the queen bees, as they kill each other until only one survivor remains, is a strategic and essential event for the colony and the species. This natural selection is completely lacking when it is the beekeeper who provides the orphaned colony with a single queen from another colony or from a farm that is even thousands of kilometres away.
- The physical, geographical separation between colonies of the same race but also between different species (e.g. between Apis *mellifera* and *Apis cerana*) is essential to maintain and select winning characteristics for specific and unique environmental conditions. The breaking down of these barriers, which has been taking place for thousands of years, in the case of beekeeping decreases the genetic variability necessary for the essential processes of evolution.
- Feeding along with the provision of an artificial nest.
- In the natural honeycomb there may be different types of cells: one for workers, one for drones and one for queens. Worker bees build cells with different sizes and characteristics on the basis of a demographic design, providing a certain number for honey, workers, and drones.

Thus, honeycomb composition also guides queen bee oviposition. Artificial wax sheets were invented by a German in 1857, so this artificial process has been altering the natural life of colonies for many years. Pre-printed wax honeycombs with only worker cells hinder or do not allow the breeding of the necessary number of drones that are essential for reproductive and selection functions (natural wax sheets are white while those recycled by beekeepers are yellow). This practice reduces the male population. The use of pre-printed wax honeycombs alters a design that is the result of millions of years of evolution. In addition, recycled wax contains higher concentrations of poisonous substances (e.g. acaricides) and may contain paraffin. By recycling wax, beekeepers obtain several advantages, such as an increased production of honey (bees save energy: they need up to 10 kg of honey to produce 1 kg of wax) and control the population project (e.g. avoiding the growth of drones).

- Another practice that decreases the drone population is the suppression of the male brood with the intent of gaining an advantage in controlling a pest, the *Varroa* mite. Beekeeping practices include replacing combs that have cells for male brood with those for female brood. It makes sense to anticipate that these beekeeping techniques will select mites that will prefer to parasitize the female brood.

- The periodic smoking to stun the bees, with the intention of making them tamer during the inspection, represents another invasion. The bees experience this intrusion as a fire alarm and therefore prepare to flee. Migration is the only strategy for survival in case of fire. The presence of smoke alters the social order.

- Sociality and communication within the colony is regulated by the production of hormones that are single chemicals or mixtures. Chemical communication within the hive is very complex and different bee ecotypes have developed slightly different mechanisms (e.g. different chemical mixtures). Genetic shuffling favours the weakening of this communication system and makes it uniform.

- The blocking of swarming is a habitual and consolidated practice in beekeeping because swarming is considered a serious economic loss. Among the techniques used, there is the elimination of the royal cells, i.e. those that will give rise to the new queen bees, and the replacement of the old queen with a new one purchased from far away (the queen may also be introduced already fecundated). [973] It is amazing that the natural event necessary for the reproduction of the colony, i.e. swarming, is considered a nightmare or a calamity by most beekeepers. Yet swarming is the way a colony generates its own offspring, just like giving birth.

- Swarming, i.e. a fraction of the colony leaving their old home to look for a new one, allows Asian bees (*Apis cerana* and *Apis dorsata*) to fight and control pests such as the *Varroa destructor* mite. The health value of swarming is underestimated. The systematic blocking of this behaviour (e.g. by using hormones), together with the overcrowding of colonies in territories and nomadism, favour the spread of one of the main enemies of beekeepers. Another practice used to prevent swarming is to cut off the queen bee's wings, preventing her from flying away.

- The continuous, often annual, programmed change of queens hinders natural selection and facilitates genetic homogeneity, weakening, and the spread of diseases.

- Bees host microorganisms that make up the microbiota and at least a dozen species of bacteria are necessary and useful. The microorganisms have evolved with the different ecotypes of bees (subspecies), but this heritage has been altered due to the mixing of honey bees all over the World and due to molecules with an antibiotic action (e.g. some medicines used in beekeeping and some pesticides used in agriculture damage the microbial flora).

- The practice of installing an anti-*Varroa* bottom to control the parasitic mite *Varroa*, i.e. a wire mesh under which a metal tray is placed, has become widespread. In this artificial bottom it is possible to control the presence of mites that fall both naturally and following the use of acaricides. Bees need an environment that can be cleaned; this system hinders cleaning and facilitates the presence of another parasite: the wax moth.

- It is possible buy hormones that are released into the hive in a controlled manner and remain active for weeks. In this way it is possible induce specific behaviours artificially. One can buy mixtures of hormones normally produced by the queen bee for different purposes: [224]

  - to reduce the production of drones;
  - to keep the bees quiet in the absence of queen bees;
  - to stop the swarming;
  - to lure bees into a queenless hive.

Many practices interfere with the biology and health of bees and other pollinators. When man intervenes in the management of nature, he causes damage which, in some cases, shows its effects long afterwards. Limits should be accepted to safeguard our future.

MONETIZING NATURE'S ESSENTIAL SERVICES: A REDUCTIVE AND ANTI-ECOLOGICAL APPROACH

THE ESSENTIAL POLLINATION SERVICE FOR FOOD SAFETY

Pollination may occur by wind (anemophilous), by other abiotic factors (rain, gravity, electrostatic forces) or through animals such as birds, bats, mammals or insects. Plants have adopted many different strategies to spread pollen and fruit. For example, a herbaceous plant native to Canada (*Cornus canadensis*), whose flowers are about 20 cm above the ground, uses a mechanical system to catapult pollen grains at a speed of three metres per second, allowing them to reach a height ten times greater than that of the flower. [986]

Wind and insects are the most important agents for pollination. Probably 135 million years ago most plants relied on the wind for pollen, wasting a large amount of it. Flowers initially hired insects to carry pollen and later began producing nectar to reward them. Plants fertilized with the help of insects were very successful, diversified, and began competing to attract them. This competitive drive helped the diversification of flowers and the specialization of the relationships between plants and insects (e.g. a 6 cm moth can have a 30 cm long proboscis, this is the case of *Xantopan morganii*). [688] Bees are among the pollinating insects par excellence, and derive from wasps that were probably carnivorous.

In the case of entomophilous pollination some plants are highly specialized in that they can only be fertilized with the help of one or a few insect species. In some cases, very specific symbioses have been established such as that of the fig plant. For some flowers, a single visit by pollinating insects is sufficient to bring in enough pollen to form the fruit. In other cases, pollen transported during several visits is necessary. In plants such as apples, pears, melons, pumpkins, kiwis, a single visit by the pollinating insect is not sufficient to deposit the necessary pollen. [469]

Estimating the value of the natural capital, i.e. trying to monetize the services freely provided by nature, is a difficult task: there is a risk of a gross underestimation. Oversimplification leads to not considering important aspects, which in many cases are still unknown. When we manage to give a monetary value to the important services offered by ecosystems, it will probably be too late, as we will be forced to quantify irreparable damage. Some attempts to quantify the benefits generated by pollinators to agricultural production are explained below.

In Europe, about 80% of crops and wild plants depend on insects and, at the same time, at least 9% of bees and butterflies are certainly seriously threatened (over 50% in some states). [260, 481] In Europe 84% of cultivated species benefit from the presence of pollinating insects and, in the World, 87 crops of the 124 used for human consumption (70%) benefit from the presence of pollinating insects. [753] In this regard, it is useful to remember that the average economic value of agricultural production which does not depend on pollinators (e.g. cereals) is about 151 euros/ton, while for those which depend to some extent on pollinators it is 761 euros/ton. In 2005 the World agricultural production of the 46 crops, which is at least partly dependent on pollinators, was estimated at 39% of the total value. [753] Pollinating insects contribute to the 2005 production for an estimated value of 153 billion euros. It is considered that among 46 crops only 6 are very dependent on pollinators (more than 90% of the harvest is lost in the absence of pollinators) and 13 have a high dependence (between 40% and 90% of the harvest is

lost in the absence of pollinators). According to this criterion, only some crops are at risk of not generating any economically interesting production in the absence of pollinating insects, while for other crops production is reduced quantitatively and qualitatively but continues to be of some economic interest (e.g. reduction of less than 30% in the absence of pollinators). Thus, the vulnerability rate is variable and may be over 90% in the case of some pollinator-dependent crops such as coffee and cocoa. Crops for which there is an unquestionable economic benefit from animal pollination probably account for less than 10% of the World agricultural production, but help to generate at least one third of the market value and, more importantly, are the main source of essential nutrients such as some vitamins. [753] To stay healthy we should eat at least 400 g of fruit and/or vegetables per day (excluding cereals), but in Europe 50% of the population probably does not reach this nutritional target; the situation is more serious in other countries.
Another estimate reports that about 87 of the 115 most important species (75%) grown by humans are benefited by the presence of pollinators, such as almonds, apples, cocoa, and coffee. These 87 crops account for 35% of the total World agricultural production. [348] A decrease in the presence of pollinators (there are at least 40,000 species, of which about 25,000 are bees) could cause a reduction in food production of at least 5-8% and a decrease in choice. [193, 481] Crops dependent on animal pollination are the main source of some important micronutrients such as vitamin A, vitamin C, and folic acid. [481] Reducing the pollination service could adversely affect the variety of foods available and the quantity of micronutrients. As a result, it could promote the spread of some preventable diseases and increased deaths (e.g. 1.4 million more deaths due to heart disease). [481] To compensate for the 3-8% loss of production of some plant species due to the decline in pollinators, the area under cultivation would have to increase by at least 25%. [855] This calculation is purely theoretical and does not consider many factors, but it gives an idea of the importance of pollinated plants in our diet.

DIFFERENT PLANTS BENEFIT FROM DIFFERENT POLLINATORS

Several strategies may be used to assess how important the pollination service is. [469] One can artificially block the possibility of flowers receiving insects by covering them with special nets. In this way it may be assessed whether entomophilous pollination is important. In some cases, even seeds of plants that can self-fertilize, when crossed with genetically different plants, produce more vigorous plants. With these experiments, it is easy to discover that plants such as almonds, apples or plums need to be pollinated by specific varieties, so the choice of the pollen type is important too. Studying the importance of the contribution of wild pollinators requires special techniques such as the use of video cameras.
The ability of an insect species to successfully pollinate a plant may be measured by assessing:

- the amount of pollen transported on each trip, per day, and the amount of pollen released in each female flower (inside the stigma);
- the number of flowers visited on each trip and the number of flowers visited during flowering;
- the insects' dispersal area;
- the number of seeds and fruits produced as a result of visiting flowers (this number may be compared with that obtained by the artificial deprivation of entomophilous pollination);
- the quality of the fruit obtained: colour, size, shape, quantity of sugars, acidity, absence of defects, turgidity, aroma and nutritional value (vitamins and mineral salts) in the presence or absence of entomophilous pollination;

- the quantity of fruit per plant per unit area;
- the ability of seeds to germinate and generate vigorous plants;
- the resistance to stressors such as pesticides;
- the fidelity of insects to a species (it is the ability to look always for the same type of flower and this is called floral constancy);
- the attraction generated and, therefore, the preference between different types of nectar and/or pollen;
- the density of insects and/or colonies needed to fertilize the flowers present in 10,000 square metres. In the case of honey bees, usually between 2 and 5 colonies and up to 25 colonies per hectare are used (e.g. in the case of blueberry blossom). [469]

This information makes it clear that assessing the quality of the pollination service and estimating the economic benefit is not straightforward. In many cases there is insufficient information on the contribution made by wild pollinators, whose role may be underestimated.

Strawberries pollinated by bees (e.g. solitary bees such as *Osmia* and to a lesser extent by honey bees) achieve a higher commercial value than those pollinated by the wind or self-pollinated. Fruits will be heavier, have less deformations, record a higher sugar content, and be more colourful. [676] Resistance to storage is increased by 11%; in this regard it is useful to remember that in the case of strawberries most of the fruits could become unmarketable after only 4 days. All these factors increase considerably the economic benefits of the pollination service for strawberry producers. An interesting aspect is that strawberries are 65% pollinated by wild bees, such as *Osmia* (52%), bumblebees (*Bombus terrestris* for 4%) and other species (another 4%). Honey bees contribute 34% and some flies for the remaining part. Thus, honey bees are not the most important pollinator for improving strawberry quality: overall at least 12 wild pollinator species, including solitary bees, are more important. Similar results have been obtained for coffee, cocoa, melon, loquat, rape, and cucumber plants. [676] Pollination by wild animals (wild solitary bees in the case of strawberries) is very important and is difficult to assess. Hoping to solve the problem of insect decline by relying on some sort of universal pollinator, such as honeybees, in the future is naive and wrong.

Insect pollination in Europe increases the quantity and quality of the production of rape (*Brassica napus*), field beans (*Vicia faba*), strawberries (*Fragaria x ananassa*), and buckwheat (*Fagopyrum esculentum*). The quantity, thanks to the insects, increases between 18% and 71%; moreover, rapeseed will contain more oil and strawberries will have a higher commercial quality. [747] Even for these crops, domesticated bees can only partially compensate for the lack of wild pollinators. On average, 13 pollinator species were recorded in strawberry fields and 11 pollinator species in fields of beans or buckwheat. The average number of pollinator species varied between 2 and 26. Improving biodiversity around crop fields increases pollinator presence, crop quality and quantity as well as making the agricultural system more resilient to changes such as the climate change.

Simplifying agricultural ecosystems and entrusting most of the plant production to a few domesticated insects is very risky and unsustainable. Universal pollinators do not exist. Confirming this fragility, evaluation of the effectiveness of different categories of insects on four different types of apples, grown in the UK, has shown that the insects are not equivalent. Some apple varieties are pollinated more successfully by some insects. [709] In general, solitary bees were found to be more important than honeybees and honeybees more effective than bumblebees. The importance of wild bees is confirmed, as they generate economic benefits for apple producers that are more than twice as high as the benefits obtained by using domesticated bees (*Apis mellifera*). In three apple varieties (*Cox*, *Gala* and *Bramley*) over 54% of the pollination service is provided by wild insects and only 25-28% by domesticated honey bees (between 13% and 21% by bumblebees). Wild pollinators are very important to support and improve the agricultural production of apples, as domesticated bees are less effective. In the

UK, the pollination service in apple orchards generates profits between €8,500 and €14,500 per hectare per year, depending on the variety. To sustain this profitability, it is important to help wild pollinators by ensuring semi-natural areas with plants and flowers that provide pollen, nectar, and nesting sites. The pesticides acetamiprid, thiacloprid, imidacloprid, thiamethoxam, and myclobutanil may be found in apple orchard flowers (in the USA), and in pollen and nectar (at concentrations of up to 70 ppb): farmers and beekeepers should establish more forward-looking symbioses. [1224]
Estimating the economic value of the service generated by pollinators such as bees has many uncertainties and does not adequately account for important ecological factors. Economic factors are also difficult to assess, e.g. the people employed in almond, cocoa, coffee and apple cultivation would lose their jobs: at least 1.4 billion people in the World are employed in agricultural work. [481] Another critical aspect that is underestimated is that generated by monopolies: to give an example, the market for cocoa production is controlled for the most part (80%) by two big companies; despite this potentially favourable condition (few players involved), it is still difficult to have information on the ecological and social conditions of the crops. [980]

THE ECONOMIC ESTIMATE OF THE SERVICE PROVIDED BY POLLINATORS

There are different estimates of the value of the pollination service which are very variable, depending on the species and conditions considered. Some species partially depend on pollination to produce quality fruit, others are pollinated by different insect species and, therefore, it is more complicated to estimate the contribution provided by farmed or wild bees. Between 80% and 90% of wild plants depend on pollinating insects, and 60% of birds feed also or only on insects.
Estimates of the value of the pollination service, offered by both farmed and wild insects, have many limitations and may be considered underestimates. To get an idea of the figures considered by experts, several estimates of the economic value of pollination are given:

- 260 billion of euro per year (all pollinators on the Planet). [196]
- Between $235 billion and $577 billion (2015) per year (all pollinators on the Planet). [481]
- The services provided by wild insects, in the USA, are quantified as at least $57 billion per year. [346, 348]
- Between 78% and 94% of the European wild flowers depend on pollination by biotic factors. The value of insect pollination in Europe is estimated to be around €22 billion per year and 84% of the crops depend directly or indirectly on pollinating insects. [13]
- 153 billion of euros per year (all pollinators for the 100 most important crops), of which €14.2 billion of euros in the European Union. [243, 864] This figure is equivalent to about 9.5% of the monetary value of all World food production in 2005 (which is estimated to be at least €50 billion for vegetables, an equal figure for fruit and €39 billion for oil crops). [753]
- In Europe, the value of the pollination service provided mainly by bees, between 1991 and 2009, generated wealth equal to 12% of the value of the entire European agricultural production. [707] In Europe, the dependence and therefore the vulnerability of the pollination service increases from north to south, in parallel with the increase in the number of plant species that require pollination: Italy and Spain are the most dependent on pollinators. The most important European crop in terms of sensitivity to pollinators is apples, followed by peaches. In the World, this dependence is lower: the disappearance of pollinators could produce a loss equal to 9.5% of the value of the entire agricultural

production (in 2005). The economic value of agricultural products dependent on pollination is closely related to the biodiversity of insects. In synthesis, it is realistic to attribute to the service of pollination an economic value between 9% in the World, and 12% in Europe, of that of the entire agricultural production. This is undoubtedly an underestimate, since only the increase in production generated by the presence of pollinators is evaluated considering the market prices of the various vegetable products. [707] Other aspects that are more difficult to quantify should also be considered, such as the damage to ecosystems that would be generated by the disappearance of pollinators (at least 2,000 species of insects in Europe), the loss of jobs, the increase in illnesses due to nutritional deficiencies (e.g. vitamins A and C), the damage generated to crops that depend on insects only for reproduction and not for production (e.g. potatoes) and the damage generated to feed production.

- In Europe, the pollination service in agriculture generates an economic value of at least €22 billion, or 10% of the economic value of food production. [733] In Europe, the pollination service is useful for 12% of the cultivated areas and is essential for at least 3% of the agricultural surface. In Italy at least 2.5 million hectares of agricultural land benefit from the pollination service provided by insects, in Germany or Greece 2.2 million hectares, in Spain 5 million hectares and in France 6.1 million hectares. [733] The complete loss of the pollination service in Europe is estimated to generate damage to food production of between €190 and €310 billion.

Below are some economic estimates of the benefits provided by pollinators in some crops (in USA dollars per hectare): [766]

- watermelons (USA; 2009): 267 - 5,400;
- coffee (Brazil; 2003): 2,415;
- rapeseed (Ireland; 2009-2011): 652;
- strawberries (Europe; 2009): 14,968;
- raspberries (United Kingdom; 2010): 7,641;
- apples (UK; 2010 and 2012): 14,000 - 25,000;
- blueberries (Canada or USA; 2013): 20,000 - 26,500;
- tomatoes (Canada; 2001): 434 - 2,344.

On a planetary scale, depending on the estimation method used, the average economic benefits generated by the pollination service range from a few tens of dollars per hectare, up to several thousands per hectare (the standard deviation is higher than the average value, indicating a considerable uncertainty factor). The different estimation methods generate values that differ by a factor of more than 10 and up to 1,000, so the results are highly uncertain. For example, estimates of the economic value of the pollination service are reported for coffee between less than $160 per hectare and over $2,400 per hectare and per year. In general, in North America or Europe, the economic value of the pollination service is estimated at over $1,500 per hectare per year. [585] An example: almonds and blueberries depend on entomophilous pollination and, for the former alone, this service generates a global economic value of about 265 billion euros per year. [585]

To have another term of comparison, bumblebees have been bred for about 30 years to pollinate tomatoes. Previously, tomatoes in greenhouses were pollinated with special sticks by teams of workers who passed over the flowers three times a week at very high costs. [688]

Simplistically, several methods were used to evaluate economically the service performed by pollinators to obtain the above mentioned estimates: [760, 763, 864]

- The cost necessary to support pollination using operators who proceed manually, in place of natural pollination.
- The cost due to the hiring of bee colonies, which replace wild insects, during flowering.

- The reduction in the economic value of the crop resulting from the lack of pollinating insects. In this case, the market value of the crop is multiplied by the quantity lost due to the lack of pollinators. Crops are classified according to the reduction in quality and quantity of fruit in the absence of pollinators. For some crops the dependence is very high: reductions of more than 60% in quantity in the absence of pollinators. In many crops it is difficult to assess the contribution from wild pollinators, so their value is often underestimated.

These different methods applied to the same crops give very different estimates and, in any case, do not consider many other ecological and economic factors. Among the weaknesses of the estimation methods it is possible to highlight that: [766]

- They are influenced by factors such as the price of renting bee colonies and selling plant products, which in turn depend on market rules that change geographically and over time.
- The work done by wild pollinators is ignored or underestimated.
- The benefits generated by pollinators to wild plant species, which are necessary to maintain important balances also for farmers, are ignored or underestimated. In many agricultural areas of the Planet, where fruit trees dependent on pollinators are grown, the number of hives used per hectare is usually lower than recommended: it is estimated that 41% of the recommended hives per hectare are used. [763] This figure allows us to predict that the shortage of domestic bees is compensated by the presence of wild pollinators, for which it is difficult to estimate the economic contribution.
- Issues such as loss of jobs, reduced availability of nutrients and reduced food self-sufficiency are not considered.

Worldwide, it is estimated that 71% of the 100 plant species that provide 90% of food have some benefit from pollination by animals and, in Europe, 84% of the 264 crop species are pollinated by animals. [585] In developed countries, it is estimated that the area cultivated with plants requiring pollination increased by 17% between 1961 and 2006. Another estimate reports that 75% of the Planet's 115 major crops benefit to some extent from pollination. [676] Wind pollination or self-pollination is sufficient for about 28 crops. Pollinated crops account for between 6% and 12% of agricultural production (in quantity) but 35% of the market value because they are more profitable than, for example, cereals (which do not require an entomophilous pollination). [676]

MONETIZING THE NATURAL CAPITAL IS REDUCTIVE AND DANGEROUS: COMMON GOODS MUST BE PROTECTED

Estimates are always very rough as it is impossible to measure the natural capital accurately. [1189] For many crops, the contribution of wild pollinators is unknown, and for some crops, the impact of the service provided by farmed insects is unknown too. [753] It may also be observed that the method of analysis applied is very anthropocentric in that it does not consider the indispensable natural services that biodiversity ensures for the survival of the Planet and agriculture. Estimating this value is difficult, but we must always remember that the survival of ecosystems is necessary to maintain a human society in peace and equilibrium for long periods. Estimates also fail to quantify all economic aspects such as jobs. For example, if the estimates include crops used to produce feed, the economic value of the pollination service could increase by at least a quarter; the economic benefits generated by pollinators to food production are underestimates but, nevertheless, are considerable figures.

It is very reductive and too simplistic to limit oneself to considering the estimate reported by some researchers according to whom, in the absence of pollinators, we will probably produce

10% less food for the human species. Although this is a negative prediction, it does not provide the correct measure of the seriousness as it does not consider the catastrophic events related to the collapse of biodiversity and the services offered by nature. At the same time, it is necessary to highlight that the current pollination service continues to show signs of suffering, as both wild and farmed pollinators are in decline: in the USA a 59% reduction in domesticated bees was recorded between 1947 and 2005, and in Europe a 25% reduction between 1985 and 2005. [758] Thus, today the demand for pollinators by the agricultural and livestock sectors exceeds the natural and artificial (beekeepers) supply. If this discrepancy between demand and supply of pollination is not stopped, a dangerous and irreversible crisis is easily foreseeable.
Another aspect that is difficult to estimate concerns the distribution within the society, among the different income brackets, of the damage caused by the reduction of pollinators. A study conducted in Brazil, one of the most important agricultural areas in the World (e.g. coffee), estimated that 68% of the 53 main crops depend to some extent on pollinators (36 crops out of 53). In the World this dependence percentage is estimated at 74%, in Europe at 84% and in Mexico at 80%. In Brazil, one of the important crops is sugar cane, which is not dependent on pollinators. In Brazil 59% of the agricultural surface is cultivated with plants dependent on pollinators (e.g. soybean and coffee) and a reduction in the pollination service could cause an alarming decrease in GDP (Gross Domestic Product); moreover, at least eight million people work in the coffee production sector which requires pollination. [758] In this country, the reduction of pollinators would mainly harm the poorest, especially in rural areas. Sixty percent of the food consumed by Brazilians depends on pollinators and one third of the World's coffee production comes from this area. So preserving natural areas, reducing deforestation, reducing habitat fragmentation, decreasing the use of pesticides (Brazil is one of the biggest users of the herbicide glyphosate), and preserving pollinators, in Brazil, helps the local population but also the rest of the Planet.

Plants that depend on pollinators not only provide food but also medicines, agro-fuels, fibres, musical instruments, and building materials that are very important. In addition, there is also a value that is difficult to monetize, such as that of psychological health due to the benefits generated by living near green areas and being able to enjoy recreational areas surrounded by greenery.

The commodification of the services offered by nature is very risky and is a welcome approach to the rules designed by economists and entrepreneurs, which are based on certain anti-ecological and self-destructive principles:

- it is forbidden to forbid;
- everything has a price;
- any natural asset can be bought, privatized and destroyed;
- the environmental costs generated by businesses must be distributed to the community;
- disasters are bequeathed to future generations;
- there are no ecological limits.

ANOTHER VISION OF THE WORLD MUST BE POSSIBLE

In this book, which began with the pretext of describing the World of bees, in order to highlight some obvious ongoing catastrophes (the bee die-off), an attempt has been made to summarize a massive amount of figures and data that are both illuminating and easily accessible and indisputable. The feeling is that the slow but inexorable and brutal change towards which we are rushing does not generate the necessary upheaval, interest and debate. The general silence is very worrying and at the same time surprising, not least because there are not many possible loopholes. Wealthier people can hope to take refuge in luxurious communities, away from the big cities where the suffering is likely to be greater and reveal itself sooner. [975] Running away and hiding from prying eyes is definitely not the solution. Neither can tragic formulas such as suicide or the choice not to procreate be solutions: how can one think of procreating with the awareness of these predictions!

We have to deal with the dynamics that tend to bring the human society to a collapse, but we must try not to be overwhelmed by what can be considered a deluge of bad news. Amazing technologies have been created but we have the same Stone Age emotions and institutions that we have retained from medieval times.

We are fast approaching a new reality that frightens us, a World in which we fear the arrival of summer, in which we are constantly insecure because of the probable arrival of extreme events (drought, cloudbursts, lack of resources), where cities, despite their high level of artificiality, retain greater biodiversity than the surrounding agricultural areas which are now devastated. Climate change and the systematic ecocide of non-humans are looming as terrifying nightmares.

The selfish human being, who by choice blindly gets submitted to economic rules, is no longer natural. The *Homo oeconomicus* has married the culture of consumerism and capitalism, destructive and self-defeating. The new World we are creating will hardly be better than the one we left and delays in decision-making are fatal. The well-being of the community depends on a good state of conservation of nature, which cannot fall below certain thresholds. When the limits are exceeded the level of social security slips into unacceptable deprivation. We are blinded and mankind's capacity to do has far exceeded our ability to understand the consequences of our actions. As a result, we are continually generating new and more serious problems, also because technological innovation offers new and increasingly powerful means of altering the biosphere; one thinks of the new frontiers of artificial intelligence or the possibility of modifying the heredity of species, including that of humans, thanks to biotechnology. The crisis is not only environmental but also moral, cultural, of civil coexistence and democracy.

The massive attack launched by humans on the biosphere will reveal itself in a collapse that is unlikely to be sudden, it is more likely to be gradual. Progressive but unstoppable negative changes anticipate a sad and inexorable end. Partial and isolated collapses warn us of a likely systemic collapse. Anticipation and waiting can be more painful than the extreme and final event.

Climate change, if you look at it carefully and in perspective, can be compared to a state of war. Like any war, it generates anger, despair, confusion, difficulty in making farsighted and altruistic decisions. We can define it as suffering from ecological disaster, as that which is experienced following the disappearance of an ecosystem and the environment in which one has lived (for example due to hurricanes, floods or desertification). Climate migrants know exactly what this is, a feeling of desolation, despair and loss of identity. Being sad after a catastrophic event can be considered a sign of good mental health. Destruction of what we hold dear may make us unable to react but it highlights a positive attachment to the Earth, which is what could save us.

The deepening of certain themes involves a high risk for mental equilibrium, especially if one is not in a position to organize with one's own strength an escape route, as some rich people can achieve. Awareness generates emotional storms that risk leading to nihilism. Knowledge combined with powerlessness easily discourages. Add to these factors one’s isolation, such as intellectual isolation, and it is difficult not to slip into sadness and negative thoughts. Being immersed in anxious information on a daily basis and at the same time being exposed to the ignorance or apathy of the community around us generates an awareness of irreversibility and impotence. To this one often adds - aggravating the unpleasant sensations - the denial, the indifference or the hostility of those who would have the means, such as the intellectual, social and economic ones, to act, at least at a local level. Trying to remain detached and neutral, as scientists would like to show themselves, is a sign of weakness and misunderstanding of the seriousness. Instead, the feeling of urgency, of concern, should be expressed and it is important to try to convey it. In many communication strategies, such as some of the scientific ones consulted to write this text, detachment and neutrality are perceived as misplaced, as emotions are indispensable in an effective communication process that touches one of the most distressing issues we can imagine: the collapse of ecosystem services necessary for the health of humankind.

Time passes, events precipitate faster and faster, and it becomes unrealistic to think of miraculous solutions, especially if one mistakenly expects them to be proposed by a few individuals, such as politicians and administrators of the public good. This is probably an unsolvable problem, like death. This level of acceptance and at the same time understanding is disarming and depressing. Backing up after suffering this shockwave is a titanic task.

We must invert the scale of values that in society is based on artificial rules, by-products of human culture, that give little value to natural resources such as raw materials (e.g. soil, wood, water), a little more to processed products (industry) and a lot to virtual products such as money and finance. We must invert the priorities of values also because soon the value of natural resources will become inestimable (e.g. that of water). We must imitate nature, bearing in mind that:

- Waste does not accumulate in ecosystems (think of forests as a simple example of circular flows).
- Almost all the energy used is solar, i.e. it comes from photosynthesis. So it is necessarily local, it cannot travel long distances (except in some cases like in the oceans and for the water cycle).
- Mutual aid, i.e. symbiosis, is essential in nature to be successful. The basic principle of personal enrichment is a loser: unfortunately we have espoused this belief for too long. Unlimited material appetite is self-defeating.
- Nature rewards diversity: it is more stable and more resilient. We systematically and unknowingly destroy it, not only the wild but also the cultural one.

If we continue not to deal with the environmental crisis, we will soon find ourselves without the means to deal with it: think for example of a Planet with a climate of + 6°C. To find ourselves in the midst of a change of such magnitude and hope to live with dignity is beyond our strength.

In order to achieve the indispensable change of vision, a general mobilization is needed. Few people seem to be aware that we have entered a war with nature and we are losing it. We are destroying the Earth that is our home and our future, just as if we were at war. As we would do in war, a massive mobilization is needed to reforest and stop climate change. We need to organize a great rapid and coordinated transition. Among the enemies that must surely be opposed, there are the oil industry, capitalism and the inaction of the powerful. To be afraid of war and to prevent it is a sign of sanity and love for one's own and other's lives; to enter the war, that is, to be willing to sacrifice oneself with the goal of reducing climate-altering gas emissions by 70%, one must be aware of the emergency. Unfortunately, most ongoing environmental collapses are slow and do not generate the sense of urgency and danger necessary to promote a mobilization equal to that of a World war. Moreover, our hierarchical social system delegates important decisions to others. It therefore easily creates the illusion that someone else will do it for us, for our good. The rigid hierarchical organization of our society is an aggravation of the current catastrophe because it promotes selfishness (every man for himself) on the one hand and indifference (someone else will take care of it) on the other. In times of crisis, cooperation and coordination at a local level are an effective defence strategy. Resilience in difficulty will depend on human relationships and, therefore, on very close ties.

Stop acting to save the Planet, in the absence of the feeling of immediate danger, is the easiest solution. Not acting today certainly means not being able to avoid violence tomorrow: the ecological crisis will bring insecurity, poverty, conflict, chaos for everybody, but decline and difficulties also for the rich and powerful of the Planet. The standard of living in Europe is sustained by the equivalent of several hundred energy slaves (at least 400). It means that each of us exploits an amount of energy equivalent to the workforce of hundreds of people, irreversibly altering the biosphere and using finite resources such as oil. If, as it is easy to foresee, energy slaves (fossil fuels) diminish, disappear or can no longer be used, our standard of living will necessarily have to change. To survive we will have to adapt to doing hard work such as farming.

Climate change may surprise us in a negative way: we Italians may also become climate migrants and seek asylum elsewhere. Let's suppose that 50% of the population is forced to find a new home, the other 50% will necessarily have to take in. We are all potential migrants. Mutual aid and altruism, in times of crisis, are winning strategies, also adopted by many examples of non-humans. Ecologists by profession teach that altruistic groups beat selfish ones. The society based on competition and accumulation of goods, above real needs, is a failure, even more so when resources are scarce. Unity is strength: a better measure of wealth might be the number of people of which I trust and who are willing to help me in difficult times. Ties and the network of solidarity will be increasingly important in a system destined for decline. Wisdom and solidarity are needed.

I have lived most of my time delving into these distressing issues in solitude, behind a screen and consulting texts, also because the environment around me is not receptive, and at times I even perceive it as hostile. When I tried to discuss these issues I found myself among people who do not want to know, refuse to believe or consider me irrational, a spoilsport, an announcer of misfortunes, not real or very distant. It is not easy to try to predict that soon it might not be possible to travel by plane or buy food from the other side of the World or that the private car will become a luxury good; they are considered uncomfortable and disturbing predictions, far from the dominant imagination. Talking about the predictable predictions of collapse is almost impossible, one is constantly censored. Materialism, competition, and selfishness prevail.

The intellectual desert, superficiality and ignorance that I often perceived were essential factors in the writing of this work. Sharing the pain of the ecological disaster, in this case with the community in which one lives, could have brought relief, created a common sense and encouraged the organization of concrete actions. Hope must not be lost and must not be accompanied by passivity, it must help to build projects and imagine a different and better future.

One of the most difficult moments is the confrontation with young people, with students, the workers of tomorrow. They listen and ask: what can we do? Why don't we do what is necessary to avoid these disasters?

An increasingly evident point of view, in a society rooted in unbridled consumerism for decades, well represents the perception of the future by some adults: raising children in this society is a selfish act because they are destined to suffer. What will young people be able to tell us when they have matured the full awareness of reality: you have exhausted most of the Planet's resources, you have exterminated almost all living species. and pollution, besides destroying the biosphere, generates unacceptable diseases and suffering.

One choice for the preservation of a mental balance might be to espouse individual voluntary ignorance. Mass ignorance could also be promoted. In the latter case it could be programmed by the state offering systems of public information, education, and training that do not address issues deemed inconvenient.

The freedom of the choice not to procreate by someone is perceived as an individual solution in the direction of eco-responsible actions to save the Planet. Population growth and the increase at an even faster rate of the Planet's degradation are crucial aspects that should be managed in a collective context, but perhaps society is not ready to deal with such delicate and private issues. What would we tell our children when we inevitably need to talk about them? How have we chosen the welfare of one generation at the expense of the next? We will have to explain that we have not been able to listen to the messages that nature and the World we live in have been screaming at us for some time. Our carelessness and voracity have blinded us: we are witnessing the ecocide, the mass extinction of non-humans, without caring, with a distressing indifference. We have to imagine and build a different society, a better one, where we would like to live and where we hope our children can live. There are no simple solutions or ready-made recipes, we have to invent everything bravely listening to nature.

DO SOMETHING NEW WITH THE OLD AND IMITATE NATURE

SIGNALS HAVE BEEN WARNING US FOR SOME TIME

For each of the anthropogenic factors that generate negative impacts on biodiversity or the ability to produce food, it is easy to think of solutions. The spread of sustainability in the coming decades, on a planetary level, will depend mainly on what form agriculture will take. Unfortunately, food shortages are easily foreseeable and may be attributed to the greed and individualism of the human species, for which peaceful solutions are not easy. The economic law of unlimited exploitation, both of natural resources and of labour, is sure to lead to a collapse. [36] Factors that have already been largely explored are chemical agriculture, loss of biodiversity, reduction in soil fertility, climate change (e.g. drought, extreme weather events) and the spread of alien species. Unsustainable livestock practices also include industrial beekeeping with nomadism, the use of medicines, artificial selection and feed.

The increased mortality of honeybees and insects in general (pollinators and non-pollinators) favours a reduction in the capacity to produce food: in the long term, both quantity and quality will decrease and the possibilities of choice will be reduced.

There are many estimates of the amount of food that depends on the service given by bees, which, by the way, is well remunerated in many parts of the World. According to some estimates, honeybees are responsible for the pollination of 71 of the 100 most important species used for consumption and generate a value of over $200 billion (between $200 and $400 billion). [198] Some scholars propose that the value of pollination by bred bees can be estimated at about 60 times that of the sum of all bee products. [199] It is difficult to predict what might happen if bees were to decline dramatically, but food security would certainly be significantly compromised. An increased bee mortality is one of the many alarm bells that signal bad choices, but we keep repeating them. The problems of beekeeping are a very worrying alarm and the only possible solution is to seek a better balance between economic interests, the biological and physical limits of the Planet, and the indispensable functionality of the services offered by nature. The answer is therefore the spread of sustainable agriculture. [609] The danger pointed out by beekeepers is accompanied by many other more worrying ones, such as air and water pollution, reduction of biodiversity, climate change, and loss of soil fertility.

No further proof is needed. We have information that leaves no doubt. In spite of this, a sort of social contagion is taking place, involving scientists and experts in the various incriminating fields in a worrying way. It is a form of epidemic that draws attention away from the facts (inconvenient or frightening, depending on one's point of view) to untraceable ghosts, such as new and unknown causes for the increase in bee mortality in recent years (see *Colony Collapse Disorder*). There is no need to discover new factors, we know enough to explain the ongoing ecological disaster and all of them can be linked to human action. [36, 529]

The adoption of compensatory measures to counter the decline in the presence of pollinators has become a priority for the agricultural system and not only. The use of pesticides, the destruction, fragmentation and isolation of natural habitats has made it very difficult for insects to survive. One of the best compensatory measures is to dedicate a portion of agricultural land to annual and multi-year wildflowers. Reforestation and the creation of semi-natural areas facilitate the restoration of eco-systemic balances that have been destroyed by chemical agriculture. Several crops are only possible due to the presence of wild or semi-natural areas in

the vicinity, which allow wild pollinators to perform their useful service, as it is the case with the Brazilian walnut or cocoa.
We have entered an era that has been called the *Anthropocene*, but to better represent the disaster we are generating we can suggest that we are entering in the *Eremocene*: the era of solitude, in which human beings will be surrounded only by the few species bred or cultivated for their own survival. Even this vision is optimistic because man cannot live without wild animals and the presumption of being able to decide with which living beings to share the Planet creates imbalances that will generate the collapse of the human society as well. Possible solutions must be shared with a serious but difficult dialogue with strong economic interests that practically dictate the rules today: the chemical industry, biotechnology, plant breeding (seeds) and producers of fossil fuels and derivatives (e.g. plastics, cars).
In conclusion, it is difficult to think that the human species will be able to starve to death if only farmed bees get extinct; however, we can realistically assume that changes that generate an increased mortality of bees and wild pollinators will cause serious problems for the biosphere and, consequently, for the human community.

THE TRAGEDY OF THE COMMON GOODS

The species that provide over 90% of food are less than 15, they are the result of artificial selections, they cannot be reproduced by farmers and are protected by anti-ecological rules that allow nature to be privatized. Therefore, foods are monopolized by patents, are sterile or are no longer able to survive without the support of the chemical industry and mechanization, i.e. fossil fuels. With the patenting of seeds, the right of farmers to save and share them is being treated as theft, in other words as a crime against intellectual property. It is enough to succeed in modifying a gene in a plant for a big company to be able to claim protection and commercial rights that will produce enormous profits to the detriment of the community and to the detriment of the future. Farmers can no longer save the seeds, because it becomes a crime, but worse is happening. If the pollen of genetically modified plants transfers the patented characteristics to non-GMO cultivations (e.g. with the wind), the farmers risk being sued by the companies that own the property rights: this has already happened. [175] In a fairer World it should be the patent holder who should have to compensate, because of the genetic contamination of originally non-GM crops. In other words, large biotech companies are being allowed to appropriate of fields planted with non-genetically modified plants.
Biotechnology has opened the way for new forms of colonization with devastating effects equal to those of a real war. Farmers are turned into criminals because they steal (unintentionally) patented genes and are forced to pay compensation to the biotech giants. In practice, common sense is being turned upside down along with one of the basic principles of environmental protection: the polluter pays, because in this case it is the polluter who is paid. The irreversible effects include a reduction in biodiversity, as well as an increase in food insecurity and poverty. In the absence of an effective collective protection system, such as that which public administrations and politicians should play, civil disobedience could be a way forward. Erroneous faith in obedience to unjust laws will continue to spread poverty, hunger, and ultimately slavery. Seeds should be protected by a true "right" as they are a living system that must be free and available to the community, without the threat of extinction through biotechnological sterilization or genetic contamination.
Biotechnology companies have managed to organize their profit plans properly in many aspects which are briefly mentioned here, such as the lack of the possibility for consumers to know what foods are used in what products derived from genetically modified organisms: think of soy derivatives, such as lecithin which is an emulsifier found in chocolate, ice cream, and many

other products, or soy oil which is probably the most widely used vegetable oil in the World, and in animal feed. It is almost impossible, for most food products, to know all the ingredients used (e.g. wine, cheese, confectionery, ice cream), to know where the ingredients used come from, or to have information on the protection of workers and the environment adopted by entrepreneurs. Why not also make public the pesticides used for each lot, soil and company?

The strategies adopted by biotechnology companies are based on a distorted vision that involves the entire capitalist system. Property and extraction rights are protected, as if they were natural, from which only the owners of large companies benefit. [845] As a result, the destructive power of multinationals, which are now economically stronger than entire nations, cannot be limited. The well-known condition of the *tragedy of the commons* has been created. When the public thing becomes a business managed according to private criteria, arrogance, cunning and, ultimately, the law of the strongest prevail. In this model the State becomes an enemy or a subject to be corrupted.

It is increasingly clear that we need to rethink human laws and their relationship to nature, as scientific knowledge is used to dominate nature and control human beings, while law is designed, increasingly, to protect the extractive freedom of the few. Instead of protecting the indispensable services that nature offers us, we have protected the right to plunder it. Through the organization of a juridical system, which dates back at least to Roman times, and which defends private property, the freedom to appropriate and destroy things that are necessary to all and that should instead be considered the property of all has been affirmed. An alliance between private interest and public power has in fact been established. The administrators of the State, through politics, can privatize the common goods, transferring resources fundamental to the survival of all from the public sector to the private sector. This process has created an imbalance, namely a constant and irreversible flow of public resources into a few private hands. Today it is very evident that public power is incapable of counteracting the possibility for multinational corporations to acquire the control of human and natural resources, at their pleasure and everywhere.

We need to rethink the relationship between man and the environment, since we currently consider ourselves the masters of nature and not a (non-essential) part of it. Leaving food security and food sovereignty in the hands of a few powerful entrepreneurs is a carelessness that will cost dearly.

As long as the system remains conditioned by a few private interests and knowledge and information in such strategic and vital matters are monopolized by a few big players, it will be impossible to design a society with less inequality and more peace. If we leave supermarkets free to think about us, they will do so, but according to undemocratic entrepreneurial and financial principles. Some useful aspects of fostering a more sustainable behaviour are often opposed, such as: [749]

- The dissemination of information on the ingredients used and their origin.
- The dissemination of information on eco-guarantees.
- The dissemination of guarantees on the protection of the health and dignity of workers.
- The encouragement of local and seasonal foods whenever possible.
- The reduction of the consumption of animal products.
- The decrease in the use of packaging.
- Not supporting with public funds strategies that are harmful to the environment and health, such as subsidies to agro-fuels and GMO production; not financing anti-ecological systems such as sterile plants or agro-technologies that increase the use of fossil fuels and pesticides. [741] Fossil energies receive enormous subsidies: probably between 6% and 7% of the World GDP. [776] Here it is useful to remember that the fifty biggest fossil fuel companies account for half of the global industrial greenhouse gas emissions. The 20 most industrialized countries in the World generated more than two-

thirds of all global emissions. At least theoretically, acting on a relatively small number of private companies and governments would be enough to bring about big changes. Among other things, some of the most important companies are, at least in part, state-owned. We could argue that an international agreement on reducing the use of fossil fuels is needed to stop climate change and unsustainable agriculture. We cannot allow the burning of known reserves, because before they are exhausted we will have turned the Planet into an inhospitable place.

- Getting serious about the polluter-pays principle. Not paying for the cost of environmental damage is a very generous and unintelligent indirect subsidy. Taxation and disincentives for pollution, such as the use of pesticides and fossil fuels, should be increased.
- Giving a value to water systems, soil regeneration, the chemical composition of the atmosphere, the richness of ecological diversity, photosynthesis, to name a few. Our society is experiencing an environmental crisis that is much greater than the current economic crisis and, consequently, the environmental disaster will have an increasingly evident impact, not only on social security and health, but also on economic and financial accounts. Therefore, it is necessary to attribute values (monetary, ethical, moral, ecological) to the natural capital and to protect it, defending it from degradation.
- Considering the ecological footprint of goods and services, comparing them and eliminating all the superfluous ones. Energy footprint, water footprint, land consumption, and energy balance are some of the ecological issues that should be seriously addressed in a systematic way for every human activity, especially those that pollute the most and are unnecessary. [36]
- Considering the health costs generated by environmental disasters and political compromises such as those on pesticide use and fossil energy (e.g. in China, health costs generated by the use of coal-fired power plants amount to 5% of the GDP). [680]
- Making scientific publications free of charge. Very often scientific publications are accessible for a fee, but scientific research that is even partially funded by public resources should be accessible to all.
- Most of the results of scientific research are not considered by politicians or farmers, who are among those who could do most to bring about the necessary change. Therefore, dissemination, in an understandable language and at an affordable cost, should be supported much more strongly.

Probably, for the first time in the history of mankind, big private companies, i.e. armies of tens of thousands of workers located in all the economically strategic points of the Planet, are able to subdue entire Nations and Continents. It happens, among other things, that groups of well-organized and trained workers, representing the interests of a few tens of entrepreneurs, very often unconsciously, fight and win a war against nature and the remaining majority of humanity. A few entrepreneurs decide the fate of producers, distributors, traders, and consumers of entire Continents. Ultimately, they are able to influence or even control the sovereignty and food security of the majority of us, especially those who live in countries where less than 10% of the population is involved in agriculture. It is a war in which the losers are the new poor, the hungry, the thirsty, the climate refugees, and those sickened by the environmental disaster. Yet, a small increase in the income of the poorest would be able to generate a much more significant increase in well-being than an increase in the income of the already rich.

Unfortunately, this is a conflict on a planetary scale, so we could compare it to a World war, the devastating effects of which will be amplified over time and will generate very sad repercussions on future generations. There is an obvious disparity of forces in the field between those who have an interest in manipulating the environment or are indifferent to it, and those who are actively engaged in defending the common good. Many more defenders of the commons are needed to hope to achieve the necessary results. It is disarming to measure the inability of the human society to limit its self-damaging power: at present the interests of a few prevail to the detriment of the community.

HORRORS OF JUDICIAL REPORTING ON BIOLOGICAL DAMAGE FROM ARSENIC

In 2011 there was a news story that underlines the inability to limit the freedom of those who damage the community and the common good. In some municipalities of the Province of Viterbo (in Italy), high values of arsenic were found in drinking water, i.e. higher than the value in derogation (20 μg/L). [36] At the same time, delays were reported in the construction or commissioning of plants to reduce arsenic concentrations. In 2011, a file was opened against unknown persons by the Viterbo Public Prosecutor's Office (Procura della Repubblica di Viterbo) and investigations were ordered for the presence of arsenic above the permitted limits in water intended for human consumption. At the judicial level, the lack of transparency in the disclosure of information to the population also had its consequences. On 22 January 2012, the Lazio Regional Administrative Court (Tar del Lazio) issued a ruling condemning the Ministry of the Environment and the Ministry of Health to pay compensation to citizens of the municipalities in the regions concerned (Lazio, Lombardy, Tuscany, Trentino Alto Adige and Umbria). In these regions, water for human consumption containing arsenic at concentrations higher than those permitted by the derogation was distributed. The compensation was set at 100 euros, per person served by the incriminated aqueduct, for a total of approximately 20,000 euros. [887] The judgement states:

> "*The wrongful act constituted by the exposure of the users of the water service to a risk factor, at least in part traceable, in terms of magnitude and time of exposure, to the violation of the rules of good administration, determines a non-asset damage overall compensable, by way of biological, moral and existential damage, for the increased probability of contracting serious infirmities in the future and for the psycho-physical stress and the alteration of personal and family life habits consequent to the delayed and incomplete information of the health risk.*"

The judgment is of considerable and particular importance because it condemns the public administration for the risk to health alone. However, the "economic" evaluation of the damage to health, in this case, is evidently too low, and is certainly insufficient to constitute a deterrent. Moreover, the following comments arise spontaneously:

1) no natural person in the public administration is identified as being liable for the damage;
2) the economic compensation is financed by the users/citizens themselves, in an unconscious and invisible way;
3) The sum of 100 euros to compensate those who have been exposed to arsenic underlines how unbalanced the system is in favour of short-term private interests.

EXPLORING NEW SOLUTIONS IS A NECESSITY

The prevailing economic and market rules are based on several erroneous principles:

- if you consume what you produce, you might as well not produce it;
- it is essential to privatize profits and socialize losses;
- postpone the solution of the ecological crisis to future generations.

Some of the immediate effects on the farmers are indebtedness and no longer being able to buy the products grown on their land; they are also forced to sell the fruits of their labour in a manner predetermined by someone else. The result is a paradox: half of the people who suffer from hunger are farmers and waste are increasing, such as using more than 10 kilocalories of cereals to obtain one kilocalorie of meat, or occupying the land to produce agro-fuels or agro-plastics. [36, 165, 741] In many cases it is sensible to argue that returning to ancient customs and practices will be a step forward for sustainability and resilience.

Anti-democratic laws incentivize hunger and malnutrition and must therefore be opposed, before apocalyptic environmental and social conditions are inevitably created. The ecological crisis will damage humanity as well as the Planet. It is better to stop before.

Society seems to have seemingly entered a tunnel with no way out especially if fossil fuels are used to the point of environmental collapse, which could happen sooner than we imagine and certainly long before the end of known underground fuel supplies. Some foreseeable consequences will be as follows:

- it will reduce the numerosity of the population;
- we will go back to living the way we lived at least one century ago;
- peace and social security will become precarious.

In the long term we are creating the conditions for a drastic reduction in the numbers of our species and the number of people who will be able to lead a decent life. It is necessary to focus our attention on a fundamental point: exploring new solutions is not a whim but a necessity.

While it is true that many obstacles remain in our minds, others seem objectively insurmountable. It is very likely that the difficulties generated by the ecological, economic and social crisis into which we are plunging will force us to seriously consider the principles of ecologically attentive agriculture and therefore dependent on nature. We will probably re-evaluate agricultural practices that are now considered obsolete because they were adopted over a century ago. We must not forget that the amount of food produced, per square metre and per calorie used, was incomparably higher in the Parisian market gardens of the 1850s than on modern farms. The efficiency was much greater but the flip side of the coin is not negligible: only a small fraction of the population could engage in intellectual work or jobs other than food production.

The diversification of the agricultural ecosystem produces benefits for the environment but also for farmers, as production becomes more resilient to change and less dependent on external resources such as pesticides and energy. Using different strategies in an appropriate way can enable more sustainable agricultural production and, at the same time, ensure the necessary profitability.

Agricultural policies should encourage the application of sustainable methods and practices in food production. A modulation of incentives could be envisaged according to the intensity of the adoption of the proposed criteria. An example is a model proposed in England (the *Environmental Stewardship*) which mainly foresees three levels of adoption of sustainable agricultural practices, with different forms of incentives. [661] Among the agronomic practices that are incentivized, there is that of leaving strips sown with wildflowers (annual and multiannual), at least 6 metres wide, at the edges of fields, and bans or restrictions on the use of pesticides and fertilizers are planned. [612, 660]

BIODIVERSITY IN URBAN ENVIRONMENTS

The first cities, with over 50,000 inhabitants, were probably built after 6000 b.C. In 2011, more than 1,400 cities had a population of at least one million, and 22 cities had more than ten million inhabitants: in the 19th century, only the city of London had one million inhabitants. Today, the Planet's urban areas occupy about 3% of the Earth's surface but are home to more than 50% of the World's population and this concentration, according to many forecasts, is destined to increase. According to some studies, the migration from rural areas to cities implies a several-fold increase in *per capita* resource consumption. [980] Agricultural environments, especially in some countries where industrial monoculture is the norm, are so degraded that beekeeping becomes difficult if not impossible, and wild species no longer exist. In general, the urban environment is also not conducive to the survival of wild species.

Cities have a considerable ecological footprint, even over five hectares per inhabitant, and are the source of most of the Planet's carbon dioxide emissions. [978] The separation between humans and nature in cities reaches its maximum expression, which is also manifested in excess consumption of resources above what is necessary to ensure a high level of quality of life. Life in the urban environment has been disconnected from nature for generations, fostering a poor attitude and sensitivity to its preservation.

Within urban areas there can be many spaces dedicated to green areas such as public and private gardens, botanical gardens, sports facilities, and urban vegetable gardens. Many of these artificial areas, from the point of view of biodiversity, are of little interest, such as the grass lawns of private gardens or sports facilities. Botanical gardens, in small areas, may have much higher levels of biodiversity than the surrounding agricultural environments, but they are limited and artificial. Increasing the areas dedicated to public green spaces generates various benefits for fauna and flora, but also for the physical and psychological health of the inhabitants. In some cases, urban green areas, being very often the result of projects disconnected from the surrounding nature, favour problems such as the spread of invasive species. In some cases, health problems are also favoured for a part of the population such as allergies (e.g. from pollen).

The artificial condition of urban environments favours the presence of few species that are adapted to the artificial ecological conditions such as mice, mosquitoes or sparrows. In some cases, these species may be carriers of diseases, such as the West Nile virus, which is transmitted by mosquitoes (e.g. genus *Culex*) and is carried by some birds (e.g. *Passer domesticus*), which promote its spread through migration (e.g. in the USA). [978]

Conditions in urban environments may favour particular species such as bats, which take advantage of artificial light to attract the insects on which they feed. Artificial lighting may alter the cycles of some nocturnal species as has been recorded for frogs and some birds.

Temperatures in the cities are on average higher than those in the surrounding countryside, and for this reason some species are attracted, such as starlings, which take advantage of this to sleep in the heat. Others are attracted by the huge amounts of food available in the waste deposits (landfills) such as the gulls.

In general, the urban environment favours a few species such as gulls, magpies, crows, blackbirds, rodents, foxes and a few insect species (e.g. flies, mosquitoes, bugs). The examination of the faeces or the contents of the digestive tract of some urban animals confirms that most of the food, e.g. of gulls and foxes, is of anthropogenic origin (e.g. waste).

Urban environments near natural areas may be the greatest cause of mortality. For example, road transport is the greatest cause of mortality for animals such as fox, hedgehog, coyote, armadillo, and white-tailed deer in Virginia. [978]

Chemical control is used to control the spread of certain species considered to be a nuisance such as rodents and insects (e.g. mosquitoes). The use of poisons such as pesticides or rodenticides also causes problems for non-target species. Rodenticides harm birds, such as some birds of prey, and other mammals such as coyotes, lynxes, squirrels, deers, and hedgehogs. In the case of coyotes, studies show that after road accidents, rodenticide poisoning is the second most common cause of death in urban environments. [978] Little is known about sub-lethal and synergistic effects. Pesticide use has also reported problems for non-target species such as many birds: the red kite (*Milvus milvus*) poisoned by carbamates (in France), the brent goose (*Branta bernicla*) and the Canada goose (*Branta canadensis*) poisoned by diazinon in the USA (due to distribution in New York meadows). Herbicides have also been reported to have adverse effects on wildlife. Molecules such as atrazine and glyphosate have produced lethal and sub-lethal effects (demasculinization) in amphibians (e.g. frogs). [978]

Another negative phenomenon recorded in urban environments is the damage generated by domestic animals that are often abandoned and adapted to live in a semi-wild state such as cats. Hundreds of millions of cats (perhaps more than 600 million) have been released worldwide and they are non-native species and potentially dangerous to biodiversity. In the USA it is estimated that the presence of several tens of millions of free-roaming cats generate many problems such as bird predation and the spread of diseases (e.g. *Toxoplasma gondii*, *Giarda* spp). Cats could be responsible for the killing of more than 3 billion birds and 20 billion small mammals every year in the USA alone. [978]

In general, increasing urban plant biodiversity, creating corridors, certain practices such as not removing dead trees, not cutting lawns, not using chemicals such as herbicides and insecticides, benefit many species and are an act of love towards the next generation.

RESTORING AGRICULTURAL ECOSYSTEMS TO A SEMI-NATURAL STATE

In the World of modern agriculture, respect for the environment is usually set against productivity, wrongly implying that nature is not productive. To give an example of greater agricultural sustainability, a forest planted with chestnut trees produces more protein than the equivalent area planted with wheat, but does not require tillage and needs less input in terms of fertilizers and pesticides. Tree orchards are more sustainable and more should be invested in their design (the so-called food forest o edible forest). The forest is one of the most productive forms that nature has designed: tropical forests produce over 40% more dry matter per square metre and year than temperate deciduous forests, and the latter produce almost twice as much as cultivated land. [738]

In general, a forest produces at least twice the biomass of crops, without human intervention. It is important to point out that in natural ecosystems annual plants were the exception or the antechamber to desertification. However, even in a forest, if more is taken than the renewal capacity or the natural capacity to increase fertility, an unsustainable system is generated.

We have destroyed nature by replacing it with completely artificial monocultures, which use the soil to transform oil into food: at least ten or twenty calories of fossil energy for every food calorie on the plate. Moreover, chemical agriculture encourages genetic homogeneity. Most crops, such as vineyards and orchards, are clones, i.e. genetically identical plants grown on huge areas, everywhere on the Planet. These plants are reproduced not by seed but by cuttings, grafting and micropropagation.

It is unthinkable to increase food production without destroying nature: no civilization can last long if it wastes more than ten calories to produce one. The Planet can no longer afford societies populated by human beings in which only a small fraction is involved in agriculture: in the USA 1% with an average age of over 57 years or 3.5% in Italy. We must also reflect on the fact that a social model that cannot be extended to the entire World is morally unacceptable. Among the imaginable solutions, in addition to demographic and consumption reduction, one can hypothesize an improvement in the link between man and nature through the self-production of food. A society based on consumption and waste, as numerous as that of the most industrialized and rich countries, where less than 4% of the population is involved in agriculture, is not sustainable. [837] There are numerous proposals and experiments in designing a more sustainable agricultural production. They go by different names, such as agro-ecology or permaculture. [738] The principles on which they are based are often equivalent, such as wanting to slow down the transformation of nature into an edible or urbanized landscape. Among the steps necessary to spread this type of agriculture, founded by small producers and highly diversified, is information and dissemination. The connection between schools and the agricultural World, between citizens and urban greenery, could encourage very useful individual and collective behaviours. The different forms of agro-ecology provide some suggestions: [837]

- implement polycultures (different plant species grown at the same time in the same plot) and annual plant rotation (annual monocultures in chemical agriculture can mistakenly be repeated in the same plot for several years continuously);
- counteract the use of plants and animals that cannot be reproduced by farmers;
- encourage the reproduction of plants and animals locally (encouraging the creation of nurseries of plants, centres for the conservation of local species and small animal breeders); encouraging self-sufficiency in the reproduction and propagation of seeds and plants is a fundamental step;
- leave a part of the surface (10% - 30%) in a semi-natural condition, i.e. occupied by wild species and without human intervention;
- do not use pesticides;
- reduce the need for fossil fuels: the elimination of pesticides alone could reduce energy needs by one third;
- replace herbicides with mechanical mowing and other strategies such as constant ground cover;
- do not use chemical fertilizers;
- encourage the recycling of organic matter such as manure and food waste;
- do not burn agricultural residues;
- compost locally and without plastic;
- ensure minimal tillage;
- do not use genetically modified plants;
- reduce the consumption of animal derivatives;
- invest in local economies such as energy and food production in the area (on average, in the USA, food travels over 2,400 km);
- protect ecosystems by involving local communities;
- calculate and advertise the energy consumption per unit of kilocalorie obtained;
- encourage tree orchards;
- reduce the need for irrigation;
- collect rainwater;
- do not use plastics (e.g. in mulching, beekeeping);
- oppose large-scale retailers especially if they do not commit to providing more information: on the ingredients used, on the origin of raw materials, on the protection of workers, on the protection of producers (e.g. of raw materials), on the environmental protection strategies adopted, on the kilometres travelled, on the water footprint, on

emissions (such as greenhouse gases), on the pesticides used, on the quantity of energy used per kilocalorie, on the reduction of packaging, on waste recycling, etc.

Our society is so detached from nature that it cannot imagine that agriculture may be a solution for many of the environmental challenges. Some of the principles of cultivation aimed primarily at energy and food self-sufficiency and, at the same time, increasing sustainability are as follows:

- dedicate between 50% and 60% of the cultivated area to plants that produce biomass as fuel, building material, feed, compost, fruit and for services offered free of charge by nature such as clean air and water, preservation of biodiversity and soil protection;
- cultivate 20-30% of the area with tubers, cereals, legumes and other crops that provide energy and proteins;
- dedicate 10% of the cultivated area to vegetables.

Throughout the Planet fortunately there are still areas of nature that if left simply to their own fate could expand and restore degraded areas in close proximity. There are semi-natural areas that have been partially degraded and could be restored to their original condition with simple interventions, such as the elimination of alien species and the reintroduction of native species. However, there are huge areas of very degraded land where ecosystems should be restored from top to bottom (e.g. the Po Valley). In these cases, as in the agricultural and urban environment, intervening is not easy and is in any case an artificial manipulation. Projects of reconstruction of semi-natural areas from very degraded areas require skills, organization, determination, perseverance, and participation, therefore they are not easy to implement. In some cases, one could learn from the past. Various agricultural techniques invented and perfected in the Mediterranean basin were based on maintaining a mosaic of vegetation consisting basically of forest, pasture and cultivated fields or, in drier areas, arboreal grazing was favoured. In this way, a certain level of compromise between ecosystem resilience and productivity useful to our species is obtained.

Refuge zones could be established for different ecotypes of bees in order to preserve unique genetic characteristics. In isolated and regulated areas, the ability of different subspecies of bees to survive in the absence of beekeeper care could be tested. A very important challenge for the future is the ability of honey bees to survive without the care of beekeepers. Protected and characterized genetic reserves could be set up and, at the same time, their ability to feed and resist diseases without human intervention should be tested. We must remember that natural selection has worked and could continue to work miracles, such as rewarding the appearance of insects resistant to insidious parasites.

Some of the simple but effective recommendations are those of crop rotation, intercropping, minimum tillage and many others already known to our ancestors. We must remember that in the past, in the general culture, waste was considered a sacrilege and many solutions were based on cooperation.

Predatory agricultural practices, as non-conservative, include tillage. The beneficial effect on pollinators resulting from no-tillage could be investigated. It must be remembered that probably 70% of the bee species build their nests in the soil. Ants may be mentioned and that, in general, the soil ecosystem benefits from no-tilling.

In annual crops and orchards, replacing herbicides with mechanical mowing has many advantages:

- the soil is protected from erosion;
- the loss of soil fertility is reduced;
- the surface water runoff is slowed down;
- biodiversity is increased;

- the community of organisms in the soil (micro-organisms and invertebrates) is not damaged;
- other animals such as pollinators and birds are not harmed;
- water is not contaminated and the food chain is not poisoned.

It should be pointed out that in some cases mowing may still be necessary after the use of herbicides, in order to reduce the dry biomass that could ignite and obstruct the passage. All the more reason not to use them.

Reducing the use of fossil energy in agriculture will require increased work efforts by a larger fraction of the population than is the case today. There is, however, another major obstacle. In order to achieve greater or almost complete energy and food self-sufficiency, it is necessary to have several hectares of fertile land per inhabitant. This aspect constitutes a considerable limitation, since, for example, in Italy we have less than 5,000 square metres *per capita*, of which about one third is urbanized and only one third is cultivated. Due to the limited availability of fertile soil it is not possible to propose an agricultural system completely independent of fossil fuels.

One evidence of the disconnection from nature is in our school educational programmes where agricultural work and food production are completely absent. We do not have the widespread ecological knowledge and agronomic culture necessary to ensure the self-reproduction of plants adapted to various environments.

INCREASING THE CHANCE OF SURVIVAL OF THE ENEMIES OF CROP PESTS

Among the practices proposed for conserving natural or semi-natural ecosystems near agricultural areas, there is the strategy of habitat manipulation to favour natural enemies of plant diseases and organisms useful to farmers, such as wild pollinators. Increasing the number of species in the agricultural ecosystem is a strategy that goes in this direction: it increases diversity to favour organisms useful to farmers. However, this is an anthropocentric view of how the agricultural ecosystem biodiversity could be improved, so it is partial and incomplete. It has the advantage of being a more ecologically sustainable proposal than the current intensive monoculture.

Environmental restoration increases resilience to changes in the agricultural system, although it is a vision limited by human economic interests. For example, some insects that eat other insects still need, at specific stages of their life cycle, to feed on pollen or plants. Therefore, the presence of plants, all year round, near cultivated fields favours the presence of refuge zones and sources of nourishment for the entire life cycle of many useful insects. In this vision, the design of the agro-ecosystem must be carried out by increasing the percentage of surface areas not dedicated to cultivation. Refuge zones for organisms useful to farmers provide alternative food sources, and constitute areas where nests can be built (e.g. in the soil that is not ploughed).

Increasing the biodiversity of the farm should provide benefits in terms of energy, resources, and pesticides saved. The agricultural ecosystem should be manipulated with the intention of receiving some of the energy and resources needed to sustain the profitability of production from natural sources, such as wild pollinators rather than farmed bees, or carnivorous insects and insectivorous birds rather than insecticides. This generates a production system that is more diverse and more resilient, i.e. able to withstand a change for longer. The increase in the biodiversity of the agro-ecosystem helps the spontaneous generation of a greater "immunity" to pests. More complex balances are generated that tend to limit the dominance of a few species such as those that feed on crops. Intensive monoculture does not constitute a suitable environment for the survival of the natural enemies of pests and implies a drastic reduction in biodiversity and a simplification of the agricultural environments which become an inhospitable desert for most of the wildlife. Food becomes abundant only in a few weeks a year and for a few species, which will be mainly those that feed on cultivated plants. Ecologists have long known that reducing the biodiversity of an ecosystem favours a few species, which often invade the environment because they have no competitors. For example, the number of herbivorous insects tends to increase as a result of simplifying ecosystems. Furthermore, pesticides such as insecticides are often more harmful to carnivorous insects than to herbivorous ones. In addition, predatory insects are often less able to develop resistance to insecticides than herbivores, which are in many cases more numerous and less mobile.

Some beneficial insects need to feed on crop pests (e.g. hymenoptera and coleoptera feeding on aphids) and need flowers and refuge areas to complete their cycle. Beneficial insects may need a complex network of plants with which they communicate through chemical olfactory signals. To facilitate the growth and development of natural plant enemies it is useful to have corridors with flowers and refuge areas in which to complete the life cycle, and to have the availability of food sources other than prey. This is the case with a wasp (*Trybliographa rapae*) that parasitizes harmful insects, such as the cabbage fly (*Delia radicum*). [644] *Delia radicum* is a small fly (Diptera) in which the adult feeds on nectar, while the larva feeds by digging tunnels in the roots and leaf petioles. [645] Affected tissues are subject to rot. The females of the wasp (*Trybliographa rapae*) that parasitize the fly (*Delia radicum*) are attracted by volatile substances produced by plants attacked by this dipteran and by substances produced by flowering plants, whose nectar is part of the wasp's diet. Therefore, the simultaneous attraction by the diseased plants and those producing nectar helps to counteract the presence of the cabbage parasitic fly. [644] The dipteran allows reproduction while the nectar feeds. In order to be successful, this type of biological control requires the simultaneous presence of both insect and nectar.

Another strategy that has been explored for several decades, but has not led to extensive applications, such as pesticide distribution, is the artificial breeding of beneficial insects. The release of bred beneficial insects in the field has been carried out for dozens of species, although the applications are not widespread and often they are only in particular situations such as greenhouse crops. However, also this practice, from the ecological point of view, may turn out to be a disaster. We must remember that one of the main causes of the reduction of biodiversity, together with the destruction of ecosystems, is the spread of alien species.

ENCOURAGING MOSAIC AGRO-SYSTEMS

Two other useful strategies to reduce pest abundance and increase biodiversity are polyculture, i.e. the simultaneous cultivation of different species, and the substitution of annual crops with multi-annual ones. For example, the simultaneous cultivation of different varieties of rice has produced among the benefits a reduction in the use of fungicides. In general:

- increasing biodiversity also by growing local varieties,
- building corridors and semi-natural islands,
- reducing the extent of monocultures,

are practices that promote a mosaic agrosystem, which is more resilient and sustainable. Polycultures are less exposed to pest damage and changes such as climatic or economic ones. Production based on uniformity (genetics) is now a major threat to biodiversity and socio-economic sustainability. The myth that productivity is incompatible with polyculture must be debunked before it is too late. Many studies show that polyculture generates more nutrients per unit area. In this regard, we should try to reason according to the criteria of health per hectare and take into account parameters that measure efficiency and sustainability, such as the ratio

between energy invested and energy obtained, the variation in soil fertility and water consumption per unit (e.g. calorie or kilogram). In many cases we are already beyond the limits of safe operation that the biosphere allows us. Industrial agriculture, in many cases, increases hunger and poverty, and reduces productivity in terms of ecological efficiency. It is worth noting that on less than 30% of the arable land, small farmers produce 70% of the food consumed in the World. While big companies use 70% of the land to produce 30% of the food. These data show that small farms are more efficient and ensure a greater agro-diversity. Small farms, especially in times of crisis, may be a valid solution to fight hunger and poverty. Unfortunately, in industrialized countries such as those in Europe, the opposite trend may be observed: between 2007 and 2010, owners of farms smaller than 10 hectares lost 17% of their arable land (an area larger than Switzerland); instead, farms with more than 50 hectares acquired several million hectares. [175] The strengthening of family agriculture, which diffusely organizes and protects cultivated areas, ensuring small communities the autonomy to survive, is a solution to be encouraged also in industrialized countries. It is necessary to re-evaluate, re-qualify, and support the development of small-scale primary production aimed at sustaining local communities.
The global production of cereals in 25 years has more than doubled (from 0.6 to 1.3 billion tons in 2014), while the use of arable land has increased much less (about 12%). [843] This figure does not consider the increase in energy inefficiency (ratio of energy invested to energy obtained), does not apply a negative value to environmental devastation (introduction of alien species, use of pesticides, exploitation of soils, deforestation, fires, etc.) and does not take into account the fact that losses due to pests and waste are likely to decrease food availability by between 30% and 50%.

One of the weaknesses is that we often lack the knowledge needed to restore pseudo-natural balances with the aim, for example, of reducing the use of pesticides. The ecological interactions between tens or hundreds of species, which live in the soil thanks to plants, are largely unknown. In most cases, crops are completely artificial plants that are alien to the original ecosystem, which has often been permanently destroyed and extinguished. In the rare cases in which the cultivations are found in their original habitat, that is in the one that has not yet been completely destroyed by man, it is possible to leave it to nature. It is sufficient to leave uncultivated areas close to the wild ones to register improvements, which are guided by ecological rules tested by natural evolution.

POLYCULTURE IS ECOLOGICALLY BENEFICIAL

The simultaneous cultivation of agriculturally important species on the same area may be a useful strategy to reduce the negative pressure of some pests. In fact, plant pests tend to be less abundant, by more than 48%, in annual polycultures compared to annual monocultures; plant pests may be reduced by more than 60% in the case of polyannual polycultures compared to traditional ones. Some examples are given: [694]

- oats grown with beans to control aphids;
- maize grown with clover, soybean or pumpkin to control various pests such as the corn borer, aphids or mites;
- melons grown with wheat to control aphids;
- peach trees grown with strawberries to counteract a moth.

The simultaneous cultivation of different species reduces the danger of some diseases, even of over 74% compared to monoculture; poly-cultivation may reduce the presence of pathogens even of 64%. [610] Intensive monoculture favours the disappearance of insects and birds, altering some balances that are necessary for agriculture too.

The simultaneous cultivation of different plants is an improvement from an ecological point of view, especially if the agricultural landscape becomes a mosaic of small plots, with different species alternating over time. It is possible to experiment with the association of different plants, one of which may constitute the main crop and others may serve various purposes such as ensuring nourishment even when one species is seriously compromised.
It is important to note that, by increasing the biodiversity of the agricultural ecosystem, it is not obvious that the presence of crop pest enemies decreases significantly. Increased diversity ensures a better resilience to change and adverse events.
We need to think in a less anthropocentric way and not with predominantly economic objectives, to be achieved exclusively in the short term. Uncultivated areas are much less disturbed by practices that make life impossible for useful organisms. In uncultivated areas the frequency of ploughing, fertilizing, spraying with pesticides, and irrigation is zero or at least lower than in cultivated areas. The increase in diversity counteracts the synchronism of plant cycles, typical of cultivated areas, improving the presence of the necessary nourishment for wild organisms for longer periods. We have sufficient evidence to sustain that increasing biodiversity in the agricultural system improves resilience, i.e. the ability to resist change. This aspect is sufficient to justify investments in this direction.

THE ANNUAL CROP ROTATION DOES NOT COMPROMISE THE ECONOMIC OBJECTIVES

Increasing the biodiversity of the agricultural ecosystem, decreasing the use of chemicals (pesticides and fertilizers), and reducing the use of fossil fuels are fundamental objectives. There are many studies showing that increasing biodiversity and adopting more sustainable agronomic techniques does not reduce or increase productivity and, more importantly, costs are drastically reduced. In cases where a high risk of crop loss cannot be tolerated and where not many economic resources are available for cultivation, diversification of the agro-ecosystem is one of the best insurances against these risks: especially for farmers who primarily do it for their own food, i.e. the majority.
The alternation of different crops over the years produces another great advantage: the reduction of the danger of certain plant diseases, such as those caused by certain fungi of rice in China or barley in Germany. [610]
An example is given of a study carried out in North America (Iowa) between 2003 and 2011: the system of a two-year crop rotation, consisting of maize and soybean with extensive use of pesticides and chemical fertilizers (e.g. nitrogen-based), is compared with more sustainable methods. [609] Three-year rotations [maize - soybean - wheat (triticale or oats) and clover] or four-year rotations [maize - soybean - wheat (triticale or oats) - alfalfa], accompanied by at least a partial replacement of chemical nitrogen fertilizers by cattle manure and a reduced use of herbicides, have yielded similar or superior economic results. [609] The use of plants to fix nitrogen (alfalfa and clover in this case) and the enrichment of the soil with the use of animal manure are strategies that have been used since memorable times. The practice of crop rotation generates another great advantage over repeated monoculture: the reduction of the number and biomass of weeds, even between 50% and 80%. [610] By increasing the complexity of the rotation and reducing the use of chemicals (herbicides and chemical fertilizers) an equivalent or higher profitability is obtained, with a lower environmental impact. Without reducing the economic advantages, the negative impacts decrease, such as water contamination, distribution of pesticides, and nitrogen-based chemical fertilizers (it must be remembered that the chemical process that allows nitrogen-based fertilizers to be obtained, starting from atmospheric nitrogen, requires very high temperatures and pressures and, also for this reason, requires a lot of energy).

Under extreme environmental conditions, such as sudden drought, cereal crops alternated with legumes and fertilized with manure have been shown to be more productive and resilient than traditional systems: 137% more production is recorded for maize and 196% more for soybeans when they are fertilized with manure and grown in rotation with legumes, compared to a repeated, chemical-supported monoculture. [610] Alternating grains (such as corn) with legumes (such as broad beans) may increase grain production by 43%. [610]

The rotation of different multi-year and annual crops is a simple system that may be used to reduce the severity of some plant diseases. The rotation of crops, such as cereals with legumes, improves soil fertility and hinders the spread of weeds. To give a concrete example, here is a significant estimate: in the USA, the substitution of the practice of crop rotation of maize with other plants, with the monoculture of maize perpetuated over the years, generates an increase in yield losses (due to insects) of between 3.5% and 12%; this negative result is recorded despite the fact that, in the case of maize, the use of insecticides (organophosphates) has increased by more than 1,000 times (maize in the USA is among the crops that use the greatest quantities of insecticides). [502] Associations, rotation, and diversification are principles of sustainable agriculture that can significantly increase the benefits also in economic terms. [530]
It must be concluded that the diversification of crop rotation and the reduction of chemical use are strategies to be encouraged as they may be pursued without compromising economic objectives.

THE SPREAD OF NATURAL CORRIDORS

The fragmentation of habitats produces a mosaic in which the distances between the nest and food sources increase and also increase the barriers, which for some animals become insurmountable: for example, bees that nest underground and the smaller solitary bees are more sensitive to the fragmentation of ecosystems (at least 14,000 species of solitary bees are known). [853] It must be remembered that the movements of some insects useful for pollination rarely exceed a few hundred metres (100 m for solitary bees such as *Osmia cornuta*) and that the preferred sites for forming nests are underground and in trees. Some insects do not move to surfaces without shelter, vegetation or food. Therefore, soil movement such as ploughing, the absence of trees, physical barriers (e.g. urbanization), and the distance between the nest and the flowers of a few hundred metres are factors that make the life cycle impossible for many species of pollinating insects.
The creation of natural corridors allows the movement of wild species between distant territories and ensures a greater biodiversity, which is the opposite of what happens in most current intensive monocultures. Therefore, a new type of agricultural ecosystem needs to be designed, with characteristics that make it more sustainable and resilient.
Guessing which species to plant in uncultivated areas is not easy, in fact the opposite effects to those desired could be recorded: refuge areas near cultivated fields could favour harmful insects or other crop pests rather than useful organisms. Precisely because there are objective difficulties, in order to try to obtain results, attention has been paid to generalist predators, such as ants that prey on butterfly caterpillars and citrus moths. A strategy adopted by Chinese farmers for over 2000 years, and still used today, is to provide straw shelters for insect predators such as spiders. [694]
In general, the presence of plants that provide pollen, nectar, seeds, and fruit favours many pests and parasitoids of crop enemies. Many insects, such as some wasps that eat other arthropods, also feed on pollen. Some useful insects such as hoverflies, wasps, and coccinellids feed on honeydew, that is, the sugary substances produced by homopterous insects such as aphids.

The presence of natural corridors has demonstrated several benefits: [694]

▪ Around apple orchards and between rows, the presence of strips with herbaceous species encouraged the presence of insects that feed on aphids. Apple trees record less aphid damage than orchards without grasses. The presence of plants (such as *Phacelia tanacetifolia*) between the rows of apple trees favours useful insects such as pollinators.

▪ The corn borer (*Ostrinia nubilalis*) is a butterfly that damages maize and it is parasitized by a wasp (*Eriborus terebrans*). Maize fields surrounded in the perimeter by a semi-natural vegetation show less damage due to the presence of the parasitoid wasp. There is a gradient effect, i.e. the protective effect of the semi-natural area, which provides shelter to the wasp, decreases with an increasing distance. This wasp has been widely used in biological control plans since 1927 in North America, where it has become an alien species. By increasing the distance, between cultivated fields and semi-natural refuge areas of crop enemy pests, the time between the appearance of the pests and the arrival of their predators increases. By increasing the possibility of access of the wasp (*Eriborus terebrans*) to nectar and honeydew of aphids, its ability to survive in cultivated fields is also increased.

▪ Increasing the fraction of uncultivated green areas around fields cultivated with grain increases the presence of spiders by a factor of four. Spiders are formidable predators.

▪ In Great Britain strips of wildflowers, six metres wide and 100 metres apart, have produced good results: wild pollinators and natural enemies of crop pests have increased. [196] Industrial agriculture has also benefited from this increase in biodiversity.

▪ In the UK, crop diversification by planting wildflowers has increased the numbers of pollinators such as bumblebees. [611] The practice of planting wild species, as opposed to leaving strips of uncultivated land, is quicker to encourage the colonization by pollinators.

▪ The presence of trees favours some ants that prey on insects harmful to coffee or cocoa cultivations, which are favoured by shaded areas created by planting trees such as coconut (in cocoa cultivation in Ghana).

▪ It is useful to plant tree species around cultivated areas that are attractive to pests of the species of agricultural interest, as was done in Brazil where the density of a citrus pest beetle (*Cratosomus flavofasciatus*) was reduced by planting a small tree (*Cordia verbenacea*), at a distance of 100-150 m.

▪ The idea of being able to plant plants that have a repulsive action against the crop's pests and plants with an attractive action for the pests of the crop's enemies is interesting. These plants have a dual action: the repulsive action against pests and the attractive action of the natural enemies of the crop’s pest. This strategy has been applied in maize cultivations in Africa.

▪ The mango (*Mangifera indica*) cultivation benefits from entomophilous pollination by many different insects, such as flies and bees, so the creation of semi-natural areas with wildflowers near the cultivated fields generates an improvement: there is a greater presence of pollinators and a lower incidence of diseases. [614] Mango flowers near semi-natural areas can be visited by at least 56 different species, including eight species of ants. At least five species of flies and bees visit both the mango flowers and those in the surrounding areas. Honeybees are also used for mango cultivation (at least one hive per hectare), although they are probably not the most suitable pollinator. A distance of 300 m between mango cultivations and the area occupied by wild species is sufficient to reduce mango production by up to 41%. In fields cultivated away from semi-natural areas, pollinator biodiversity is reduced by 74%. [614] These data confirm that the absence of pesticides and the simultaneous presence in proximity (less than 300 m far) of semi-natural areas or, even better, wild habitats, increases the number of species visiting mango flowers and the productivity of this crop. Restoration of semi-natural areas increases the productivity of the mango plant by about 1-5 kg of fruit per tree. An interesting aspect for farmers is that establishing semi-natural areas - in at least every 4 ha of mango trees - is economically viable even though it reduces the number of productive trees. The economic analysis shows that the benefits of the increased productivity resulting from the restoration of semi-natural areas are 6-7 times greater than the costs. In conclusion, restoring wild areas around crops that need pollinators is convenient for the farmer and useful for the environment. These positive results were obtained in mango crops planted at the distance of 50 to up to 150 m from semi-natural areas of 25 square metres, where 30 wild species were sown. A small area restored by planting wild plants can produce benefits for an area of up to 3 or 4 hectares of mangoes, improving the biodiversity and resilience of the entire agro-ecosystem. The costs involved in creating these small compensatory measures for pollinators are outweighed by the benefits. The results also highlight another aspect: the importance of not using herbicides and not hindering the growth of wild flowers, as this benefits the crop too.

- Crops that benefit from the service offered by beekeepers such as cherries (*Prunus*) benefit from the presence of semi-natural areas that provide refuge zones for wild pollinating insects. Increasing the proportion of the area occupied by wild species or semi-natural environments, (within a radius of less than 1 km) by between 20% and 50%, increases cherry production by 150%, regardless of the density of honey bees kept. [616] The service performed by wild pollinators, when it exists due to the presence of non-cultivated areas, exceeds that of bred honey bees. This analysis confirms that the presence of wild areas around cultivated fields (in this case less than 1 km away) also generates significant benefits for farmers and that the pollination service provided free of charge by nature exceeds that obtained with the help of beekeepers. Similar results have been obtained for the cultivation of watermelons: if they are located far from areas occupied by wild plants, the biodiversity of pollinators is reduced, damaging also the cultivation. [619]

- The increase in plant biodiversity in the vicinity of rape (*Brassica napus*) cultivations, within a 0.5 km radius, has led to an increase in seed weight of 18% and an increase in the market value of 20%: oil obtained from seeds originating from entomophilous pollination contains less chlorophyll and more oil per seed, and the seeds are bigger. [617] It must be remembered that rape plants are able to self-fertilize, but they benefit from the presence of insects that feed on pollen and nectar, such as honeybees and other insects (e.g. hoverflies[I] and bumblebees). These results confirm that the presence of wild pollinating insects improves the quality of production and, in this case, the market value of rapeseed from which oil is extracted, even with the simultaneous intervention of honeybees. The service offered free of charge by

[I] The hoverflies (Syrphidae) are a vast and cosmopolitan family of insects of the order Diptera, comprising more than 6,000 species. They are characterized by a marked heterogeneity, both in morphology and in the ethology of the juvenile stages. The importance of this family is due to two distinct aspects: the adults are among the most common and widespread pollinating insects, second in importance only to Hymenoptera Apoidea; moreover, the larvae of many of its species are very active predators of phytophagous insects, especially of aphids. They can make long migrations and cross stretches of sea.

nature achieves the best results when the semi-natural or wild area is less than 500 metres from the oilseed rape plants, rather than 1 km. [617] As the proportion of the area occupied by oilseed rape plants increases, compared to semi-natural areas, entomological biodiversity decreases.
Equivalent results are obtained with a plant of the same genus which, however, needs cross-pollination and, therefore, requires the presence of pollinators. The plant *Brassica rapae* is not self-compatible and is therefore more sensitive to the lack of pollinators. In plants that can be wind-pollinated, these improvements are probably the result of several factors such as the greater amount of pollen carried by insects and the greater likelihood of crossing between genetically distant plants. It should be remembered that plants of the *Brassica* genus (such as those just mentioned, i.e. *Brassica napus* and *Brassica rapae*) have also been genetically modified, for example, to resist the herbicide glyphosate. [619] The measurement of the abundance of wild pollinating insects in three types of *Brassica napus* and Brassica *rapae* crops (in Canada) gave the following results: those cultivated with traditional methods, those cultivated according to the principles of organic farming, and those genetically modified for herbicide resistance showed that the pollination service was very deficient in the genetically modified crops, was deficient in the traditional crops, while the best results were recorded in the fields managed with the method of organic farming. [619] The number of bees in decreasing order was: organic farming > traditional farming > cultivation of genetically modified plants. These results can be explained by the fact that in organic farming, pesticides are not used, such as insecticides that kill beneficial insects and herbicides that destroy plants necessary for their survival. In the cultivation of genetically modified plants, greater quantities of herbicides such as glyphosate are used, which destroy the sources necessary for the survival of useful insects: insecticides were also used during flowering (except in organically cultivated fields). Among the insects captured, honeybees represented less than 2% of the insects surveyed. Therefore, pollination by wild species was very important. An interesting fact about the entomological biodiversity that emerged from this research is that during flowering, in the case of organic farming 342 species of insects were collected, 230 species in the fields cultivated according to traditional agriculture, and 101 species in those occupied by genetically modified plants; the entomological biodiversity was correlated to that of the plants. A conclusive consideration is that wild pollinators are able to perform the function needed by farmers in the case of organic farming, but this natural service is insufficient in fields managed according to traditional farming methods and is worse in those occupied by plants resistant to the herbicide glyphosate, which have the lowest abundance of wild plant species.
In rapeseed cultivation, the presence of uncultivated areas in 30% of the area has led to an increase in production and a reduction in costs that justify this strategy in agronomic terms. [586] Agricultural production of rapeseed improves in the presence of uncultivated areas at a maximum distance of 750 metres and for an extension equal to 30% of the cultivated surface. [586] The presence of uncultivated areas occupied by wild plants increases the number of wild pollinating insects, providing an indispensable service to crops. These refuge areas could increase the farmers' independence from pollination by farmed species such as *Apis mellifera*. [622]

▪ Many arthropods have a low mobility, in fact the planting of alfalfa near the cotton crop ensures a significant increase in predators up to a few metres away. It only takes between 10 m and 50 m distance of the crops from the refuge area to record a halving of the number of some predators. Some guidance in cotton cultivation reports that alfalfa strips should be less than 300 m apart and 8-12 m wide. [694] In addition to alfalfa, strips of other crops such as chickpea and caiano (*Cajanus cajan*, a legume that is a type of bean) have been successfully tried in cotton cultivations.

Establishing what is the minimum fraction of area that should be left uncultivated to encourage crop pest enemies is not easy. In the case of cereals and oilseed rape some research suggests that if the percentage left uncultivated is less than 20% of the area there is a significant difference in the density of crop pest enemies between the perimeter (near the refuge area) and the centre of the area. If the percentage of area devoted to the refuge area exceeds 20% the difference in density in the cultivated field is much smaller and, in general, parasitism by natural enemies of plant pathogens is much more efficient. In conclusion, for cereals and oilseed rape a percentage higher than 20% may be the value to suggest. It must be remembered that many insects, in the course of their life, are able to move a few hundred metres. Therefore, a strategy that may favour the presence and movement of useful arthropods can be to form strips of semi-natural areas in the middle of cultivated areas. Creating corridor areas promotes movement. For many wildlife species sensitive to habitat fragmentation, the percentage of non-cultivated or natural areas should be greater than 30%.

Therefore, regarding the minimum fraction of agricultural land that should be allocated to form semi-natural areas, there is no conclusive answer. Some studies suggest leaving at least 10% of the area uncultivated. In this regard, it is worth remembering that usually farms dedicate less than 3% of their surface to uncultivated areas. This surface area is often the minimum necessary to guarantee the presence of access roads, electricity lines, protection zones for watercourses and urbanized areas.

Some European strategies to support agriculture in the past have suggested to diversify agricultural production for ecological purposes in 5% of the land parcels. [773] This fraction is too low to achieve the desired results. Several researches, for example on solitary bees which are useful pollinators, establish that it would be very effective, in order to protect them, to dedicate at least 20% of the cultivated area to semi-natural areas with wild flowers. [853] It is not possible to establish a universal threshold, but as it is logical to expect, the greater the surface area, which provides shelter for nests and food, the greater the benefits for biodiversity. Many indicators show improved honey bee health if the availability and variety of pollen and nectar increases. Among the positively correlated indicators, in addition to the condition of the colony (number of dead and live individuals, number of larvae, amount of honey, etc.), there is the amount of fat in the body and the amount of certain proteins such as vitellogenin. [858]

The European Commission, in 2020, included the following target: [1182]

> "*To allow wildlife, plants, pollinators and natural pest regulators the space they need, there is an urgent need to allocate at least 10% of agricultural land to landscape features with high diversity, for example buffer strips, full or rotational fallow, hedgerows, non-productive trees, terraces and ponds, all of which help to intensify carbon sequestration, prevent soil erosion and depletion, filter air and water and support climate adaptation.*"

Dedicating between 10% and 30% of the agricultural land (perimeters, canal and river banks) to wild areas is a realistic and at the same time an effective goal. It is also important to build ecological corridors that support dispersal. The presence of ecological corridors supports biodiversity and adaptation to changes such as climate change. [621, 1182] The fragmentation of habitats does not allow species to move (e.g. amphibians and reptiles) and, in any case, the speed of change in some cases is too rapid to allow many species to survive (the European Commission proposes: *in order to have a truly coherent and resilient trans-European nature network, it will also be important to create ecological corridors that prevent genetic isolation, allow species to migrate and preserve and reinforce the integrity of ecosystems.*). [1182]

Once the minimum area for renaturation has been established, it is necessary to plan what species to favour: which and how many plants to sow? 20, 40 or 100 different types of seeds? Annual, biennial, and polyennial plants could be chosen: but in what proportion? Some institutions have tried to provide indications, such as in Switzerland (the *Swiss Federal Research Station for Agroecology and Agriculture*). [694] Sowing wildflowers at the edge of fields has resulted in at least six times as many arthropod species and a density in semi-natural areas of up to 1,300 arthropods per square metre.

Small populations suffer from inbreeding, therefore semi-natural areas must ensure the survival of a consistent number of individuals in order to favour genetic exchanges between geographically and genetically distant communities. Protected areas of a few hectares are not able to protect rare insects, as they support too small and isolated populations.

The spread of natural vegetation corridors to reduce habitat fragmentation, decrease physical barriers and encourage an increase in biodiversity is also a useful condition for farmers. Planting plants in a way that produces semi-natural areas permits to achieve many more results in exploited agricultural contexts. Simply leaving areas uncultivated favours an increase in biodiversity but, in some cases, better results can be obtained by sowing appropriate plants, as has happened in England, with the intention of protecting and favouring the diffusion of species of bumblebees that are becoming increasingly rare. [660] In order to increase the survival of insects that are favoured by the presence of flowers with a longer corolla, as they have a tongue suited to this type of flower (e.g. some bumblebees), suitable plants can be sown (e.g. leguminous plants such as the Fabaceae). Throughout Europe there has been a decline in the presence of some species of bumblebees and the plant species on which they feed (e.g. *Trifolium pratense* and *Lotus corniculatus*). [660] The sowing of particular plants in small areas near cultivated fields can counteract the reduction in biodiversity generated by modern agriculture. The sowing of suitable plants (e.g. *Trifolium pratense*, *Trifolium hybridum*, *Lotus corniculatus*, *Onobrychis viciifolia*, *Festuca rubra*, *Poa pratensis*, *Centaurea cyanus*, and *Cynosurus cristatus*) has shown to be able to increase the presence of insect species that have become rare. The selected plants were sown at a density of 20 kg of seed per hectare and neither fertilizers nor pesticides were used. The presence of these small plots in semi-natural conditions increases the number of bumblebee species, compared to the control areas (uncultivated areas). A total of 10 bumblebee species were observed, in descending order: *Bombus lapidarius*, *Bombus pascorum*, *Bombus hortorum*, and *Bombus terrestris*. [660] Eight of the ten species were also surveyed in sites where these semi-natural areas were not present, but at a lower density; *Bombus muscorum* and *Bombus humilis*, which are considered rare species, were absent. Plant biodiversity is also increased by the semi-natural areas, as 221 species of flowering plants are classified, of which at least 50 were visited by bumblebees: the plants *Trifolium pratense*, *Trifolium hybridum*, and *Lotus corniculatus* were found to be those most visited by these insects. The results confirm that small interventions are sufficient to register significant benefits. In this case a significant improvement in the biodiversity of pollinators and plants was obtained, within the range of the bumblebees (less than 1 km), from plots sown with flowers measuring 2,500 square metres.

Incentives could be used to improve the agricultural landscape and increase biodiversity (e.g. of plants, insects, and birds). The planting of new species could be encouraged by supplying trees, plants, and seeds free of charge, with the involvement of citizens and schools.

Probably at the current rate of demographic growth, which is accompanied by an even faster increase in the exploitation of the Planet's limited resources, none of the proposals designed to save insects, biodiversity, and humankind will save us. Deciding whether to dedicate 10% or 30% of the agricultural land to natural or semi-natural areas is probably a political rather than a scientific choice, as it may not be sufficient. It may be necessary, in order to safeguard biodiversity and the Planet's capacity to maintain billions of human beings healthy, to dedicate at least 50% of the Earth's surface to a natural state. [693] Currently, less than 15% of the Earth's surface and 4% of the oceans are occupied by areas that are, at least formally, protected: these are areas protected by specific rules for the safeguarding of biodiversity (*World Database on Protected Areas*). [795] In spite of the recognition of the vital importance for the conservation of biodiversity, only a small fraction of the areas considered essential are seriously protected and safeguarded.

IMITATING NATURAL ECOLOGICAL SUCCESSIONS

An ecological succession is the evolution of an ecosystem due to the alternation of different communities in the same area, in relation to the modification of the physical environment. The succession process tends to reach a stable ecosystem, or *climax*, where the system's capacity to absorb external perturbations, whether natural or induced by man, will be at its maximum. In the course of a succession, some general trends can be observed, such as an increase in the number of species, an increase in total biomass, an increase in soil fertility and depth, and an increase in the interactions between different organisms. In the best-case scenario, in the *climax* the total biomass reaches the maximum value and the species are in equilibrium with their competitors. It can take a long time for a forest to reach this stable phase. It takes more than 2000 years for a seed to produce a giant sequoia with the size of the tree named *General Shermann*, which is found in North America. Unfortunately, some of the most common agricultural systems on the Planet are monocultures consisting of grains, which in the wild are easily destined to become desert.

Where there are stable communities because they are maintained by human action, it is difficult to speak of a *climax*, but one could propose the expression *anthropogenic climax*.

One idea that could be interesting to develop in the agricultural system is to program a series of ecological successions, so as to try to achieve the same results: increasing biodiversity and the resilience of the ecosystem to disturbing factors. It might be possible to move from annual monoculture to annual polyculture that could include plants that fix nitrogen in the soil. Then short-cycle multiyear plants could be added, and later long-lived plants such as orchards. Initially the areas could alternate between areas planted with trees and areas with herbaceous plants and multi-year shrubs. After several years the trees could become the main part of the plant biomass. At this point one may think of reintroducing, in localized areas that constitute a fraction of the mature agro-ecosystem, disturbing elements such as cutting trees and planting annual crops. This ecological design of the agricultural system would yield many benefits, such as increased biodiversity, improved soil health, and increased resilience to change. The maturity stage of this model is a mosaic in which the multiyear species are dominant.

CHEMICAL FERTILIZATION FAVOURS CERTAIN CROP PESTS

Chemical fertilization such as nitrogen fertilization favours certain pests such as aphids and mites. For example, the green peach aphid (*Myzus persicae*) is favoured by increasing nitrogen in the leaves of the peach tree. [694] In the cultivation of cabbage (plants of the *Brassica* genus), inorganic nitrogen-based fertilization favours insect herbivores. One phytophagous insect, the western flower thrips (*Frankliniella occidentalis*), is very damaging to horticultural, fruit, and flower crops, as it can cause both direct damage (stings on leaves and flowers) and indirect damage (transmission of viruses). The increase in the presence of this insect pest in the tomato cultivation has been correlated to nitrogenous fertilization. The presence of the mealybug (*Pseudococcus comstocki*) in apple orchards, the presence of psyllids in pear trees, the presence of the corn borer in maize cultivations are all favoured by inorganic nitrogenous fertilizers. The application of nitrogenous fertilizers (urea) in rice cultivation favours mosquitoes.

The use of organic fertilizers (manure and compost, as a replacement for synthetic fertilizers) increases soil fertility and, at the same time, reduces water contamination by nitrogen and phosphorus, with the consequent reduction of problems such as eutrophication and the presence of nitrates in drinking water; the water retention capacity of the soil is also improved by between 20% and 40%.
Switching from chemical to organic fertilization has shown to reduce the presence of herbivorous insects and mites. Thus, soil quality also promotes balances that limit crop pests. In the case of maize, the egg-laying capacity of the corn borer in fields fertilized with organic substances was 18 times lower than in fields fertilized conventionally, i.e. with chemical fertilizers. Organic fertilization (manure, compost) also promotes biodiversity and fertility, which can be helped by growing legumes, a practice whose benefits have been known for hundreds of years. Keeping the soil covered with vegetation at all times also has many positive effects such as those on wildlife.

REDUCING THE USE OF PESTICIDES

Farmers who use pesticides are, in the production chain, the weakest and often the poorest even in industrialized countries. Farmers are exposed to toxic molecules throughout their lives: colon cancer, prostate cancer, malignant non-Hodgkin's lymphomas, Alzheimer's disease, Parkinson's disease, and infertility problems are higher among French farmers and their families than in the general population; furthermore, farmers have a higher suicide rate than the rest of the population (e.g. 20% in France). [230, 292, 293]
Among the most obvious and simplest solutions, there is the reduction of the use of pesticides. The European Community proposes to reduce the use of chemical pesticides of 50% by 2030; this target must be supported by the implementation of pollinator protection initiatives. [1182]
A forward-looking society should be able to find alternatives to spreading mixtures of toxic substances over millions of hectares. It is useful to point out that probably less than 0.3% of the active molecules used by farmers in the field manage to reach the organism and the target site. The remaining part is distributed in the environment and some persistent molecules (such as some organochlorine insecticides) may be found in the food chain and in human tissues after decades and thousands of kilometres from the site where they were used. The number of treatments and the quantities distributed should be reduced; in fact, the fate of the Planet depends on the ability of the human society to set limits to its destructive capacities towards pollinators and not only, as has been widely discussed.
Among the actions that could be taken with the aim of reducing pesticide use, several possibilities are worth mentioning:

- Surveillance and encouragement of compliance with rules, such as not treating with insecticides during flowering. Treatments against aphids that produce the sugary substances sought by bees (and other animals) should be reduced.

- Census and registration of all treatments carried out in agriculture and beekeeping. This obligation has been in place for years (in Italy), but, acually, there are very few records. The traceability of the sale of any pesticide by producers, traders, sellers, users, and disposers should be guaranteed. Various information could also be recorded through the internet (e.g. lots, quantities sold or used per unit area, per plot and per crop). In California, the place and date of use of pesticides are recorded, thus providing very useful information also for epidemiological studies: e.g. to correlate the increased frequency of occurrence of certain nervous diseases following the occupational exposure to pesticides. [294]

- It would be useful to extend the requirement for all pesticides to be prescribed by qualified personnel (e.g. veterinarians or agronomists) belonging to public services (e.g. health service). [375] As in the field of human health, farmers also need plant doctors. Too often the advisors of farmers are the sellers of pesticides.

- Encouraging sustainable and organic forms of agriculture, as it already happens in part in Europe. Increasing the size of the areas dedicated to organic and sustainable agriculture, and reducing those cultivated with chemical agriculture is no longer a choice but a necessity. We can no longer afford to continue producing food without taking care of soil, water, and biodiversity. In Europe, organic farming prohibits the use of pesticides and, in general, the use of synthetic chemical products (e.g. fertilizers). Organic farming is therefore a form of cultivation that should be encouraged, replacing the traditional one. Organic crops obtain lower yields but have a lot of advantages, such as a lower economic investment, a reduction in environmental damage and consequent health costs, which are not accounted for today. According to some researches, organic agriculture generates 19.2% lower yields than traditional agriculture: this average was obtained by comparing the results obtained from studies conducted in 38 countries, on 52 species cultivated over 35 years; the range of difference in yields was estimated between 15.5% and 22.9%. Differences may vary considerably from country to country and with crops; for example it may be as high as 180% in developing countries. The difference in the quantity of harvest, from organic farming compared to traditional farming, may be reduced in some crops by using simple cultivation practices, which among other things are good agricultural practices, such as rotation and the simultaneous cultivation of different crops. Rotation consists in not cultivating the same plants for several years consecutively on the same soils, as it occurs with cereals. Rotation between different crops instead of a repeated monoculture cultivation on large plots has many environmental advantages, such as the lower probability of selecting pesticide-resistant super pests: insects or weeds. Crop rotation interrupts the life cycle of organisms linked to a crop and the use of legumes enriches the soil in nitrogen. The yield difference between organic polyculture and conventional monoculture may be 9% (9 ± 4%). [530] The simultaneous cultivation of several plants is an ecological strategy to reduce the differences in quantity between conventional and organic farming. Differences in yield and other drawbacks should be evaluated comparatively, trying also to quantify the environmental damage and the health risks to farmers, the population living nearby, and consumers, which is not done. Organic farming generates a lower reduction in biodiversity which adds further value that should be used in economic estimates.

- Among the food production methods considered less harmful to the environment and less dangerous to health, there is the so-called "integrated production". One of its main principles is the preventive assessment of the presence of pests and the economic evaluation of possible damage. Through the definition of economic thresholds of intervention, the levels of infestation are established, below which it is not necessary to intervene. This assessment should be carried out before the use of any pesticide, exactly the opposite of what happens today with the use of seeds coated with insecticides such as neonicotinoids or genetically modified plants that produce biological molecules with an insecticidal action. In practice, we are systematically making plants wholly poisonous for all their life, even before we know whether the pests will be there and whether they will do any major damage.

- A ban on the use of pesticides in public green areas, sports fields, cemeteries, and private gardens, as was recently done in France.

- Restricting the sale and purchase of pesticides. Currently anyone can buy pesticides for non-professional use, even in 20 L bottles on the internet (e.g. glyphosate herbicide). It is estimated that 82% of the American households use pesticides at home, using between 3 and 4 different active molecules. [505] Also because of this erroneous habit, some studies detected 7 different active molecules in the blood of 83% of the mothers and children examined. [505] The domestic exposure may be particularly dangerous for children: it is known that the domestic exposure to pesticides, both of parents (before and during pregnancy) and of children immediately after birth, increases the probability of registering leukaemia in children (these are tumours that may occur in children under 5 years of age). [515] Banning pesticides for household use and, therefore, banning the marketing in supermarkets and large-scale retail trade is a very useful and effective objective to reduce health costs and unnecessary expenditure.

- The ban on the use of pesticides to improve the appearance of fruit. A fraction of pesticides is used to improve aesthetic aspects that are irrelevant from the point of view of nutritional quality. Therefore this type of application should be banned, as it has already happened in some nations such as Canada (in 2009 in Ontario). [517] Commercial aesthetic standards should also be changed to encourage a reduction in the use of poisons for this purpose.

- Increasing the mandatory information on pesticide labels such as the chemical class to which the molecule belongs, the compounds present in addition to the active ingredient (100% of the ingredients should be indicated), and the known chronic effects (e.g. as endocrine disruptors).

- Informing consumers about the pesticides used by farmers for each batch of food products. Still today in many commercial networks in Italy it is difficult to get information that has been compulsory for years, such as information on the origin of fruit and vegetables and the name of the plant variety.

- Monitoring the quality of water that may be highly contaminated by pesticides, such as herbicides. In areas where clean water is scarce and the water available is contaminated, protection zones could be set up to prohibit the use of any active ingredient (as, in some cases, it is regulated for the protection of water from contamination by nitrates from chemical fertilizers).

- Crop rotation should be applied, together with intercropping, so as to reduce the problems resulting from intensive monoculture repeated over time. An example of mixed cropping is applied in Mexico where wheat, beans, and pumpkins are grown together. [175]

- All diseases should be registered and catalogued using computerized tools, so that the desired information can be easily collected. For example, population cancer registries in Italy collect information on cancer patients residing in a given territory. Unfortunately, in Italian hospitals, both public and private, the data relating to the diagnosis and treatment of cancer are not always filed in a detailed and systematic way. If we want to monitor the progress of oncological pathologies it is therefore necessary that someone takes on the task of actively searching for information, coding it, archiving it and making it available for studies and research. The Italian Cancer Registries have this function and have been established since the late 1960s. [572] It would be useful to provide for the registration by all doctors and health care facilities, both public and private, of registered diseases, including cancers. Specific registers should be designed for occupational diseases (e.g. of beekeepers and winegrowers) and for particular categories (e.g. hereditary diseases). Today's computer tool means make it possible to carry out these records and catalogues quickly and easily, so this is an opportunity that should not be wasted. The absence of the collection of this information slows down the progress of knowledge and the society's ability to gather the evidence that is essential to try to reduce or stop risks generated by private interests, such as the agro-chemical industry.

- Promoting the culture of organic and ecological agriculture. Scientific research in this direction should also be encouraged, favouring more sustainable and self-sufficient systems. The principles of circularity of flows (e.g. recycling of organic substances such as fertilizers) and of autonomy, such as that regarding plant reproduction, should be addressed with great energy and determination.

- Training and assistance to farmers and beekeepers. Independence from consulting by big companies is a prerequisite for the spread of an agro-ecological culture. [271] It is necessary to build a new network that spreads the principles of sustainable agriculture and promotes an agronomic culture different from the one currently dominant in industrialized countries.

These are some of the good practices that should be encouraged also through Universities and the professional training for farmers. [271] An ancient system to maintain soil fertility was the rotation of different crops, such as cereals and legumes (e.g. wheat, turnips, barley and clover, in Great Britain more than two centuries ago).

It is now at least 30 years since the first studies showed that reducing pesticide use would not lead to a decrease in crop yields. In the USA, in the 1990s, it was estimated that a 50% reduction in the use of pesticides in agriculture would generate a 0.6% increase in production costs and no decrease in quantity. [538] However, this estimate did not evaluate, in economic terms, all the benefits of reducing the negative health and environmental impacts of a 50% reduction in pesticide use.

A complete ban on the use of pesticides is the ultimate goal that must be set and that must necessarily be achieved with the development of a new agricultural culture. It is necessary to move from chemical agriculture to sustainable agriculture, and in order to do this the current agricultural World needs support and knowledge that is not available today. Farmers depend in all their most important choices on a culture that has been monopolized for over 50 years by multinationals and their technologies. Therefore, it is necessary to build and spread cultural alternatives, and to do this we need the strong intervention of the State. We must move from a model in which profits are privatized and environmental costs are ceded and distributed throughout the community, to a system in which the collective interest and sustainability must be the key principles.

Until it is demonstrated that the use of pesticides generates indispensable and essential benefits and, at the same time, does not cause disastrous effects, their use should be suspended. [449] Unfortunately, while the problems generated are now known, the advantages of some procedures such as distributing insecticides all over the Planet (e.g. neonicotinoids) are much less clear before knowing whether the pests will be able to create unacceptable damage. It is like ingesting medicines such as antibiotics for all our life before any symptoms or premonitory signs of an imminent danger.

THE LIMITS OF THE TOXICOLOGICAL EVALUATION

Tests for the assessment of acute toxicity have many limitations:

- They are greatly influenced by the pesticide companies and in most cases are carried out only by their in-house and affiliated laboratories. In fact, the public control and authorization bodies judge the documents and results produced by the same companies that manage the commercial deal. This is clearly a very dangerous conflict of interest situation that should be countered. Some of the unpleasant and dangerous cohabitations between controllers and controlled are denounced in dozens of publications and documentaries. [260, 280]

- Experimental methods involve measuring toxicity using doses that generate measurable effects in a few hours, so there is no information on sub-lethal and long-term effects. To understand better, it is like trying to know whether cigarettes can generate a carcinogenic effect by exposing volunteers to dozens of cigarettes for a single day and then recording what happens over a week. The principle of toxicology according to which small doses repeated over time can produce negative effects equal to or greater than those generated by a single dose, even if higher, is not considered. The lethal dose that kills 50% of the exposed individuals by ingestion or contact is measured. According to the World Health Organization, chemicals that have a toxicity of less than 5 mg/kg body weight, for solids, and 20 mg/kg, for liquids, can be considered extremely hazardous. If a pesticide registers a lethal dose (LD) for humans of 20 mg/kg it means that 1,400 mg can kill a 70 kg man; it is useful to make comparisons between the LD_{50} of some substances: 11,900 mg/kg body weight for vitamin C, 3,000 mg/kg body weight for table salt, 15 mg/kg for the organophosphorus insecticide parathion, 10 mg/kg for the organochlorinated insecticide DDT, 0.5 mg/kg for cyanide or nicotine, and 0.02 mg/kg for dioxin. [280]

- A well-known fact in toxicology is that the chronic effects of a poisonous substance, in principle, do not have a threshold value (e.g. endocrine disruptors and mutagenic substances).

- Synergistic and additive effects cannot be easily measured because thousands of tests would have to be done and, in any case, it is impossible to predict all combinations of possible exposures. Often exposures are confused by shifting the focus to what are the consequences and not the causes. To give an example, some pesticides weaken the insect defence system so that pests take over. It becomes easy to shift the focus to a parasite such as a mite, and the viruses it transmits, as they are well evident in the colony.

- In the case of field trials, artificial colonies of about 10,000 bees, i.e. much less numerous than the colonies in spring, are placed for a few days in the vicinity of a treated field, ranging in size from 2,500 m^2 to 10,000 m^2 (1 ha). [260, 857] This area is too small and therefore underestimates the real exposure: worker bees every day can potentially move in an area of more than 100,000,000 square meters (100 km^2). Thus, their exposure can be underestimated by a factor of more than 4 orders of magnitude. To reduce this source of errors and inaccuracies it has been proposed in France to use exposure areas of at least 2 hectares. [857] However, it is impossible to prevent bees from moving within a radius of several kilometres and, consequently, it is impossible to prevent multiple and uncontrolled exposures. It follows that there will be no significant differences in exposure between the control bees (located far from the experimental field) and those participating in the test (it is like trying to measure the damage generated by smoking between two samples of people consisting of both smokers and non-smokers: since there are no differences, the results allow the conclusion that smoking does not generate variations in the incidence of cancer).

- Some types of assessment are also difficult to implement because they are unforeseen or not considered. For example, sowing seeds containing pesticides (e.g. neonicotinoids) has been shown to harm bees in the vicinity. Yet bees do not eat the seeds, but the operation distributes pesticides into the environment that are so powerful that they damage the insects in the few minutes during which they fly over the field where the sowing takes place.

- Acute toxicity tests are carried out mainly on adult insects and for a few hours, neglecting the juvenile stages and the effects on the balance of the whole colony.

- Very often the active ingredient that is tested on bees, and in other toxicological studies, constitutes less than 5% of the weight of the formulation that is distributed in the field. Therefore, the other components of the mixture, which in some cases may be more hazardous, are not evaluated. A fraction of the mixture ingredients containing pesticides may be considered secret, i.e. protected by laws and patents. An example is the herbicide based on alachlor, which could be carcinogenic to humans (according to the UN), whose commercial name was *Lasso* and which contained "secret" substances (7%) and hexachlorobenzene (it represents 50% of the product and is neurotoxic). [280] The industrial secrecy has repercussions on human health and the environment. The synergistic effects of more than 100,000 synthetic molecules that we have been distributing in the environment for more than 50 years should also be evaluated, with its well-known consequences: contaminated water, air, and food; reduced biodiversity and an increase in chronic diseases in humans. An unprecedented chemical madness is underway.

- Metabolites are not considered, i.e. molecules - deriving from of the original molecules - which are produced by the spontaneous degradation or within living beings. Some metabolites, as already explained, are equally or more dangerous than the original molecules. Moreover, they can be much more persistent in the environment and act with different mechanisms and in different sites from those for which they are used (e.g. herbicides with antibiotic action and insecticides with hormonal action).

- The effect of pesticides on wild insects is not assessed, so no information on this is estimated in the authorization procedure (also because it would be almost impossible). Many studies have shown that honey bees are more resistant to pesticides than some wild insect species, partly because bees have a formidable helper: the beekeeper, who feeds them artificially, moves them around, cleans the hives but uses pesticides too.

In conclusion, the mechanism underlying the marketing of pesticides is very intuitive: the risks are distributed over the community while entrepreneurs receive the benefits. The knowledge already available should be a warning. A holistic approach to science is often lacking. Reductionism has gone too far: the number of specialists has grown and fewer and fewer are trying to provide a systemic and complete picture of reality. The lack of capacity of the scientific community to communicate clearly what risks we are facing is a weakness too. These weaknesses are also evident in many other areas, such as climate change, the destruction of biodiversity or water pollution.

AUTHORITATIVE DISCLOSURE, THAT IS SCIENTIFICALLY INCOMPLETE AND ETHICALLY INCORRECT

In conclusion, it is possible to state that agriculture is probably the main cause of the problems generated in the World to bees and pollinators. Paradoxically, the greatest beneficiaries in economic terms of the pollination service (farmers) and honey production (beekeepers) are the main cause of bee mortality and, in general, of the reduction in biodiversity. It is a system that has been blindly self-harming, constantly for decades. The agricultural system is a mosaic of monocultures that constitute a food desert and a toxic environment, for bees and insects such as pollinators (and others). The only ones who can be temporarily advantaged are crop pests, which instead have the availability of a massive supply of food in the absence of competitors and limiting factors (pesticides aside which, however, select the most resistant pests).
An insidious danger is misinformation associated with ignorance. The dissemination of inaccurate and incorrect information can cause a dangerous delay of the necessary social action. Collective awareness and the positive actions that may result are feared and hindered by strong economic powers. Market, economic, and financial rules are often presented as dark forces from which it is impossible to defend ourselves. We must never forget that they are nothing more than a product of human culture and not an inevitable asteroid. Some of the messages that are propagated to spread and cultivate doubt with the consequent nefarious effects are the following:

- The pesticide is only dangerous if good use practices are not adopted. [869] In this view, the adverse effects on insects are partly explained by the poor skill of the worker who used the molecule. Ineffective proposals to reduce risks to bees and the general population include the use of appropriate protective equipment during the application and adherence to appropriate behaviours and lifestyles (e.g. no smoking). These actions, even if applied to the best of the available technology, may help to reduce the exposure only of farmers and other operators professionally exposed to these poisons. Unfortunately, some protection devices are expensive or very uncomfortable to wear in real conditions.

- One of the most powerful strategies used to inculcate doubt is to equate dozens of very different factors, as is the case when trying to explain the sudden death of bees. Among the messages that have been spread by little or no scientific disclosure on the causes of the increase in bee mortality, we recall again aircraft flights and electromagnetic fields, such as those produced by mobile phone antennas. [241, 482] It has been discovered that bees have iron in their abdomen (granules connected to neurons) which allows them to perceive the Earth's magnetic field and participates in the flight orientation mechanism. [243] Perhaps one day it will be demonstrated that these factors (e.g. the flight of airplanes and antennas) can also significantly affect the life of bees, but it is certainly better not to be distracted: we have sufficient evidence. [254] We need to focus on obvious and established problems, such as the deaths of bees (and other animals such as birds) generated by neonicotinoids and other insecticides. The instrumentalization of science to sow and cultivate doubt is now a very fine art (and a well-paid trade). It is a very ingenious social weapon: spreading doubt outside the company to get the molecule accepted and inside the companies to keep its production going.

- It is the dose that makes the poison, according to Paracelsus' principle: small amounts are close to nothing so there will be no effects. As has already been explored, for many mechanisms of action this is not true, such as for mutagenic (potentially carcinogenic) substances, endocrine disruptors or in the case of defence system inhibitors.

- We need more studies and more research. The view that in the absence of hard scientific evidence nothing should be done is very worrying. The mere suspicion of any adverse effects should trigger the application of the precautionary principle. Until it is proven to be harmless, the product should not be used. Unfortunately, the opposite happens, in fact the pesticide is used until its dangerousness has been confirmed by people outside the manufacturing companies. This work is very expensive and cannot be left to the goodwill of small research groups. In this way the big companies gain time and face few obstacles.

Another interesting aspect concerns the semantics of pesticides transformed from pest exterminators to plant protection products and, finally, to phytopharmaceutical products. Over time, the toxicity of marketed molecules has increased, from 10 to 1,000 times for each new generation of molecules (e.g. if we compare fungicides based on copper sulfate, used in vineyards as early as 1885, and those used today such as chlorothalonil, which damages DNA, or epoxiconazole, suspected of being carcinogenic and toxic to human reproduction). [281] The World of agro-industry has tried to reassure us with semantics. A clever play on words that is intended to deceive and divert attention. We must always remember that pesticides have the function of killing living beings and in the quickest way possible (man included, as many molecules are derived from the research in the military field and have been used in war, on several occasions). The art of avoidance is intended to distract attention from these basic concepts.

ORGANIC FARMING HAS SEVERAL ADVANTAGES

The diversification of the agricultural ecosystem can only generate beneficial effects on biodiversity (e.g. pollinators), soil quality, soil water holding capacity, the amount of carbon sequestered, plant nutrient management, control of plant diseases and pests, and agricultural productivity. [610] The diversification of farming systems generates a lot of positive effects such as those on natural disease control and improved pollination service. A winning strategy is to encourage research and the application of this knowledge to increase resilience and adaptability to changes such as climate change. Food security requires increasing the sustainability of production systems. Increasing the biodiversity of the agricultural system can be achieved in several ways such as:

- diversification of crop rotations;
- the cultivation of different plants (e.g. varieties adapted to local conditions);
- intercropping;
- the non-cultivation of areas close to agricultural fields (e.g. uncultivated strips or edges);
- the reduction of tillage;
- reducing the use of pesticides;
- the use of organic fertilizers instead of chemical ones;
- the cultivation of orchards instead of annual crops (agro-forestry systems).

Promoting agricultural biodiversity means enhancing the natural services provided by ecosystems that also benefit food production, such as beneficial insects like pollinators. Organic farming, i.e. farming that should not use synthetic pesticides, has been shown to increase biodiversity locally compared to traditional farming.

The presence of mycorrhizal fungi and, in general, of those symbiotic with plant roots is favoured by organic farming, allowing an improvement in plant growth and production. Cultivation according to sustainable criteria reduces soil erosion by up to four times and improves the content and availability of nutrients for plants.

In organic farming in general, organisms are 50% more abundant and the number of species increases rapidly by more than 30%. [610] There is an increase in the number of predatory arthropods (natural enemies of plant pests) and soil decomposers (organisms that recycle organic matter). Organic wheat farming compared to chemical farming in Germany increased pollinator richness by 60% and abundance by over 130%. [610]

Organic canola cultivation, in Canada, compared to chemical and genetically modified plants (they use pesticides such as herbicides) has seen an increase in the number of seeds between 3 and 6 times, mainly due to an increase in pollinators. [610]

The conversion of traditional strawberry cultivation to organic farming produces significant advantages in pollination success. In organic farming, already in the first years after conversion (2-4 years), there is a higher pollination success: 45% of strawberry plants are fully pollinated in organic farming, compared to 17% in conventional farming. [618] The cultivation method that does not use or reduces the use of pesticides increases the service offered by pollinators (e.g. increases the number of butterfly species) and also benefits farmers. In fact, the number of fruits with defects, in fields cultivated according to the method of organic farming, is lower than those obtained by traditional agriculture. Similar results have been recorded in other crops such as watermelon. [618] The watermelon (*Citrullus lanatus*) pollination service needs insects to transport pollen from male to female flowers, which are present in the same plant. Watermelon flowers are active for only one day and must receive, during daylight hours, at least 1,400 pollen grains. A study conducted in the USA found that 65% of watermelon growers (in some areas) either own bee colonies or hire the service offered by beekeepers: 4.5 hives of *Apis mellifera* are used per hectare, at a price between 60 and 75 dollars per colony. [760] Despite this help, which is an additional cost, researchers have shown that the transport of pollen in watermelon flowers is carried out for 62% by wild bees: bred bees contribute only 38% and cost more than 250 dollars per hectare every year.

Small variations in environmental conditions may greatly affect the pollination service. Almonds in California are known to be pollinated by honey bees. Research has found that in the presence of wild pollinators and in the absence of wind, bees prefer to visit the top flowers and wild species the bottom ones. In the presence of wind, bees prefer to visit the lower flowers, and if the wind exceeds 2.5 metres per second, honey bees do not visit any flowers, while some wild insects continue to do this useful work for the farmers. [613] This simple observation confirms how useful the presence of wild pollinators is, as they supplement and compensate for the work done by the farmed bees, making the agricultural system more resistant to change.

The important species of wild pollinating insects in Europe are many, for example: *Andrena flavipes*, *Andrena labiata*, *Anthophora plumipes*, *Bombus hortorum*, *Bombus lapidarius*, *Bombus pascuorum*, *Bombus terrestris*, *Megachile rotundata*, *Osmia cornifrons*, *Osmia cornuta*, and *Osmia bicorni*. [733] The importance of the presence of wild or semi-natural areas to ensure pollination by wild species is confirmed by the fact that crops that are more than 1.5 km far from biodiversity refuge areas receive insects in their flowers with 50% less intensity than areas close to refuge areas. [733] Many studies show that this relationship is important. For example, dedicating a tenth of the area cultivated with watermelons to semi-natural areas ensures the survival of pollinators capable of satisfying more than 40% of the pollination service required for this crop. [733] The maintenance of natural areas, together with the management of the agricultural system in a more sustainable way (e.g. without pesticides, as is the case with organic farming), ensures the survival of essential balances for the agricultural economy.

The European Union has ambitious goals: [1182]

"*Agroecology can both provide healthy food without altering productivity, increase biodiversity and soil fertility and reduce the footprint of food production.*

> *Organic farming, in particular, offers great potential for both farmers and consumers: it is a sector that not only creates jobs and attracts young farmers, but also offers 10-20% more jobs per hectare than conventional farms and creates added value for agricultural products. To make the most of this potential, at least 25% of the EU's agricultural land must be used for organic farming by 2030.*"

Organic farming, if it is based only on the reduction or non-use of pesticides, cannot meet all the expectations that are desired from a sustainable system. Organic farming has many weaknesses, such as:

- The use of the same plant varieties as in traditional agriculture: 95% of organic farming uses the same crops as chemical farming.
- It does not prescribe the use of other good agronomic practices such as rotations, polyculture, no tillage, soil always covered by vegetation, no chemical fertilization, etc.
- It does not encourage diversification. In fact, there is evidence that small plots growing different species are more productive than the same area occupied by monocultures and, at the same time, they are more resilient to change. Organic farming often does not exploit local factors that would make it more efficient, resilient and competitive than conventional agriculture.

Another critical aspect is that organic farming, in Italy, is controlled by a few private entities (about 14) which in turn are managed by a single private body (ACCREDIA). The controls are carried out by private organizations economically supported by their controlled companies. The condition of conflict of interest is very evident. It is a self-referential system that does not stimulate improvements in the adoption of new agro-sustainable practices. There is no incentive for experimentation and comparison: it is a rigid system that encourages the homologation of behaviour and lacks transparency. It is not surprising if there are some doubts about the ecological nature of purchases considered virtuous, such as those of organically certified food. This could be another form of advertising and trade that does not alter the pre-established order and encourages the purchase of more expensive products. We are still talking about the same monocultures, often cultivated on large extensions and in mono-succession, sold in the same temples of the market: supermarkets and large-scale distribution. How is it possible that traditional agriculture, which grows the same plants, cannot manage to abandon pesticides (and vice versa)? Let's hope that natural food does not remain a dream. Perhaps natural agriculture has never existed and cannot exist in a modern society. There are no painless solutions, just as dramatic is the idea that we cannot change our vision.

ECOLOGICAL AGRICULTURE CAN HELP MITIGATE THE CAUSES OF CLIMATE CHANGE

Intensive agronomic practices increase climate-altering gas emissions, worsening the already serious situation. Useful agronomic practices to reduce climate-altering gas generation include not leaving land free of vegetation cover, not tilling the soil, and letting annual plants grow between the rows of arboretums. Not tilling alone could increase the amount of carbon sequestered by about 31 g per square metre per year.

In general, organic farming uses less energy and is more efficient when considering the ratio of energy invested to energy obtained, compared to chemical farming. It therefore has a much lower impact on the climate. In fifteen years of organic farming, the organic substance in the soil may double compared to conventional farming (it increases the organic carbon in the soil). [610] Sustainable agriculture has the potential to sequester in the soil an important portion of the carbon dioxide emitted by human activities: it is estimated between 5% and 15%. [610] Other

studies estimate that a much larger fraction of current emissions can be sequestered in agricultural soil and vegetation by modifying production systems. [1181] Some field trials have shown that the application of sustainable practices, such as those of agriculture defined as regenerative and organic, could allow to take three tons of carbon per hectare from the atmosphere every year. If these practices, such as the non-use of chemical fertilizers, the non-use of pesticides, no-tillage cultivation, keeping the soil covered with vegetation, the use of organic fertilizers (compost, manure), rotations and intercropping, were extended to the entire World agricultural surface, it is hoped that large quantities of climate-altering gases could be moved from the atmosphere to soil and vegetation. [1181] The application of these agronomic practices could even increase yields and decrease the need for fossil energy.
Another agricultural practice that consumes energy and generates various direct and indirect costs is irrigation. The choice of agricultural systems that save water consumption will become a priority condition in many ecosystems due to ongoing changes. The diversification and diffusion of agriculture make the production system more resilient to extreme conditions such as drought.

Finally, some of the objectives proposed by the European Community are summarized as follows (EU nature restoration plan: main commitments by 2030): [1182]

1. By 2030: large areas of degraded ecosystems are restored.
2. Reverse the trend of declining pollinators.
3. Reduce the risks and use of chemical pesticides by 50%.
4. Allocate at least 10% of agricultural land to landscape features with high diversity.
5. Use at least 25% of agricultural land for organic farming and significantly increase the uptake of agro-ecological practices.
6. Planting three billion new trees in the Union, fully respecting ecological principles.
7. Achieve significant progress in the remediation of contaminated soils.
8. Bring back at least 25,000 km of free-flowing rivers.
9. Reduce the number of Red List species threatened by invasive alien species by 50%.
10. Reduce losses of nutrients in fertilizers by at least 50% by achieving at least a 20% reduction in fertilizer use.
11. Provide cities with at least 20,000 inhabitants with an ambitious urban greening plan.
12. Eliminate the use of chemical pesticides in sensitive areas, such as urban green areas in the EU.
13. Substantially reduce the negative effects of fishing and mining on sensitive species and habitats, including the seabed, with the aim of restoring them to good environmental status. The EU should also use its diplomatic leverage and mobilisation capabilities to facilitate an agreement on the designation of three marine protected areas in the Antarctic Ocean.
14. Eliminate bycatch of fishing or reduce it to a level that allows for restoration and conservation of the species.

The only way to preserve the quality and continuity of human life on Earth is to protect and restore biodiversity.

INTEGRATED AND ORGANIC PRODUCTION IN BEEKEEPING

The World market for foodstuffs mainly consists of a few categories: traditional foodstuffs (pesticides are permitted), genetically modified foodstuffs (again, pesticides are permitted), organic foodstuffs (in which case synthetic pesticides should not be used) and integrated foodstuffs (in which pesticides should only be used after economic thresholds have been set). Agro-ecological or more sustainable alternatives do not exist or are not important in industrialized countries. Organic farming alone occupies a niche position and does not have all the ecological requirements that should be needed: it is still a monoculture. Even organic beekeeping employs artificial methods that alter the biology of the colony and, even in this case, chemical products must be used (e.g. oxalic acid that decomposes into formaldehyde and formic acid). To give other examples, the blocking of male brood and swarming are allowed, i.e. practices that alter the natural cycle. [973]

The principles of integrated production have also been adopted by beekeepers. Some of the principles contained in the specifications for integrated honey production are as follows: [746]

- *A minimum linear distance of 1,000 m must be observed from large urban centres, motorways, ring roads and expressways, industrial areas, sugar refineries, landfills and waste incinerators.*
- *The location of the apiary within urban centres is prohibited.*
- *It is advisable to locate the apiary within plots of land that are conducted organically or with low environmental impact farming techniques. In the absence of farms and/or cultivations conducted according to the aforementioned methods, and/or in addition to the above, it is advisable to prefer farms with natural components for rebalancing the agro-system (hedges, groves, etc.).*
- *The use of plastic for the construction of structural components inside the hive is prohibited. It is also forbidden to use paints of any kind inside the hive.*
- *Artificial feeding of the family is permitted up to 15 days before the start of harvest. The use of starch hydrolysates is prohibited at all times.*
- *Bacillus thuringiensis and sulphur dioxide, however produced and/or administered, may be used to protect (stored) combs from wax moths (Galleria mellonella and Achroia grisella).* It should be noted that the bacterium *Bacillus thuringiensis* could be a biotechnological product or a genetically modified organism.
- *It is recommended to replace the nest wax at least every five years. Only sterilised beeswax should be used to renew the wax.* Wax accumulates pollutants that are dangerous to bees, but how can one get uncontaminated wax? Heat sterilization eliminates parasites, but many active molecules are resistant to high temperatures.
- *High temperature (steam, direct flame), radiation, caustic soda and sodium hypochlorite solution (bleach) may be used to disinfect hives.*
- *The pharmacological treatment must meet the requirement of indispensability, i.e. it must be carried out when preventive measures prove inadequate and technical interventions other than pharmacotherapy are inadequate. Furthermore, pharmacotherapy must not be given a preventive role.* In practice, the use of medicines (e.g. acaricides and fungicides) by beekeepers is permitted.
- *In the case of pathogens such as American foulbrood (Paenibacillus larvae) or European foulbrood (Melissococcus pluton), provision must be made for the destruction by incineration of colonies and the disinfection of hives and equipment. Fumagillin may be used to control nosema (Nosema apis), and bromopropylate, thymol, eucalyptol, menthol and camphor may be used for mites (Aracapis woodi), provided that the withdrawal period between treatment and harvesting is observed.*

To control Varroa jacobsoni, substances such as coumaphos may also be used. It should be noted that the obligation to destroy colonies probably discourages self-reporting and that very dangerous active molecules, such as coumaphos, are permitted.

- *During all operations, from the extraction to the potting of honey, it is forbidden to work with a temperature higher than 40°C.*
- *The use of plastic containers for potting or storage is prohibited.*
- *The honey must be stored for a maximum of 24 months from the date of extraction (minimum shelf life).*
- *Pesticide residues should be less than 0.01 mg/kg.*
- *Hives should be placed above 900 m altitude.*

The rules just outlined allow for many considerations. For example, the specification for the integrated production of honey prohibits placing the hives in urban centres, but many beekeepers argue that urban green areas are healthier for bees than fields used for monocultures, where bees die because of pesticides and the absence of flowers. The use of new control systems is authorized, such as toxins produced by the soil micro-organism *Bacillus thuringiensis*, which can be modified by genetic engineering. Pesticides such as acaricides are authorized and no economic thresholds for intervention are set. The integrated production specifications also lay down minimum quality requirements for certain categories of honey. Robinia or acacia honey (*Robinia pseudoacacia*) comes from a plant with thorns, deciduous leaves and white flowers in clusters, introduced into Europe in the 17th century from North America, initially cultivated for ornamental purposes, it is now a wild plant throughout the territory, and is often a real pest. In acacia honey there should be an average of 8,500 pollen grains of this plant every 10 g, which should represent at least 15% of the pollen contained in the honey. [746] In the case of citrus honey there should be an average of 8,900 pollen grains per 10 g, which should represent more than 10% of the pollen present; in the case of chestnut honey (*Castanea sativa*) or eucalyptus honey (*Eucalyptus camaldulensis*) 90% of the pollen grains should come from each of these plants.

More restrictive production regulations are adopted in organic beekeeping. Unfortunately, even in beekeeping the attempts to spread the ecological culture are still ineffective and very weak. For example, hives placed in chemical monocultures to support pollination maintain the status of organic hives, but the product cannot be sold with the reference to organic farming. Another ambiguous rule, as it is difficult to enforce, requires that in order to have honey certified as organic, hives must be located in the centre of an area of at least 3 km cultivated according to organic farming principles. Finding such large areas without chemical agriculture is a difficult mission, in fact the specifications included expressions such as the following: [751]

> "...the crops within *a radius of at least 3 km from the apiary are composed ESSENTIALLY of organic crops and/or wild flora...*"

In addition, hives should be located more than one kilometre from urban centres, highways, landfills etc. In some cases, a minimum altitude over 900 m above sea level is also suggested. In most agricultural environments these conditions are non-existent. A good idea could be to map the areas where it is certainly not possible to obtain organic honey and those that are suitable. As a matter of fact, hives may be placed near traditional crops if they are not in bloom. Nomadism is also allowed for organic beekeeping. Basically, pesticide contamination of so-called organic honey cannot be avoided. Pesticides are not allowed in organic beekeeping, but some chemical molecules may be used, such as those against mites (*Varroa destructor*), like formic acid, lactic acid, acetic acid, oxalic acid, menthol, thymol, and eucalyptol. These substances, which are considered less dangerous, are nevertheless harmful both to insects and to the operators who use them. For example, the information in the documents on products containing oxalic acid is not very reassuring: it is harmful if swallowed or if it comes into contact with the skin; it causes serious eye damage. Consequently, during use it is necessary to protect the eyes (with tightly fitting safety glasses), the skin and the hands (gloves must be made of nitrile rubber 0.11 mm thick and guarantee a permeation time of over 480 minutes, therefore very protective). It is necessary to wear for the protection of the respiratory tract the mask (with filter type P2), with medium retention capacity, for irritating or harmful solid or *aerosol* particles. With strong heating, oxalic acid forms explosive mixtures with the air. Among the decomposition products, listed in the oxalic acid data sheet, there is formaldehyde, formic acid, and carbon monoxide. Formaldehyde (or methanal, CH_2O) is a potent bactericide: in 2004 it was classified by IARC among the group I compounds, i.e. among certain carcinogens. [921] Since 2016, formaldehyde has changed from the European classification of "*suspected of causing cancer*" to "*may cause cancer*". [922] Formaldehyde causes severe skin burns and serious eye damage, can cause an allergic skin reaction, can irritate the operator's respiratory tract and is suspected of causing genetic alterations (information given in the safety data sheet). [923] So even products authorized in organic beekeeping can be very dangerous.

Feed based on sugars, molasses syrup is allowed as long as it is organically produced. It would be interesting to investigate how far this restriction is respected, as certified feeds, like organic sucrose, are much more expensive.

Other weaknesses in these mechanisms are the costs of certification and controls, which are borne by the beekeeper and/or farmer, in a private-sector negotiation. Therefore, private companies (at least 14 in Italy) with the official role of control bodies are in fact clients of those they control. [752] This mechanism generates situations of conflict of interest and undermines the necessary impartiality.

Beekeeping that claims to be more environmentally friendly is based on principles that have been developed by several organizations. *Bee permaculture* aims to provide the bees with the least artificial conditions possible. It tries to espouse the philosophy of doing nothing, that is, of letting bees do what they do. [973] After all, how can bee ecotypes suitable for different environments and resistant to parasites be developed if all biological phases are continuously and systematically interfered with? Modern beekeeping practices prevent the species from defending itself. Continuous interference with the life cycle of bees and in agricultural ecosystems contributes to bee decline. Some of the principles for sustainable beekeeping are summarized below:

- Favouring sedentariness (nomadism is countered).
- Not marketing queen bees or buying bees from far away.
- Encourage natural ways of colony reproduction (e.g. natural swarming only) and with local ecotypes. Not hindering the natural swarming necessary for the natural reproduction of the colony. The suppression of queen cells is forbidden.
- Not performing artificial insemination.
- Not blocking the male brood.
- Not joining weak colonies with the aim of obtaining fewer but stronger ones. This practice is used by beekeepers to prevent weak colonies from succumbing: entire frames are moved from one hive to another.
- Not using pesticides, antibiotics or other synthetic products.
- Not recycling the wax and, therefore, not providing the colony with preprinted sheets (wax sheets and mobile frames are not used).
- Reducing the frequency of hive inspections. The use of hives (such as straw hives) in which it is not possible to inspect the inside of the colony is prohibited in many

countries of the World; this is justified by the impossibility of easily diagnosing the presence of parasites.

- Not using metal or plastic parts to build the hives (only wood is used).
- Reducing the amount of feed to less than 8 kg of sugar per colony per year, or using only honey or pollen. There is a tendency to avoid or prohibit the use of feeds.
- Reducing the number of interventions to the lowest possible number (e.g. do not using the mechanical queen excluder system to block egg laying with the intention of controlling pests such as mites; not cutting off the queen bee's wing to block swarming; not using smoke; not destroying the queen bee's cells to inhibit swarming; making as few inspections as possible).
- Reducing the density of colonies per unit area.
- The hive must be placed high above the ground (2-5 m).
- No movement of combs or bees from one colony to another.
- Reduction in honey withdrawal so as not to weaken the colony and avoid having to provide feed.
- Increasing plant biodiversity in areas near hives: growing to feed bees.
- Not placing hives near traditional agricultural areas: monocultures that use pesticides.

This is a series of measures aimed at making less artificial what is still an intensive farming of animals that cannot survive without the support of the beekeeper. Entrepreneurs consider the application of these principles to sustainable beekeeping to be a failure from an economic point of view.

MAKE THE COMMUNITY ECO-LITERATE

The improvement of the educational and training system can foster a culture more sensitive to environmental issues and, above all, more aware of the need not to exceed the limits imposed by finite resources. We could talk about environmentalist literacy, considering that by now, most of the population lives in artificial places and ignores the seriousness of the environmental emergency. It is evident that cities are completely disconnected from nature and, also for this reason, environmental ignorance is rampant. We need to reconnect with the World of Nature and rediscover its rules and limits. Being able to diffuse, in the general culture, even a few valid moral and ecological principles, will be able to instill an enormous amount of sustainable behaviour, not obtainable with thousands of laws. The present apathy towards environmental problems is very counterproductive. It is necessary to reconfigure the social and cultural rules both in the family environment and in the school system, in the sense of a greater knowledge and a more careful respect of the principles that govern the natural balances on which we depend.

Consumerism and the economic rules by which we govern ourselves are "cannibalistic" in nature, destroying environmental resources and exploiting human beings, based on the existence of great differences in the access to opportunities. For example, if water is treated as a common good, everyone is interested in saving it and avoiding its pollution. If, on the other hand, water is treated as any other commodity or sold under a monopoly, the main objective will become to maximize profits.

One of the most important resources to counter consumerism is a cultural growth through an educational system that stimulates freedom of criticism and a sense of responsibility in the choice of values and models of life. Greater investment in the seriousness, rigidity and selectivity of public education can produce much more fruit than monetary investment. Perhaps we can consider fortunate a system that can be improved, first through its management and

then, eventually, with greater economic resources. But the way of thinking and acting of a generation is neither simple nor quick to change.
Environmental illiteracy, which reigns and spreads more and more in our society, is fostered by a system of dissemination of information, education, schools and universities, which is often incapable of promoting the necessary cultural change. We can choose whether to invest resources in culture or in weapons or concrete, that is, we can perhaps still decide how to prepare the future generation for unprecedented and worrying changes and challenges. More culture also means having to explain to young people that often the damage has been generated by the same institutions to which we have historically turned to seek solutions: the market, the politics, the education system, the administrators.

The modern capitalist and industrial society are transforming the commons into capital, destroying them. A consequent result has been that most of the World's population lives in cities, with an alarming level of ecological illiteracy and, at the same time, high levels of alienation and loneliness. Most of us, and especially children, do not experience the changing of the seasons through the transformations of plants or animals, and know nothing about the production of food and goods. Illiteracy is so dangerous that it completely disconnects the urban man from the environmental crises experienced in the area where one lives, such as water shortage or soil degradation. Without knowledge, urban people have no incentive to implement ecological behaviours and cannot make choices that will affect the community and politicians. Without environmental literacy, the community cannot defend and protect its territory. On the contrary, consumerism educates to destructive extraction and spreads the illusion of limitlessness. It is necessary to revise the culture in ecological terms, otherwise the necessary change cannot be planned peacefully and in time. In the current political and economic World order it is naive to believe that governments will act in the collective interest or in the survival of the Planet. Change must be promoted from below and will not happen without an adequate environmental culture. The distinction between public and private is increasingly blurred and tends to mask the failure of current democracy to protect the environment and reduce inequality. As long as we do not vigorously oppose the dictatorial system of modern private companies, we can only worsen social and environmental security. Changing bad habits bottom-up requires the cultivation of environmental education, diversity, social networks, altruism, and resilience.

The process needed to provide social and ecological benefits needs more eco-education. [988] A new awareness of ecological issues needs to be developed and education in this regard is crucial. Building partnerships and increasing transparency, such as the mandatory disclosure of information about the environmental impacts of what we buy, are equally important. The community could defend itself against the enormous power of big corporations by collecting and disseminating information on the internet, for example about the sustainability guarantees of individual operators. This form of selection of the most forward-looking and environmentally sensitive could be a major incentive. Different aspects of the quality of the work of producers and sellers could be assessed, not only ecological ones but also those relating to guarantees on workers' conditions. The time has come for a peaceful, civil but determined mobilization to protect future generations. Companies must make public the hidden costs and externalities of their activities. The State should also operate in a more transparent way, for example, by publicizing direct and indirect subsidies to the activities. The State should ensure a better management of public resources by favouring the most ecologically virtuous ones. This has not happened in agriculture, just as it has not happened in the production of agro-fuels. [741] The State should make a greater effort to establish a dialogue, a public communication on how to solve problems. These objectives could also be achieved by making appropriate changes to education and training in the cathedrals of education such as schools and universities. Fostering dialogue and debate, even at local level, is a fundamental step: everyone must be involved. We need to make clear the interconnections between quality of life, wellbeing, and the ecosystem services offered for free by nature. Eco-education could promote the citizens' control of the territory and hinder waste and failed choices, such as the privatization of nature and its services (e.g. water management). A project to improve ecological education must also involve food education. Through food choices we contribute to safeguarding our own health and that of the Planet.

Eco-education could encourage the adoption of sustainable practices more widely, could help make the invisible visible and prevent unintended consequences. It is imperative to reconnect with nature and to do so we need to understand how nature sustains our lives.

Industrial agriculture and capitalism have produced several crises: an agonizing Planet, sick citizens, and stifled farmers. Spreading the knowledge necessary for cultivation, among citizens and in schools, can be of considerable help and certainly looks to the future. Through urban gardens and projects in which agriculture enters schools, an educational content with interesting and far-sighted reflections on the quality of life and self-sufficiency can be conveyed. Environmental education, according to this vision, is a prerequisite for the design of a sustainable economic and social order and is necessary to ensure a more democratic food security and sovereignty. On the other hand, an obvious paradox can be highlighted: the irreversible consumption of the Planet's resources, pollution, climate change and the reduction of biodiversity are generated by the most educated and schooled fraction of the Planet, which constitutes less than 20% of the inhabitants.

Science or, more precisely, the small fraction of humanity made up of thousands of scholars and researchers, proposes predictions based on numbers and demonstrations, which no lobby, multinational or political class can disprove using objective and democratic methods. Yet, these predictions that should frighten us are not used to change the way our society is run, not even by the most educated fraction of humanity. How could we possibly convince the smallest fraction of the Planet's inhabitants, but also the most educated, that leaving fossil fuels in the ground is perhaps the most useful thing we could hope to do for future generations? Even if we were able to turn off all the engines, the climate would continue to change, warming up because the force of inertia in the system is such that it will take a long time, before the climate finds another equilibrium. Let's be honest, stopping engines to arrest emissions is something no one wants to hear about. Intelligence and rationality do not help us save the environment, and technological innovation accelerates the process of an efficient and unsustainable exploitation of natural resources. The scientific information available to us suggests that we are not behaving any better than the societies and cultures that destroyed themselves before us and that continue to do so today in a bloody way in many areas of the Planet.

We have the choice of preventing or slowing down the catastrophe that has been foretold, or of letting ourselves be overwhelmed by the events, which are expected to be very negative: poverty, inequality, a decrease in individual freedom, a worsening of the quality of life, but also of life expectancy. Already today the reality is very cruel for many communities, as it is ravaged by wars and famines. Current scientific predictions warn us that we are running dangerously towards an ecological precipice and an increase in social insecurity.

Some predictions about the eventual post-apocalyptic life are very sad, as it could become more solitary, poor, and brutal, i.e. governed by instincts no longer contained by ethics and morals. The ruthless ethics of profit, dictated by a capitalism without rules, will be supplanted by the primitive and frightening laws for survival. In practice, human error, in this pessimistic view, although easily predictable and perhaps still preventable, is accepted and therefore becomes inevitable. Unfortunately, however, we cannot delude ourselves, we have to build our way out by actively engaging all of us. The first step should be to oppose the economic and financial laws that, in fact, force us to remain ignorant, and to damage the environment, even when it does not serve to improve the quality of life: we are subjected to a consumerism and capitalism that are blind and disproportionate. We should spread more ecology in education and economy, before it becomes too late.

We can affirm that "*we are our brain*" and that conglomerates of cells write books that will be read by other conglomerates of cells. [890] The industrial revolution that has allowed, in the last 150 years, at least 98% of USA inhabitants (but also the majority of Italians), to stop doing tiring agricultural work, is a success in many ways. In a few years, society and life patterns have been overturned. The human species has never experienced such a rapid change. However, the structural constraints are a major obstacle to change, derived from biochemistry and physiology, which are the same as 200,000 years ago: genetic, cultural, and social adaptation are much slower than the pace of technological innovation. The brain is the same as our primitive ancestors, so it is unprepared for self-induced changes. The brain has specialized in having to deal with and solve short-term problems, as the future is uncertain anyway, and if you go a little further, it does not exist, except in the genes and culture passed down to the children. Also, the brain is better predisposed to seek rewards in the short term, rather than over longer periods of time. These aspects mean that, arguably, the brain tends to devalue the future. Commitments that produce long-term effects are often underestimated. Will our brains be able to ensure that we do not continue to burn and eat our descendants' futures?

A laptop computer has more computing power than the most powerful computers that existed just a few years ago. Technological progress has not been followed by physical, behavioural, and social adjustment. Consider, for example, the wars and the necessary investments. War, poverty, and inequality are a manifestation of the incompleteness of humanity's evolutionary process.

Paradoxically, our cultural system does not account for the cost of soil and water pollution, but values the business of de-pollution (drinking water treatment, soil remediation), and the treatment of consequent diseases. So, pollution and sick people increase some economic indicators positively: a collective madness, apparently unstoppable. Even the consumption of fertile land and the unsustainable use of water are not accounted for, while the actions necessary to artificially bring fertility (e.g. fertilization) and to make water reach where it is needed (e.g. with the construction of dams and distribution networks) increase the economic indicators of wealth of a State (such as GDP).

Unfortunately, wealth and poverty depend on each other, the first cannot exist without the second. The knowledge that intelligence allows cannot but make us pessimistic, but with the will to do we can try to find the strength to change. The destiny of peoples is closely linked to the environment and we hope that necessity, the mother of inventions, generates the necessary strength for improvement. The philosophy of life "*do what you like today because you do not know if you will be there tomorrow*" has its advantages but also many limits, especially for future generations. Our brain is well equipped naturally to deal with imminent threats, but stumbles when it has to prevent problems that affect the future and the community. Continuing to apply only short-term strategies is a recklessness that we should not allow, not least because we will continue to build a completely inefficient and non-self-sufficient society. Perhaps some of the weakest points of the present system is the inability to take important decisions, for the safeguard of the environment and of the collective interests, in a short time, and to succeed in having political choices not subdued to the interests of a few.

Another major limitation of the human species is its greed for power and wealth, such as no other species has ever recorded. Not even the certainty of the arrival of a catastrophe can curb the lust for profit.

Society is blind: it stubbornly refuses to change its relationship with the environment, which has become self-defeating and unsustainable. Selfish behaviours (genes) prevail. Apparently, humans look very different, but we have most of the same genes. These genes are mainly for building and leading an orchestra, made up of millions of proteins. We must hope that the "less

selfish" genes that are more useful for our survival are already there, and will be expressed as soon as possible. If we have to wait for the course of evolution to favour them, we will not have any chance.
Everything we have been able to do is owed to billions of neurons (subdivided in many different cellular types), which form trillions of (synaptic) connections among them. These brain cells consume like a 15 W light bulb to function (very little), being crossed by action potentials that travel at speeds between 1 and 100 m/sec. [891] In the course of an adult's life, a substantial number of new nerve cells are not added and, therefore, learning generates and strengthens new connections. The connections between neurons constitute the elementary units of memory. Long-term memory can be consolidated by anatomical changes that require the synthesis of new proteins and the formation of new connections. These structural changes, in the brain, take place more easily in young people than in adults. For this reason, too, cultural change must necessarily involve young people.
Understanding such a complex organ, with a network of connections of about one hundred thousand kilometres, is difficult. However, any human action could be reduced to a number of neurotransmitters that move and a weak electric current that is generated and flows through networks of nerve cells. Capitalism and the financial rules that govern society are derived from weak electrical currents that are generated in our brains, regulated by genes that, over millions of years, have been selected to reward selfish behaviour. Perhaps, a democracy different from the current one, with less corruption and more environmentalism, can try to curb this structural limit: the human nervous system. Even moral rules, which aim to promote mutual support through the imposition of constraints on individuals, are the result of interactions between neurons and movements of neurotransmitters. Perhaps, one day, we will be able to enhance the areas of the brain dedicated to altruism by boosting specific genes.
Scientific discoveries increasingly allow the simplification of every individual human action, bringing it back to a very complex combination of neurons and chemical molecules, coordinated by a series of information present in the DNA (the genes). The scientific method allows us to give many explanations but, at the same time, it withers the thought we have of ourselves. Actually, it is becoming more and more evident that the possibilities of choice, intrinsic in free will, are bound to physiological rules and environmental stimuli. Human nature, like that of other living beings, must submit to structural limits that we perhaps underestimate, and that are the cause of our behaviours that we arbitrarily classify as useful or disadvantageous to ourselves. The evolutionary process that led to the selection of such a complex brain could be a loser, because of the strength with which it was able to generate changes in the environment. The invention of technology constitutes a very critical phase: think of weapons of mass destruction, the ability to modify the climate of the Planet or the ability to program our genetic destiny. Change is so rapid that it is difficult to keep up with the time required for any genetic changes and physiological adaptations, probably necessary for the very survival of the human species, and therefore of the brain. Today's system of social regulation involves taking important actions, such as those that would be necessary to implement today, only during an emergency. But this way of operating is a loser, as it is activated too late. Probably the society will not take a serious and useful self-limitation to the use of fossil fuels, or to the reduction of biodiversity and pollution, until the disaster will not be impending or already evident: too late!
In the final analysis, even if the investment in a cultural improvement on environmental issues succeeds in producing positive effects, we cannot wait for the formation of a new generation of politicians and entrepreneurs, more informed and sensitive to ecology. Unfortunately, action must be taken very quickly if concrete results are to be achieved. As a result of the ecological disaster, industrial civilisation could collapse within a few decades. Someone could rightly argue that we are just passing through and even if we tried we would not be able to extinguish all the life from the Earth which, therefore, will continue without us. We do not have

technologies able to solve cyclopean problems such as climate problems, without generating others.

THE CONCENTRATION OF FOOD SUPPLY

A phenomenon that favours unequal distributions of power and wealth is that which leads to the concentration of the food supply in a few big distributors. The so-called large-scale organized distribution (GDO) in some countries manages more than 50% of the market: at least 76% in Germany, 70% in the United Kingdom, and 55% in Italy. An imbalance continues to be generated in the bargaining between producers and the final sales network, which is managed by a few operators. The economic system has thus generated an oligopoly. This state of affairs means that many food products are bought by the commercial network at prices lower than the minimum prices needed to produce them. This discrepancy is partly compensated for by economic aid distributed through public resources, such as those distributed through the Common Agricultural Policy in Europe. This strategy of public economic support was introduced in 1962 to help the agricultural system: between 2014 and 2020, more than 410 billion euros have been granted. [1185] Approximately 40% of the European budget is allocated to agricultural support (in 2019: 58.4 billion of the total of 161.7 billion euros). [987, 1185] Despite this market-destroying and distorting support, many groups of farmers face economic problems, such as owners of less than 10 hectares, who constitute 78% of the farmers in Europe, but they manage only 23% of the utilized agricultural area, which totals 174,613,820 hectares; 68% of the utilized agricultural area in Europe is managed by less than 7% of farmers who are owners of holdings of more than 50 hectares (Eurostat data from 2013). In the 2021-2027 period, a substantial continuation of agricultural support is foreseen: probably more than 1,279 billion euros. [1185] It is to be hoped that these resources will be used to restore and safeguard the environment, biodiversity, protect the soil and combat the climate change. This economic aid is substantial and should be provided in exchange for guarantees that sustainable farming practices will be adopted.

The situation is alarming for many categories of products and producers. A few examples can be highlighted to bring the reflection back to concrete cases. In Italy, durum wheat is cheaper than it was thirty years ago and, moreover, it is cheaper than what farmers pay to produce it. Therefore, farmers are in crisis and consumers have no benefit because the price of bread has risen or remained the same. One thousand kilograms of durum wheat is worth the price of two pizzas. [987] Unbelievably, the price of some types of meat (e.g. chicken meat) can be the same or lower than the retail price of bread. So there is something wrong, producers are not getting the right compensation, selling prices do not reflect production costs, and the system favours the last links in the food chain that are those who risk less. Not to mention the hidden and indirect costs, such as environmental and social costs, which are cleverly concealed.

The tendency to lower the quality of information is clearly visible to anyone who reads labels carefully. Ingredients are not listed in wine, and several hundred cheeses list only three ingredients: milk, salt and rennet. Companies marketing breakfast confectionery products pay much more attention to highlighting ingredients that are absent, or present in small quantities according to arbitrary and unobjective criteria (e.g. sugars and fats), than to indicating the ingredients used. It is almost impossible to know the origin of most foods or of their ingredients.

Shortening the chain between agricultural producers and consumers has several advantages:

- it decreases dependence on distant countries;

- it distributes earnings more evenly and prevents them from being concentrated on the commercial network which, among other things, bears lower costs and risks than those borne by producers;
- it improves the possibility of interaction between companies and customers, making it easier to design mutually beneficial paths that are more respectful from a moral (e.g. protection of child labour and prevention of illegal work) and environmental point of view (those who consume live close to those who produce);
- the population is more likely to influence political and market choices;
- it decreases the likelihood of establishing monopolies;
- it reduces impacts from transport and packaging.

Advertising is increasingly governed by economic principles that serve the interests of a few big companies. The end result is the production of an infinite number of misleading messages that aim to increase sales (e.g. by generating interest) rather than informing authorities and consumers: informing consumers about the environmental impacts generated by the production of goods and services should become mandatory.
The current logic is a desperate one that leads to the superficial error of letting people believe that what they do not know cannot harm them. The industrial and financial sectors promote the strategy of collective ignorance and, even worse, cultivate a subculture of blind and unconscious consumerism. The economic system invests enormous amounts of capital to publicize misleading information and spread doubts in order to increase sales, at any environmental and social cost. This logic may temporarily delude us into believing that we can avoid tackling the problem.
Ecological ignorance has an enormous social cost, which strangely enough does not concern us as much as it should. We should consider the vital importance of the fallout of the current ecological disaster.

LABELLING AND ADVERTISING

One strategy to reduce and counteract the reluctance of big companies to protect the environment and our health is to encourage transparency, with the aim of making good reputation a lever. In a transparent marketplace, if consumers are empowered to make choices, they might favour companies that can offer food and products derived from a sustainable management and attentive to crucial issues, such as environmental health and food safety. Consumer engagement can push the market towards an environmentally sustainable direction. As some economists argue, we choose what kind of society we live in every time we make a purchase. To some extent this statement has substance even if it does not take into account that consumers who are ignorant, uninformed, poor or plagued by misleading information can be guided at will and subjected to the artificial rules of economics and finance. [12, 569, 752]
One of the strategies to encourage more eco-sustainable choices and increase consumer awareness is to advertise messages about the environmental impacts generated by the production of food and beverages. An added value to a food should be represented by advertising the environmental efficiency with which it is produced. Through this way, more sustainable behaviours could be incentivized; companies more sensitive to these issues could get more visibility and recognition from consumers. The paradoxical thing is that the market distorts prices so much that there is no correspondence between them and quality. It cannot be taken for granted that a more expensive foodstuff pollutes less or is healthier or does not exploit workers.

Eco-labelling is not widely used in the food sector and is often used to provide unmeasurable, non-objective and non-comparable messages. Unclear and confusing

information is communicated, such as "natural", "ecological", "sustainable", which are increasingly used in a free and unregulated way. Some well-established strategies, such as organic farming, focus on very few, albeit important, aspects, for example the non-use of pesticides, leaving out important aspects such as safeguarding biodiversity, protecting the soil, reducing the use of irrigation and reducing the consumption of non-renewable resources (e.g. fossil energy, packaging, kilometres travelled).
In some sectors, such as packaging derived from cellulose, international strategies are established, such as sustainable forest management.
One strategy to foster the necessary change could therefore be eco-labelling as this form of advertising can promote a greater transparency and communication between businesses and consumers and, at the same time, encourage more forward-looking business choices. A good eco-labelling strategy should: [165, 752]

- Publicize additional, specific and guaranteed aspects in a transparent and objective manner. Additional guarantees should be easily measurable and comparable.
- The application should be modular and specific per production sector (e.g. rice or beekeeping) and per single environmental aspect to be protected (e.g. biodiversity rather than water protection).
- Do not oblige (third party) certification costs as this significantly increases costs and encourages conflict of interest conditions, which may cancel out the desired benefits. The control methods adopted could be self-certification and/or under control by national authorities.
- For certain aspects and in certain contexts, additional quality reporting should be mandatory and regulated (e.g. pesticides in drinking water and food).

In the agricultural sector, examples of indicators to be considered for the design of environmental labelling could be the following:

- Improvements in managing the chemical risk from pesticides or fertilizers (e.g. preferring organic fertilizers, such as compost and manure, to chemical fertilizers).
- Strategies adopted to safeguard soil fertility: reducing tillage and soil movement to a minimum, keeping soils covered with vegetation all year round, not using fire in agricultural fields, rotating different varieties and species, applying a system of growing different varieties and species at the same time (intercropping or polyculture).
- Implementation of measures to safeguard biodiversity: no use of genetically modified plants, leaving a part of the agricultural area (at least 10%) uncultivated and covered by wild species (e.g. for pollinators, birds), reduction in the use of pesticides.
- Reducing the use of non-renewable energies: shortening transport routes and, in general, reducing the distance between producers and consumers, cutting the use of energy-intensive techniques (e.g. irrigation, ploughing, use of plastics).
- Reducing the use of packaging.
- Reducing the effects on climate: increasing the organic substance in the soil (e.g. avoiding ploughing and the removal of non-edible parts of the plant, which will increase the organic substance of the soil, recycling organic waste).
- Reducing water use.

These messages could be accompanied by other indicators, such as the ratio of energy invested to energy obtained, the water requirement per kilocalorie supplied, the amount of climate-altering gases per kilo of product (throughout the process including transport, fuels, packaging, equipment, cold chain, waste disposal and machinery used).

Among the information that should be disclosed by food (and other) advertising and labelling, there is:

- The *Carbon Footprint* calculated over the entire life cycle (e.g. per kilocalorie and per kilogram).
- Water consumption (*Water Footprint*) calculated over the entire life cycle.
- The energy consumption (*Energy Footprint*) calculated over the entire life cycle. The type of energy source used could be indicated: renewable and non-renewable. The ratio between the energy invested and the energy obtained is another useful indicator. For some time now, various studies have shown that, for example, in the USA the refrigeration, transport and storage of food consumes eight times more energy than that provided by the food itself. [7]
- The amount of waste generated at the various stages, including the final stages not managed by food producers. The way in which waste is managed.
- The amount of pollutants released into the different matrices: water, soil and air.
- Land consumption.
- The kilometres travelled calculating all ingredients and packaging.
- Guarantees on the protection of workers' and mothers' rights and on the non-exploitation of child labour.

Theoretically, given that most of the food trade in Europe (and elsewhere) is handled by a few big distribution and sales chains, it would be enough to act on a relatively small number of entrepreneurs to improve labelling and, in general, the quality of information.

In the case of honey, it might be interesting to have guarantees not only on the geographical origin, but also:

- on the pollinated species;
- on the miles driven and the number of trips;
- on reducing the use of practices such as artificial feeding;
- on the absence of industrial practices such as the use of pheromones, pesticides, antibiotics;
- on the absence of practices such as blocking swarming (e.g. by clipping the wings of the queen bee);
- on the absence of the use of queen bees obtained by biotechnology (e.g. from artificial insemination).

Better information and advertising may encourage choices that will reward the most environmentally sustainable products. In a transparent market with few inequalities, informed consumers, through their choices, hold a considerable influence.

Another aspect that can certainly play an important role in making food production more sustainable is that of bringing producers closer to consumers. Being able to decrease the distance between production systems and the consumer community (customers/citizens) can positively influence ecological choices and increase the possibility of directing companies in the food sector towards an eco-sustainable production.

THE GLOBALIZATION OF INDIFFERENCE

We have espoused the religion of the ultraliberal market, without rules, where the banks are the cathedrals, the companies are the faithful, the prophets are the "experts" of finance, of economy, of the Stock Exchange and the martyrs are the majority of the inhabitants of the Planet and, too often, the workers. [976] Among the sacrificed, there are the unemployed, the exploited workers and the poor. When we talk about poverty, we do not have to go too far. One of the agri-disasters tolerated and often ignored is the exploitation of hundreds of thousands of agricultural workers outside of legality: this is a modern form of slavery in agriculture that, in

2014, in Italy involved at least 400,000 people (these are precarious, underpaid, dangerous, tiring and socially penalized activities). [985] In Italy, probably at least one food product out of five, which arrives on our tables from abroad, comes from places where workers are not protected (in the World probably at least 100 million children are employed in agriculture). [987] For at least one third of the products sold in supermarkets it is not possible to know the origin of the ingredients and therefore it is not possible to trace the possible exploitation of workers.

A large part of society lives beyond economic means. To redeem sins one must pay debts with interest, that is, one must give back more than one has received, forcing necessary growth. Advertising is one of the most relevant prayers and consumerism is the gospel: the foundations are built on the faith in profit. The most feared sins are the reduction of consumption and of investments: the reduction of the gross domestic product. The artificial rules that regulate the markets - we could say the heavenly law of the market - are based on an ultraliberal free competition: the strongest wins (a natural principle, like the laws of natural selection revised in an anthropocentric perspective). The sacred holidays to be celebrated are the end-of-season sales or the Christmas holidays. There are also some forms of earthly paradise: the fiscal ones. The logic of financial religion runs, without taking care of ethical or environmental aspects, overwhelming everything and everyone in its path: it cannot slow down, indeed it must go faster and faster. The suffering and injustice that affect most people are transformed into wealth, the obsession of our civilization, of a narrow circle of entrepreneurs and unscrupulous actors. The rule of unscrupulous selfishness becomes the basic principle of social life, a sort of law of the jungle. Competition is a luxury sport because it requires much more energy than collaboration and, therefore, it is only possible in times of abundance. In periods of crisis, of scarcity, like the current ones, to continue to believe that free competition without rules should be the cardinal rule, to be expressed in systems such as economic, environmental, social, means having to accept mortal danger.

Such an unbalanced system becomes constantly unstable and the crisis ends up being permanent. This is a veritable theology of progress that can be considered a dictatorship of the markets, whose invisible hand influences everyone's life: it is constantly proposed as an unchangeable natural law to justify the suffering that a small fraction of humanity inflicts on the majority. The poisoning of water, atmospheric pollution, the degradation of the soil, the systematic ecocide of non-humans become side effects considered inevitable to which we must remain indifferent. Not to mention human victims such as the unemployed, climate migrants and people living below a universally acceptable threshold of dignity. Nature becomes a commodity that can be privatized, traded, degraded without limits, if not those of financial rules.

Agriculture has been transformed into a war industry where agricultural machinery (tractors) are the means of attack, pesticides are the nerve gas, chemical fertilizers are the explosives, genetically modified organisms are the unconventional biotechnological weapons, the chemical industries are the places where the high ranking officers reside (where decisions are taken), the seed industries manage a new strategy of colonialism and slavery (they expropriate peoples of food sovereignty) and the farmers are the soldiers (in many cases mercenaries or slaves). This is an unprecedented crime against humanity and the biosphere. Subjugation, indifference and silence are ubiquitous despite the degradation being so evident. The symptoms of a serious planetary disease are very evident as is the globalization of indifference.

People do not want to realize that, unfortunately, we live in a limited space and consequently humanity must accept limits. We are probably the last generation to be able to choose whether to build a sustainable society before the apocalypse: it is our duty to avoid falling into the dead-end tunnel of barbarization, of social chaos. Too many people are convinced that this is an unresolvable generational conflict; instead, we need to imagine a new vision of the World with more wisdom, farsightedness, solidarity, altruism and equity. Society is adrift, in the grip of a self-destructive materialism governed by the unacceptable and false religion of infinite economic growth. If the machine of consumerism and capitalism is not stopped, the nightmare of decline will inexorably come true. In extreme terms we must ask ourselves if we want to change or disappear, even if it is unlikely that even the worst ecological collapse generated by humans within this century could cause our complete disappearance from the Planet; however we must reflect and act before it gets too late: [977]

- Probably over hundreds of thousands of years, according to some scientific findings it is millions of years, the average temperature of the Planet has never exceeded ±2°C. Since the beginning of the industrial era (1850) the average temperature of the Planet has increased by more than +1.1°C and in the next decades changes are expected that, in the worst realistic forecasts, could push the increase of the average temperature over +4°C (IPPC), before the end of the century (in the next thirty years we could extract more than half of the oil used from 1850 to today: since 1970 the World primary energy consumption has increased by more than 2.5 times).
- Extreme weather events are on the rise: in the USA alone, the costs generated by climate change (cyclones, droughts, heat strokes, floods, fires, storms and hurricanes) have increased more than 66 times, from 1980 to 2017; on the Planet, these costs have increased at least 7 times. At the planetary level, these costs, if the temperature increases by more than +1.5°C above the pre-industrial average, could increase another 15 times; if +3.7°C is exceeded, these costs could increase 150 times. These are estimates that do not consider a probable domino effect that would further aggravate the situation.
- In the last ten years, negative climatic events have occurred on a planetary level, never before recorded in millions of years: melting of glaciers, frequency and extension of fires, deforestation and mass extinctions, soil degradation, desertification and ocean acidification.
- Renewable energies will not be able to keep up with the levels of consumption allowed today by the extraction of fossil energies: even renewable energies need non-renewable resources, such as oil and rare earths. Therefore the only way forward is to design a society that consumes far fewer resources and ensures a decent quality of life for all.
- Estimates of the number of inhabitants of the Planet who will soon find themselves living in places that will become uninhabitable are impressive: even more than 1.4 billion in less than 30 years (in the most catastrophic scenario, an increase in the average temperature of over +3.7°C, compared to the pre-industrial era, could mean that 75% of the World's population will find themselves in places that are, at least for some months of the year, inhospitable). Unfortunately, migration is often a way out that few can afford and, in general, an ecosystem with limited and collapsing resources cannot withstand it if it is of this magnitude.

The phenomenon of the alarming acceleration in the decline of biodiversity can no longer be ignored and it is not an experiment whose solution is restricted to the scientific circle: it must be tackled with strong and courageous social and political choices. New knowledge is not needed to understand the obvious decline in biodiversity, and the measures to be taken depend on collective and political choices. Nor are new technologies needed to save biodiversity. We are perpetuating an ecological crime with an alarming general lack of interest. We must change our attitude and try to return to a harmony with nature.

Some safety thresholds within which we should fall in order to hope for a foreseeably better future have been widely exposed above, but it is useful to report at least two of them: returning to a concentration of carbon dioxide in the atmosphere lower than 350 ppm (while writing this book the concentration of 414 ppm has been exceeded) and reducing the destruction of biodiversity to less than 10 species per million species per year (i.e. from one hundred to one thousand times less than the current rate of extinction).

How can we hope to secure fundamental rights such as air, water, food, health, if we do not recognize nature's fundamental right to exist? Even if we limit our vision to the very selfish anthropocentric one, we see that nature provides indispensable ecosystem services, even to the economy. Protecting the vulnerable and fragile biosphere is a prerequisite if we hope to continue to experience a democratic system and avoid barbarization. A rapid and broadly planned ecological transition is needed. If we do not implement it, we will soon find ourselves overwhelmed by catastrophes and a real revolution that is not at all reassuring.

The logic of destroying natural resources to create value is losing. Unfortunately, society does not seem to understand the urgency of the necessary change. As already argued in the pages of this book, in order to cope with this foreseeable decline, it is necessary to plan a collective resilience based on solidarity, on mutual aid that can be realistically planned only through collaboration and the recognition of the planetary limits. The voluntary reduction of energy and resource flows is the only way out. This planning, shared and managed collectively, is the most reassuring and least distressing way we can imagine.

STOPPING THE WHIRLPOOL OF THE INESSENTIAL

The writing of this book took advantage of the mandatory isolation caused by the pandemic (by coronavirus). Many of the daily activities performed in the pre-coronavirus period were stopped. Isolation, reduced work commitments, and the inability to do most of the activities performed prior to the pandemic suddenly generated a lot of free time to spend in homes. Probably without this (unwanted) help the publication of the present book would have been more difficult.

Radical measures were taken to counter the spread of the virus, which drastically reduced freedom. Decisions had to be made as to what things need not to be produced or done, in order to reduce the number of sick and dead people. Various inessential activities were almost stopped, such as the car industry or the tourism industry. As a result, the consumption of fossil fuels has also decreased. The rhetoric of war has pervaded the entire pandemic story, and yet climate change or the destruction of biodiversity pose far greater dangers to humanity than Covid-19. Global warming, if not stopped, will destroy the foundations of human life and that of many other species: Covid-19 can do nothing of the sort. No one will be able to heal from the climate collapse and the destruction of nature. Unfortunately, the perception of the danger generated by climate change or the irreversible destruction of biodiversity is not such as to promote even more undesirable and unpopular actions than those implemented to slow down the pandemic. Yet the environmental crisis is much more serious and, among other things, it may promote epidemics worse than Covid-19 (overcrowding increases the risk of species jumping, just as overheating promotes the spread of certain zoonoses). [1276, 1278] Preventing environmental collapse will save many more lives, but it is inconceivable that applying unpleasant measures like a *lockdown* will save the climate and future generations.

We need strong actions such as the reduction of emissions, which has been constantly diverted, postponed, reformulated in a thousand ways with the intention of not achieving any really necessary result. Why not reduce climate-altering gas emissions by 50% in a month or provide for severe penalties, up to life imprisonment, for those who destroy primary forests or eat wild animals? In reality, we have failed to maintain climate-altering gas reduction targets of 5% or 10% per year, because they are considered unacceptable extremes, when, in any case, they are not sufficient to solve the climate crisis. The defeats in the fields of climate change prevention and the decline of essential services provided by nature are countless: deforestation continues instead of reforestation, hunting and fishing cannot be curtailed (the paradox is that pseudo-medicines made from the horns of rhinos and other rare wild animals are sold with the intention of curing coronavirus), soils continue to lose fertility, plastic pollution is devastating, ocean acidification destroys marine life, and during the writing of these lines yet another negative signal comes: the speed of negative changes is greater than the speed of writing and publishing. In June 2021, the proposals to ban pesticides and to strengthen measures to reduce climate-altering gas emissions, which were put to the vote in a referendum, were rejected in Switzerland. This attempt to reduce climate change was also defeated and chemical agriculture won: out of five million four hundred and ninety thousand voters, 36% voted in favour of chemical agriculture and won; 60.68% of voters were in favour of the use of pesticides and against the limitation of the government to give subsidies only to those farms that do not use pesticides. [1277] This is a worrying defeat, another great opportunity missed: the alarm signals sounded but were not heard.

When capitalism encounters nature it does not stop to admire it but captures it, cages it, knocks it down, privatizes it and throws it into the market: in 1700, 95% of the emerged lands free from ice could still be considered in a wild state, in 2000 the proportion was reversed. The driving principle of the capitalistic vision is that if you stop destroying nature, the multiplication of capital stops, where the multiplication of capital ad infinitum is mistakenly understood as indispensable. We should, instead, have the strength to stop capitalism very quickly, with a speed greater than the application of anti-Covid measures (e.g. the consumption of fossil energy, deforestation and chemical agriculture, the privatization of natural capital such as drinking water; instead, tax havens continue to remain untouchable and inequalities increase). We must also have the courage to stop all non-essential activities: it is the only way out. To hope to save many lives and future generations it is necessary to give up something.

The current major economic, environmental, and health crises are a consequence of overproduction, overaccumulation, and finance. This is an insufficiently considered contradiction of capitalism, since the over-exploitation of nature causes self-destruction, even though capitalists hope to be the last to die while they will see the lives of workers slowly destroyed. The facts that a ruined Planet does not generate monetary wealth and any form of complex and organized human society becomes impossible are not adequately considered. Threats to the health or even to the survival of the population must be prevented, so one cannot be left free to do as one pleases. Restrictions must be adopted urgently and swiftly, as was the case with the anti-Covid regulations, which even included tracking the movements of all citizens.

There is an urgent need to design and implement an emergency plan to address the environmental crisis and the destruction of the ecosystem services on which we depend. Many crises, such as the climate crisis and the reduction of biodiversity, as they worsen, risk to trigger a self-perpetuating deterioration: once +3°C is reached, it will slide even faster towards +5°C; the decline in biodiversity is self-perpetuating to the point of annihilation. We are fostering the conditions for a progression of vulnerability, both socially determined vulnerability and vulnerability caused by the collapse of ecosystem services, i.e. resulting from the destruction of nature. The conditions created in the *Capitalocene* become increasingly conducive to increasing insecurity and accelerating the onset of disasters that cannot be considered natural, as they are caused by humankind. When ecological disasters overwhelm us all, the only thing that will count will be survival, there will be no time for meditation and peaceful reorganization of our society: it is urgent to rethink the way we treat the environment and organize an alternative to capitalism (anti-capitalism, programmed degrowth and the elimination of the inessential). Protecting nature and its services is equivalent to defending the human species. As things stand, it seems that what is necessary for this will never occur because mass mobilization is lacking, even though the environmental crisis resembles a World war in that it heralds the arrival of a catastrophe: will we let mass activation occur after the disastrous events that will overwhelm everyone, or will we manage to organize a strategic prevention of collapse? The first choice will generate the worst consequences, so collective mobilization is needed to ensure an emergency transition that protects social security and rights.

IT IS NOT YET TOO LATE: we are the architects of our own destiny

Observing the World that surrounds us, it is easy to measure the level of alteration of nature, largely justified by artificial rules such as those of economics, finance, and capitalism. Consumerism continues to advance unstoppably and the Planet becomes less and less rich and beautiful, while the limits become more and more evident. Humanity is proceeding very fast on a suicidal trajectory. We have let the World be ruled by money hunters, ruthless people without rules. The engine is fueled by selfishness, greed and ignorance on matters of survival.

The recent history of mankind is characterized by the increasing recording of alarm signals, both environmental and in the fields of health and economy, resulting from the commercialization of chemical products (and not only). At the same time society has not had the capacity to react and intervene responding to the calls of these alarms. The most deafened have been those with the most information and knowledge, i.e. those responsible for introducing new products and technologies such as pesticides. The time has come to nurture hope, even with bans and restrictions.

The history of man and bees, over the last 70 years, has been characterized by an increase in the chemical potency used to counteract diseases, largely generated by man himself. Instead of preventing problems by applying reasoning and intelligence, which a normal high school student already possesses, we have developed intellectual strategies to disbelieve what we know, especially when what we know disturbs us all and, in particular, the bearers of large economic interests. We have not been able to accept the limits of the biosphere and respect them. We have replaced the laws of biology, chemistry and physics, which are not necessarily at the service of human beings, with the rules of the market and the economy which are a product of our consumerist culture. The result is a predictable environmental catastrophe. It is no surprise to record continuous alarm signals, such as those from the agricultural system and suffering pollinators. To satisfy blind economic interests, several kilos per hectare of chemicals such as insecticides, herbicides and fungicides have been spread everywhere on the Planet for at least 70 years. Over the years, the molecules that have been marketed have always been replaced by others that have a higher order of magnitude of toxicity. To give an example, one gram of neonicotinoid insecticides, which appeared after the 1990s, is capable of exterminating the same number of insects that have come into contact with several kilos of the famous (organochlorinated) insecticide DDT, which has been banned for decades. Pesticides kill insects at concentrations of the order of magnitude of millionths of a gram and can generate sub-lethal effects (e.g. they can damage the immune system, reproduction, orientation abilities and memory) at concentrations a thousand times lower, equal to billionths of a gram: one kilo of active ingredient per hectare is capable of exterminating billions of insects. If the insect dies and the molecule remains active, it returns to circulate in the environment and, assuming it is absorbed into the biosphere (e.g. by plants the following year), it will reproduce the same effect even if we have stopped distributing new quantities (they are persistent). Sub-lethal and synergistic effects, whose mechanisms are largely unknown but whose consequences are clearly negative, are generated by even lower doses. It follows that one kilo of active ingredient can potentially harm a thousand billion insects every year. This theoretical calculation is provocative but quite realistic, if you think that in some crops more than 30 kg of pesticides per hectare per year, have been distributed for decades (e.g. between 15 and 25 phytosanitary treatments per year are made in the vineyards in Italy, while in the apple orchards between 30 and 40 treatments are accomplished). Chemical weapons are used for the systematic destruction

of the environment, and are even now being used before we know whether the pathogens will pose a real problem for cultivation (neonicotinoid insecticides in maize, sunflower, cotton and rape seeds). So there is no need to look for an answer to the question: how come pollinators disappear? This is a hypocrisy fostered by pseudoscientific popularization, in which science is exploited to persuade, distract and inculcate doubt. One of the products, son of the great economic interests at stake (chemical and seed industry), is a disease with an ambiguous name: the *sudden death of bees* or *Colony Collapse Disorder*, but it would be more correct to call it the syndrome of *Conscious Blindness to Planned Disaster*. Unfortunately, dozens of researchers all over the World have joined the search for these ghosts, a real waste of resources.
It does not require much perspicacity to foresee that the systematic and constant use of molecules that are poisonous to insects, which are also persistent, bioactive and bioaccumulative, perpetuated for tens of years, would generate the destruction of ecosystems and many other negative phenomena, such as the increase of certain diseases in humans. So why should we be surprised if insects and plants disappear when we use the most toxic molecules at our disposal with the intention of killing them? This imprudence will cost us dearly.
It should be very easy to understand the causes and quickly select the best solutions for bees and man. Incredibly, the gigantic knowledge at our disposal is invariably interpreted by health authorities and politicians as incomplete and insufficient to limit the freedom of selfish economic interests, in obvious conflict of interest.

In conclusion, it is to be hoped that the dangers highlighted in this book, summarized in a deluge of bad news, will serve to make people think more deeply and ask the right questions, before deciding to continue along ruinous paths. Each of the hundreds of scientific papers summarized in this book represents a small part of a puzzle. Examined in isolation they are less representative of reality than an attempt at synthesis such as the one made in this publication. The overall picture provided by this overview describes a situation that is much more alarming and more disturbing than it may appear. The overview shows that we already have sufficient knowledge to justify at least the application of the precautionary principle: if we do not act, no one else will do so for us. The ubiquitous use of pesticides in great quantities harms bees, but it also compromises the ability of ecosystems to render services essential to the survival of the biosphere. No ecosystem is spared and so aquatic organisms, soil microorganisms and wildlife are subjected to lethal and sub-lethal doses throughout their existence. Many pesticides are persistent, bioaccumulate and their metabolites can be more dangerous than their starting molecules (adjuvants, which may constitute the largest quantity in commercial pesticide mixtures, may be more dangerous than active ingredients). Therefore, even if the production and use of these molecules is stopped, we will continue to record their presence and effects for a long period of time. If this massive chemical attack on the biosphere is not reduced, the negative effects, including on our health, will grow in a worrying and intolerable way.
In the light of what is known and partly set out, we should find the strength to stop using pesticides. Without this we will continue to implement a sort of pasteurization of our countryside, that is, the destruction of most forms of life. Our indifference to this catastrophe will make the World more inhospitable and will not be able to be regenerated by accumulated monetary wealth. Other important changes are also necessary, such as making us responsible for climate change, which could turn out to be a real apocalypse. We have a duty to cultivate hope even when it seems futile, and it is this feeling that has sustained the effort required to complete the work of critically assembling and linking the scientific information reported in this publication.
We need more ethical programming that better expresses moral and survival instincts, and addresses concerns. Interactions between different actors in our society need to be improved so

that ecological limits are accepted and strategies are implemented that reduce obtusely selfish and unsustainable behaviours.
Any scientific knowledge is incomplete and is subject to being modified, changed or distorted by subsequent knowledge, but this should not superficially suggest that we ignore it in order to postpone the actions that constitute the most far-sighted precaution. The energies lavished on this informative work will hopefully serve to raise awareness and involve people, because information is necessary so that no one can exclude themselves by saying "*I would never have imagined, I didn't know*".

The totality of what has been summarized began to be known decades ago, yet not enough has been done, the political will and the will of big businesses, the ones that count the most, have been lacking. What we know is enough to push us to act in a responsible World, which is not the one we live in. The human race is lethally predisposed to harm itself unconsciously and has, among other things, technological tools capable of generating changes in the biosphere greater than those generated, in our day, by many forces of nature, such as the recent volcanic eruptions. Ultimately, the human population is too numerous and too effective in parasitizing the Planet to be able to hope to survive safely and in conditions of well-being for much longer. The biosphere is more and more polluted and the climate is more and more pushed by human activities towards an equilibrium that is not conducive to life, except for a few species that will be able to take advantage of it as probably some microbes, jellyfish and fungi. In the meantime we struggle with misinformation, greed and selfishness misguided by stupid economic rules and the unsustainable growth of unrestricted consumerism.

Geological history teaches us that mass extinction events, resulting from disastrous events, engage the biosphere for millions of years to achieve recovery. We can highlight our fragility with simple rules explained in survival courses to military personnel: without air we survive for three minutes, in the cold and without adequate shelter or clothing we survive for three hours, without water for three days and without food we hardly survive the third week. This simple "*rule of threes*" could inspire us on the choice of priorities for the protection of the biosphere: air, water, energy (food) and biodiversity. Today we may be able to protect most living species. Despite this, we continue to have a reckless propensity to destroy and replace a large proportion of them.
To cover the costs of reducing greenhouse gas emissions by 50%, it may be necessary to spend only a fraction of the world's gross domestic product (GDP), which is less than is invested in military spending in peacetime. [104, 106] Paradoxically, the decreasing availability of cheap natural resources exacerbates conflicts and increases the investment in military spending. The wrong investment, such as in weapons, is part of the failed strategy that "*you run to try to lose for last*".
Before the ecological collapse there will be social hardships, such as an increase in poverty and civil wars. Services considered indispensable, such as health care, will become unavailable. Negative social events may temporarily distract attention from the drama of diminishing natural resources, such as unpolluted water or fertile soils. The current short-term view of politics is therefore a loser, as it continues to consider investment in reducing environmental disaster and poverty to be far less profitable than producing superfluous consumer items and services, including weapons.

Collapses of past societies due to environmental misbehaviour are well known, resulting in ecological suicide. Among the causes of these "*collapses*" there were: [91]

- deforestation and habitat destruction;
- poor soil management, which has led to the loss of fertility;
- mismanagement of water resources;
- overhunting;
- overfishing;

- the introduction of new species (remember the disasters caused by rabbits in Australia or the smallpox epidemics brought by western conquerors to the New World);
- excessive population growth relative to the availability of resources;
- the increased ecological impact of each individual.

In addition to the causes of destabilization and collapse, other causes can be added, such as climate change, the depletion of energy resources, pollution and the end of trade with neighboring countries.

The decadence and extinction of societies of the past tend to follow similar paths, repeating the occurrence of consequent events: birth, growth, maturity, old age and death of a people. Violence and war were the natural consequence of moments of crisis.

Today, at least 170,000 litres of oil, 100,000 cubic metres of gas and 250 tons of coal are consumed every second: how long can we continue at this rate, or even faster? The race in the direction of an overall increase in the consumption of finite resources will generate an irreversible damage to health, environment and climate, even before non-renewable energies are exhausted. We should prevent our great-grandchildren from asking the same question that arises today when we think of the stories of past societies that destroyed themselves (what happened to the inhabitants of Easter Island is exemplary): how could this happen?

Time is of the essence, and it is time that is lacking in the modern World, for our children to look back and show gratitude, a feeling that will be good for their health.

Hopefully, at the end of this reading there are no more doubts left; exploring new solutions is not a whim but a necessity. The unsustainability of the current consumer society has condemned itself and we are therefore forced to change in order to move towards a new future. Many of humanity's most important innovations seemed initially relegated to the World of dreams. What seem like utopias today may surprise us tomorrow, if we break out of our current immobility. We have no guarantee that we will succeed in avoiding negative situations by changing our way of life, but if we do not try, failure is guaranteed.

THANKS

This part of the book is only available in the paper version.

BIBLIOGRAPHICAL REFERENCES INDICATED IN THE TEXT