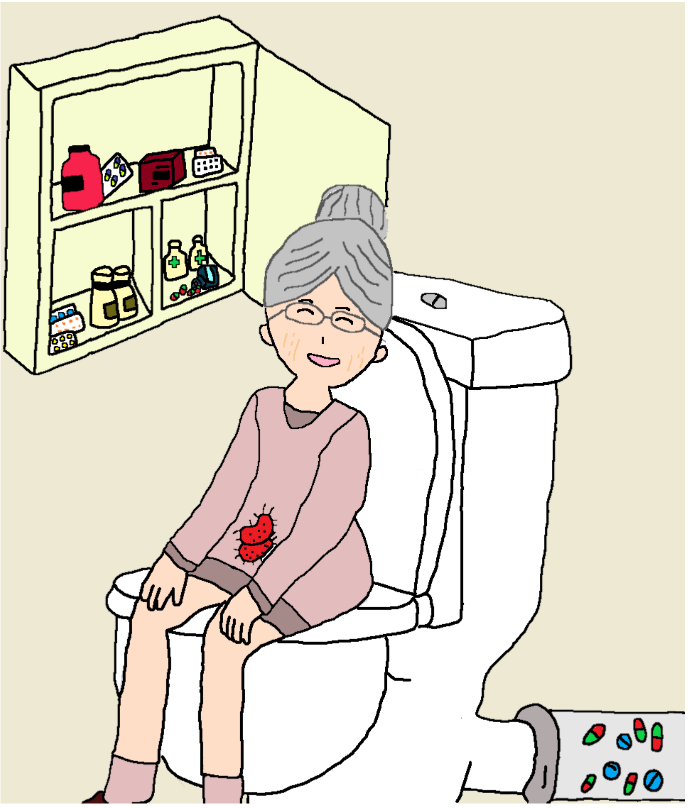
Antibiotics and antibiotic resistance in the environment

Granny: you take sooooo many medicines each day!
Do some of them pass out into the environment?



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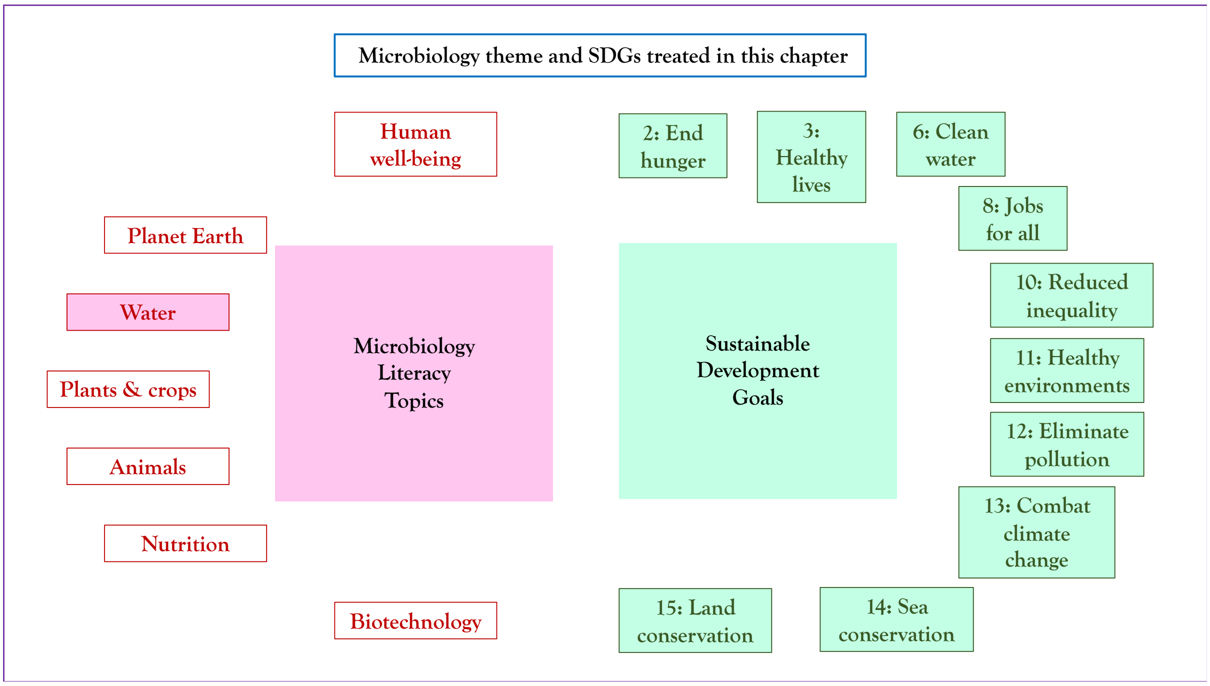
Antibiotics and antibiotic resistance in the environment

Storyline: A sword and shield story in the microbial world

Medicines are essential to human wellbeing. They are mostly highly powerful bioactive compounds which, at very low doses, can have a dramatic effect on target cells. However, bioactive compounds may also have harmful effects, not only on us, but also on other members of the **biosphere**. And, some of each medicine we take passes through us into the biosphere when we go to the toilet. In the case of **antibiotics**, very large amounts are also used in the production of food animals and farmed fish, most of which also ends up in the environment. Even though medicines in the environment are usually present at very low concentrations, their high activities – including harmful ones – affect susceptible forms of life exposed to them. Moreover, the presence of medicines in soil and water may constitute evolutionary stressors for microbes that favour the emergence of resistance to them. In the case of antibiotics, this leads to the development and spread of antimicrobial resistant microbes, one of the major human health issues of the XXI century. Nature is threatened and the act of taking medications has not only implications in health; it has also serious costs for sustainability.

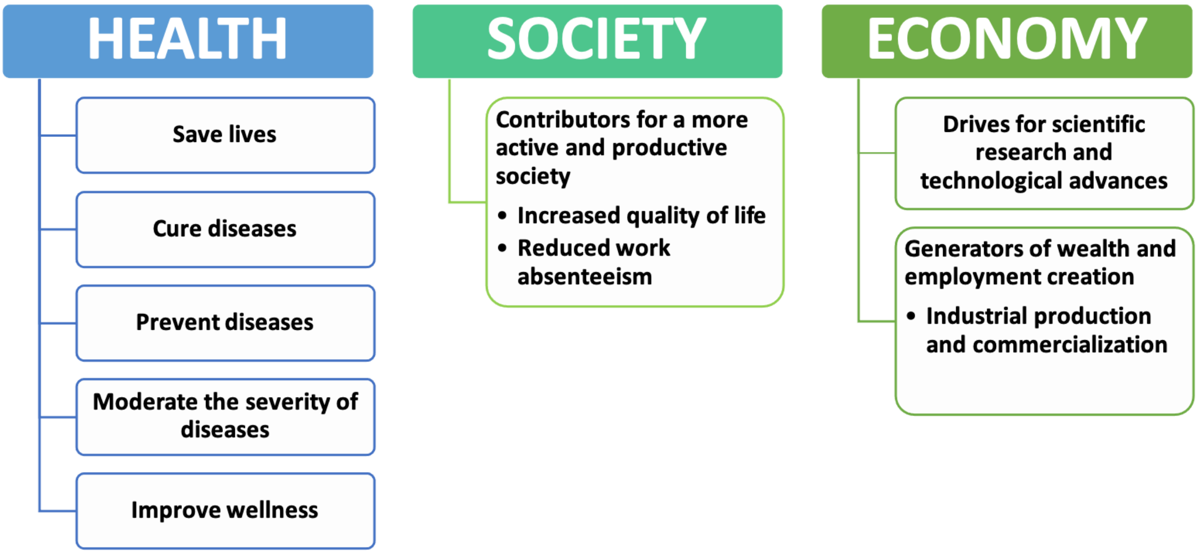
The Microbiology and Societal Context

The microbiology: antibiotics; natural bioactive products; biosynthesis; biotechnology; pathogens; biodegradation; antimicrobial resistance. *Sustainability issues:* health; well-being; equality; economy and employment; innovation; environmental pollution; water and sanitation.



Medications in the environment: The Microbiology

1. Medicines are part of our lives! Medicaments are used to save lives, to cure, prevent or moderate the severity of diseases or simply to improve wellness. Medications are also important pillars of the economy. On one hand, their use contributes, in general, to a more active and productive society, with increased quality of life and reduced work absenteeism. On the other hand, their development is an important stimulus for scientific research and technological advances, and their industrial production and commercialization are important generators of wealth and employment creation.



While the appeal to control pain and treat health conditions is probably an innate human ambition, medicines are nowadays the result of science-based research of academic institutions and pharmaceutical companies, intensified over the last century. These efforts have permitted the discovery and development of a multitude of natural (biosynthetic), semi-synthetic and synthetic active compounds that can be used as medicines (also known as pharmaceuticals) to diagnose, treat, lessen or prevent diseases - not only in humans, but also in animals, mainly pets and food-animals, and even plants/trees.

It is important to realise that some medicines do not cure us, but rather help us cure ourselves. For example, our immune system reacts to infections by killing or inhibiting the growth of invading microbes. Often it defends us without us even realising we have an infection. On the other hand, sometimes a **pathogen** multiplies very rapidly and can overwhelm our natural defences. This is where antibiotics can help, by inhibiting or killing some of the invading microbes, slowing the infection, and allowing our immune system to clear away the pathogens. The antibiotic gives us the advantage in a fight with a pathogen.

Medicines have changed the way societies are organized and live. Non-communicable diseases, such as diabetes, schizophrenia and even the viral infection responsible for AIDS, that were once life-threatening or life changing, can now be controlled by medicines, and patients with permanent medication may have a good life quality. Over the last 50 years, pharmaceuticals had profound impacts in society, such as birth control, a major decrease in child mortality, and a substantial increase in life expectancy. The availability and use of medicines are, however, associated with wealth and development so, unfortunately, they are quite unequal around the globe. According to the United Nations Secretary General, more than 2 billion people in the world still do not have access to essential medicines. This lack of access to safe, effective and quality medicines and vaccines, and other inequalities around the globe, were major motivations for the establishment of the 2030 Agenda and the definition of 17 Sustainable Development Goals.

2. Bioactive substances with potential pharmaceutical activity are produced by microbes
In 1928, Alexander Flemming, a Scottish physician with interest in microbiology, discovered accidentally that fungi, such as *Penicillium chrysogenum,* produce a compound, penicillin, capable of killing pathogenic bacteria.

Since then, many other soil microorganisms have been screened for their ability to produce substances of interest for the pharmaceutical industry, mainly for human use. These intensive exploratory screenings led to the discovery of many medicaments, mainly antibiotics, produced by different microbes dwelling in soil. Filamentous actinobacteria, a group of bacteria that resemble fungi, include numerous antibiotic producers, such as those producing tetracycline, gentamicin or erythromycin. Also, bacilli, a group of soil bacteria characterized by producing the most resistant **spores** in nature (**endospores**), include antibiotic producers, such as one producing colistin.

3. The discovery of biosynthetic antibiotics strengthened biotechnology. At the beginning of the XX century, infections were the primary cause of mortality around the world. Therefore, the recognition that penicillin could control infections caused by some bacteria, such as *Staphylococcus aureus*, encouraged the production of these medications to fight infections worldwide. Hence, since 1944, the year penicillin was first industrially produced, many other microorganisms, producers of natural antibiotics, were cultured under conditions that favoured the synthesis of these compounds for further purification for clinical use.

The production of large quantities of antibiotics in short periods of time was then a priority, which stimulated numerous studies and efforts to optimize microbial growth, antibiotic production and excretion from the producing cells. Hence, nowadays, the producing microbial strains are no longer the original microorganisms, i.e., they were selected and/or genetically modified to produce higher antibiotic yields or to release them from the cell. In simple words, we can say they have been domesticated, as were the tomato, potato and cabbage that we eat!

Besides improving the yields or producing new active compounds, recombinant DNA technology made also possible to use microorganisms to synthetize “foreign” proteins. Hence, bacteria and fungi are used as cell factories that as workhorses produce protein-based drugs. Examples of these medicines include insulin, human growth hormone, antibodies and vaccines.

4. Because antibiotics inhibit or kill living organisms, antibiotic-producing microbes are resistant to the antibiotics they synthesize. There are different possible explanations for the fact some microorganisms are antibiotic producers. Assuming that such a property represents a benefit for the producer, it has been widely argued that antibiotics may function as “weapons”. In this case, a natural antibiotic would improve the survival of its producer in a given habitat as it would fend off, inactivate or kill at least some of the neighbouring microorganisms. In this battlefield, antibiotic producers conquer space and nutrients with increased possibility of survival and proliferation: they have a competitive advantage.

Antibiotics are weapons that target and inactivate specific structures or functions in the bacterial cells. Therefore, to survive their own weapons, antibiotic producers must have protection mechanisms. A good example of this can be given for penicillin, whose activity consists of the inhibition of the synthesis of the peptidoglycan, a compound that provides the cell wall with rigidity and protection against environmental stresses. As penicillins are produced by fungi, with a different cell wall structure and composition, the producers are not inhibited by the antibiotic: they lack the target of the antibiotic.

5. To overcome the adverse effects of antibiotics, susceptible bacteria may become resistant. When a bacterial cell finds itself in adverse conditions, there may be four different outcomes:

a. some bacteria are intrinsically able to cope with such adversities, for instance because they produce resistance forms, such as spores, which allow them to enter into a dormancy state until conditions become favourable again (in a certain way, a spore resembles a plant seed, which we can store sometimes for long periods, but that germinates when sown);

b. others will live in a hibernation state, living with extremely low metabolic activity, and with no reproduction;

c. yet others cannot tolerate the adverse effects, and eventually die; and

d. the cells modify the genetic information, for example by **mutation**, to create specific cell structures that enable the progeny to resist the stress conditions.

6. Antibiotic resistance is mobile and spreads rapidly! To become adapted to changing environments and avoid extinction, organisms need to evolve by creating new **genes**, for example by **mutation**, or new combinations of genes. Animals and many plants create new combinations through sexual reproduction, which re-sorts gene combinations donated by both parents.

Bacteria do not undergo sexual reproduction, but they also obtain new genes and create new combinations. Bacteria obtain new genes through horizontal gene transfer, which means that they receive new genetic material from neighbouring cells (horizontal transfer), and not from parent cells (vertical transfer) as is the case in sexual reproduction. Horizontal gene transfer is fundamental for bacteria to acquire new traits, some of which will enable them to survive under adverse conditions.

There are three different mechanisms of horizontal gene transfer.

a. One, called transformation, involves the capacity to uptake genetic material released from dead cells into the environment. Hence, if an antibiotic susceptible bacterium uptakes a stretch of DNA containing a gene coding for antibiotic resistance released when a resistant cell died, it will gain that resistance trait.

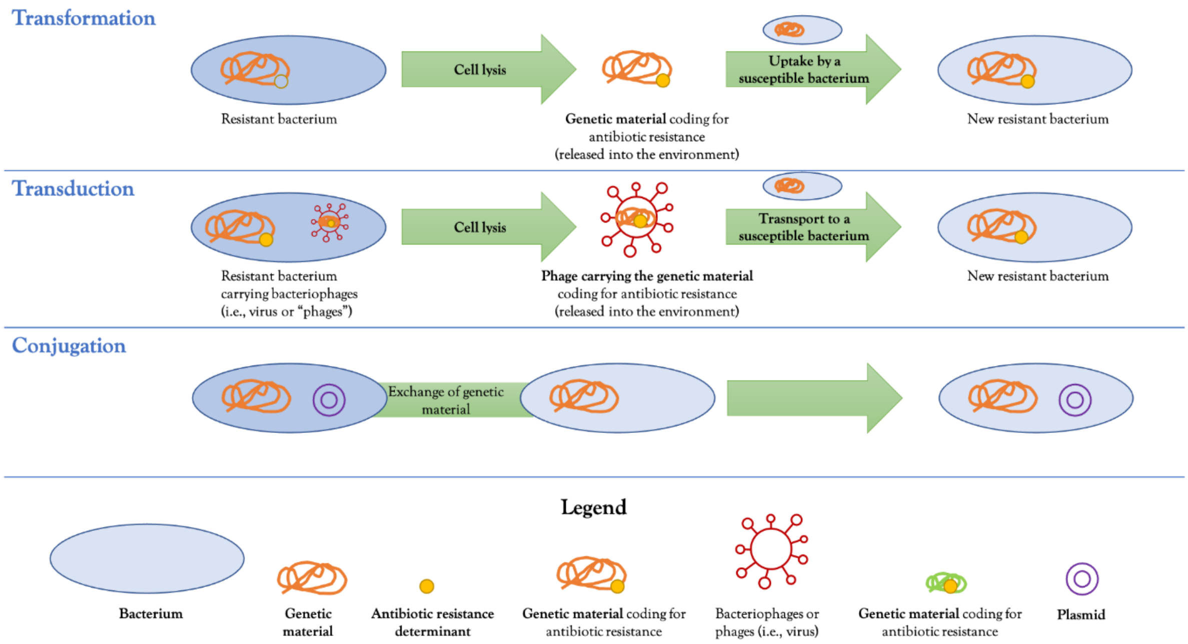
b. Another, called transduction, involves a virus, the simplest microbe, which depends for its reproduction on a cellular host. Viruses of bacteria are called **bacteriophages**, or "phages" for short, which are remarkable shuttles of genetic information.

Phages may have different lifestyles. There are so-called filamentous phages which are able to multiply within the host and be released continuously, without killing the host. But most phages can either multiply rapidly in an infected cell and kill it: this is called the lytic lifestyle because the phage lyses the bacterium. Alternatively, they may integrate their genome into that of the host and remain dormant for a period of time, or indefinitely. This is called the lysogenic state – the virus genome is passively duplicated along with the host genome - and it has been shown recently that most bacteria in the environment are lysogenic.

Lysogenic phages may change lifestyle at any time and become lytic. The trigger for this is not always clear but stresses of host bacteria, particularly DNA-damaging stresses that induce DNA repair systems, are well established inducers of the transition from lysogenic to lytic states. Irrespective of being lytic or lysogenic, when a phage is induced to multiply, by mistake the new viruses formed may incorporate part of the bacterial genetic information into their own genomes. When such a modified virus infects a new bacterial host, it transfers the DNA of the former host into the new one. If the DNA transferred encodes resistance to a given antibiotic, the new bacterial host will acquire this trait.

c. Finally, some bacteria can exchange DNA with other bacteria through a process known as conjugation of a **plasmid**. Plasmids are similar to chromosomes, but usually encode non-essential cell functions (non-essential under normal conditions), including resistance to antibiotics and toxic metals, the ability to degrade and use as food exotic compounds, like environmental **pollutants**, etc.

Most plasmids can either transfer themselves, or be transferred with the help of others, from their host cells to new cells by conjugation, which involves cell:cell contact. The new host thereby receives the entire plasmid and all its genes, including any genes encoding resistance to antibiotics. Although horizontal gene transfer by all of these mechanisms is normally an infrequent event, conjugation can increase markedly under selection pressure, such as the presence of an antibiotic, leading to so-called *epidemic transfer*.



7. Antibiotic resistant bacteria are like weeds in the garden and frequently are resistant to multiple antibiotics! Like weeds, most bacteria that acquire antibiotic resistance genes can multiply rapidly and almost everywhere, as long as some nutrients are available. So, when a diverse population of bacteria are exposed to inhibitory concentrations of antibiotics, the susceptible cells will be inhibited or die, while the resistant ones will survive. With an advantage! Now they have more space and nutrients and will be able to multiply rapidly.

And: they are often resistant to multiple antibiotics, that is: they can survive treatment with different types of antibiotics, i.e., antibiotics targeting different cell structures/functions. This means that an antibiotic, normally used to combat an infection, if not correctly prescribed or used, will have exactly the opposite effect. This is the reason why it is so important for the physician to know which microbe is causing the infection and which antibiotics work against it, i.e., to have access to microbiological and antibiotic susceptibility analyses before prescribing, and that the patient follows accurately the recommendations for taking antibiotics. If among the infecting bacterial cells, only a few are resistant, it is necessary to assure that the antibiotic and the immune system will act together to inactivate those nasty bacterial cells. If the antibiotic is not adequate and/or the administration does not follow the correct time intervals, the resistant bacterial cells will reproduce and eventually transfer the resistance determinants to susceptible cells in the microbial community.

8. Medicaments, especially antibiotics, are pervasive in the environment. It is exactly when people are taking antibiotics, which happens mostly in healthcare facilities like hospitals, that un-metabolized antibiotics and other medicines, active metabolites produced by metabolism of the medicines in our bodies, and antibiotic resistant bacteria are excreted (e.g., in faeces, urine) into the municipal sewers. In most regions of the world, domestic sewage is treated in specialised facilities – wastewater treatment plants – in which our wastes are degraded and mostly rendered safe. However, some fraction of the antibiotics and other medicines we consume, their metabolites, and antibiotic resistant bacteria, may survive the process and reach the receiving waterbodies.

But this is not the only source of these chemical and biological pollutants in water bodies. Indeed, it is estimated that more than 70% of antibiotics produced are used for animals, in particular food-producing animals (poultry, livestock, fish farms). These also excrete large amounts of un-metabolized antibiotics, active metabolites and antibiotic resistant bacteria that contaminate waters.

Moreover, the waste they produce – faeces and urine – are often used as manure to fertilise crops, and this in turn contaminates soils and the food webs they house. Sooner or later, streams, rivers, lakes, sea, groundwater and wildlife will subsequently receive these self-replicating microbial contaminants!

Some of these bacteria will not adapt to thrive in such environments, because they will not find the required environmental conditions to survive and multiply. But some ubiquitous bacteria are capable of living in a wide array of conditions, no matter if it is a pristine or polluted site, fresh food produce, or the gut of a human or other animal. While this should not cause alarm, and we should not blame bacteria for being dangerous because we create these conditions, it should make us think about our responsibility as citizens – from the food products we give preference to, and to the way we take our medication!

9. Medicines in the environment can be bad for us. Medicines are mostly highly powerful **bioactive compounds** which at very low doses can have a dramatic effect on target cells. However, bioactive compounds may also have harmful effects, so we should only use them when absolutely necessary: if the wrong medicine is given, or too much, it can damage us. This is the reason why doctors are very careful prescribing medicines, and why most are not available without a doctor's prescription.

Doctors "target" the medicine to the individual patient and to the patient's medical condition. However, a proportion of the medicines we take pass through us, and others are processed by our bodies to other compounds that may also have powerful activities, all of which end up in the environment when we go to the toilet. Another important pathway to the environment is the improper disposal of unused medicines in the trash can or down the sink.

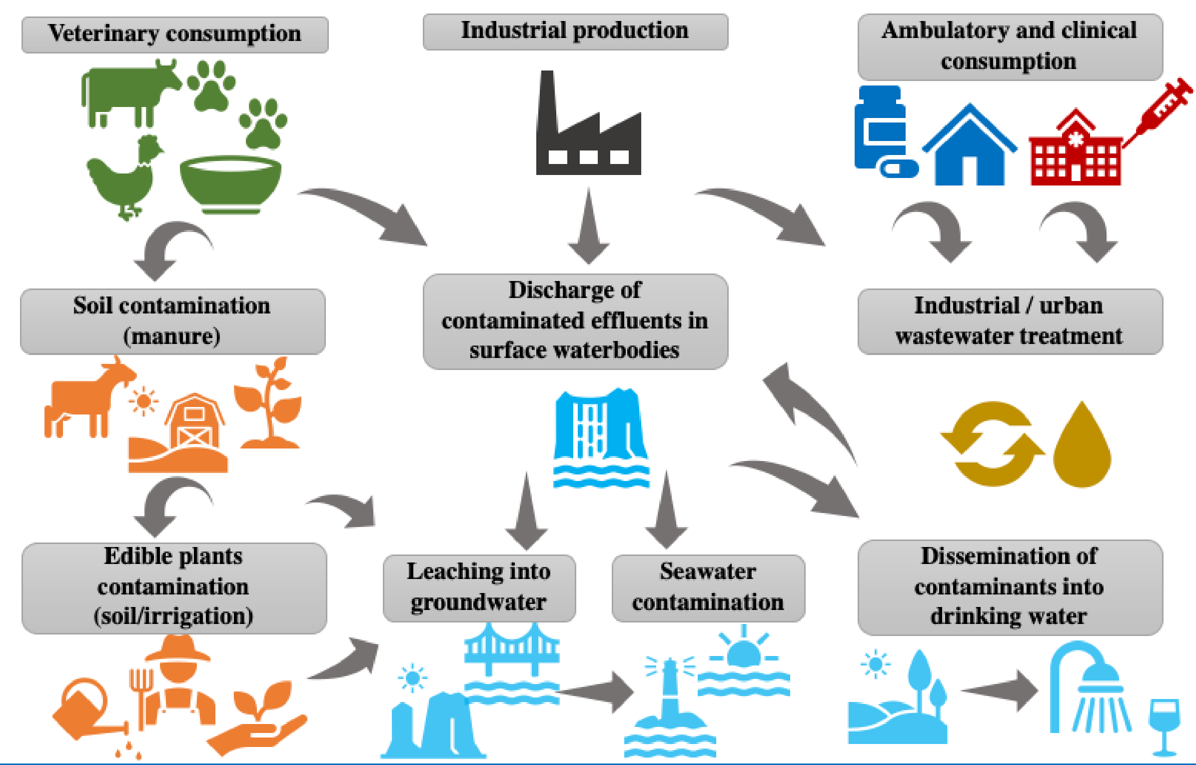
In the case of antibiotics, the largest amounts manufactured of some of them are used in the production of food animals and farmed fish, most of which also end up in the environment. Once in the environment, medicines can get into drinking water, and thereby be taken up by everyone. And, because they are so active, even though very dilute, initial "targeted" medication of a patient by a doctor becomes "non-targeted" mass medication of everyone, with any negative effects they may have. For instance, some of these substances can lead to endocrine disruption –

disturbance of the normal hormonal controls of our physiology – and/or chronic toxicity to all organisms in nature.

Moreover, their presence in soil and water may constitute evolutionary stressors that favour the emergence of resistance to them. Here, they can also affect microbes, for example antibiotics can select the evolution of **antibiotic resistant** microbes. If these can cause disease, or transfer resistance to others that can cause disease, then treatment of infections becomes a major challenge.

Besides their direct effects on living organisms, pharmaceutical contaminants can also be accumulated in plants and other organisms (**bioaccumulation**), leading to **biomagnification** through the food-web.

Box 2: Resource & Environmental Footprint of Medications



10. Some microbes degrade medicines. Microbes are the major inhabitants of the planet. To obtain energy for living, they are able to feed on an incredible myriad of organic and inorganic substances that they efficiently metabolise. Like in big cities, the crowd of microbes is composed of groups with specific functions - some take care of the sulphur compounds, others of nitrogenated molecules, etc. And some, maybe all, are quite innovative and learn how to feed on molecules that are not used by complex living organisms like animals.

Medications, natural or synthetic (hence called xenobiotics, because they are not produced in nature), once released into the environment as pollutants, disturb the ecosystems and are toxic for a wide diversity of living organisms. But the invisible and quite active microbial world is able to clean the environment from many of these pollutants, either by complete degradation to CO_2, or simple transformation to another compound that has a reduced toxicity level.

In the case of some antibiotics, the ability to break or modify the structure of antibiotics relies on the fact that the degrading bacteria are resistant to that antibiotic. Good examples are the beta-lactamase producing bacteria, which produce enzymes that break the activity-essential beta-lactam ring of a wide diversity of beta-lactams, one of the most prescribed antibiotic class that includes penicillin. By degrading these antibiotics, resistant bacteria are practicing charity with their neighbours that are susceptible to penicillins (a process termed bioprotection)! Charity, common in nature, is another interesting lesson that we can take from biology!

11. Medicines have large production and pollution footprints. Medicines need to be used carefully, in the correct doses, in small quantities, over short or longer periods of time. Some are not very stable and have short shelf lives. To keep medicines safe and fresh, they are manufactured, formulated and packaged, often in very small doses, in vast quantities in mostly plastic containers, which eventually end up in the environment as plastic waste containing residual amounts of highly active substances. Medicines therefore are associated with high production and pollution footprints. Since plastic packaging is a central component of medicament safety, replacement with alternative, ecologically sustainable forms of packaging is unlikely in the near future.

12. Possible ways to remove some medicines from the environment. Pharmaceuticals are components of a wider group of pollutants, also known as **micropollutants** due to their very low concentrations in the environment. Micropollutants result from many origins, including domestic, industrial and hospital wastewaters, runoff from agriculture, livestock and aquaculture, or landfill leachates. Such natural or synthetic substances comprise pharmaceuticals and personal care products, hormones, illicit drugs, pesticides, industrial compounds, and so on.

Despite their residual levels, micropollutants in general and pharmaceuticals in particular have been raising a huge concern due to their pseudo-persistence, i.e. the degree of removal occurring in the **wastewater treatment plants** and in the receiving environment is less than their input, thus leading to long term exposure and potential adverse effects on human health and ecosystems. Therefore, the scientific community has been studying novel advanced treatments to upgrade the conventional wastewater treatment plants, aiming to transform micropollutants into less harmful compounds or even to degrade them completely. Advanced water treatment processes include adsorption (e.g. on **activated carbon**), **membrane separation,** and **advanced oxidation technologies (AOTs)**. Another option is the implementation of natural attenuation systems, namely **riverbank filtration**, **aquifer recharge** and recovery, and **constructed wetlands**.

Relevance for Sustainable Development Goals and Grand Challenges

The microbial dimension of medication residues in the environment relates to several SDGs (*microbial aspects in italics*), including

- **Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture** (*end hunger and malnutrition, increase agricultural productivity*)**.** Veterinary medications permit the treatment of diseases not only in pets, but also in farm animal husbandry and aquaculture. Particularly in the case of antibiotics, they have been used not only to treat and prevent infections (**prophylaxis/metaphylaxis**), but also as growth promoters in some world regions. Another group of medicines that has been administrated to animals are hormones. Because the number of farm animals greatly surpasses that of humans, higher amounts of veterinary medicines are used annually than for humans. Consequently, the benefits of the productivity increase (with consequent availability of agricultural goods at lower prices) contrast with the environmental problems raised by the intensive use of veterinary medicines: the spread of the chemical (medicines and metabolites thereof) and biological (antibiotic resistant bacteria) pollutants.

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages** (*improve health, reduce preventable disease and premature deaths*)**.** Almost all of current clinical practices rely on the utilization of medicines. They are used to diagnose, prevent, lessen or treat diseases. Hence, medicines greatly contribute to improve health and well-being and to increase the human lifespan. However, their intensive use, and sometimes misuse, contributes to the chemical and biological contamination of the environment, which in turn can entrain chronic toxicity and a compromising of health. In the specific case of antibiotics, their overuse promotes the development of antibiotic resistant bacteria and thus, paradoxically leads to their own ineffectiveness.
- **Goal 6. Ensure availability and sustainable management of water and sanitation for all** (*assure safe drinking water, improve water quality, reduce pollution, protect water-related ecosystems, improve water and sanitation management*)**.** The massive production of medicines results in the release of industrial wastewater contaminated with medicines. There are many reports of industrial wastewaters containing antibiotics at therapeutic concentrations, mostly in developing countries. China has been the world's top producer of active pharmaceutical ingredients, followed by the US, Europe and Japan, collectively sharing 90% of world production until the mid-1990s. In turn, India plays a dominant role in the formulations segment, being the third largest producer of medicines in the world by volume. Besides supplying 20% of global exports of "generic" drugs, India is the major supplier of pharmaceuticals to countries of the Southern Hemisphere. The environmental control of these industries is inadequate and thus water quality is deteriorating.

  Beside contamination with pharmaceutical industrial wastewaters, the intensive consumption of medications by humans and other animals leads to excretion of un-metabolized medicines or transformation products thereof. The conventional wastewater treatment systems are not able to completely remove these chemicals from domestic sewage. Hence, treated wastewater effluents may contaminate the receiving waterbodies. In fact, countless studies have demonstrated the contamination of freshwaters by subtherapeutic levels of many classes of pharmaceuticals, namely anti-inflammatories, anti-depressants, cardiovascular drugs, etc. Medicaments accumulating in water may reach surface and underground drinking water supplies. Moreover, wildlife and food-products may become contaminated if in contact with polluted water. The presence of these pollutants may impose selective pressure on the living organisms, with disturbance of the ecosystems. As example, the reduction of the microbial diversity may lead to the loss of ecosystem services (recycling of C, S, N, P, etc). The emergence and dissemination of antimicrobial resistant organisms is another important consequence of the environmental contamination with medicine residues.
- **Goal 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all** (*promote economic growth, productivity and innovation, enterprise and employment creation*)**.** The benefits of medicines trigger numerous research studies carried out not only at academic institutions but also in pharmaceutical companies, giving rise to new knowledge on the processes underlying diseases, as well as new sustainable industrial processes. Hence, medications promote significant diversity of

businesses (e.g., industries, distributors, pharmacies) and a high number of a wide variety of employment.

- **Goal 10. Reduced Inequality** (*attenuate infection disease burden in low income countries*). Access to medications as well as environmental protection measures are closely related with socioeconomic factors. It is thus urgent to implement fair global production and trade principles for pharmaceutical industries and to promote equal access to medications at a global scale. The lack of access to vaccines and antibiotics in low income countries leads to high rates of preventable mortality caused by infections.
- **Goal 11. Promote sustainable cities and communities** (*improve infrastructure and services such as waste collection and water and sanitation systems*). More than half of the world´s population is living and/or working in cities and metropolitan areas. Hence, medication residues excreted by consumers (in ambulatory or clinical settings) end up in sewage, where they can reach high concentrations. In the case of antibiotherapy, antibiotic resistant bacteria are released into sewage, together with antibiotic residues. Conventional treatment systems are not designed to remove these micropollutants. Hence, treated wastewater effluents still contain high concentrations of these chemicals (e.g., the analgesic tramadol; the anti-inflammatories diclofenac, ibuprofen and naproxen; the antibiotics azithromycin, clarithromycin, and erythromycin) and biological micropollutants (e.g., multi-drug resistant enterobacteria and enterococci). Consequently, these pollutants contaminate the receiving surface water bodies and may leach into groundwater. Because surface and/or groundwater are frequently used to produce drinking water, medication residues have also been found in tap water (e.g., diclofenac and clarithromycin). Also some ubiquitous bacteria, with opportunistic pathogenic character such as *Pseudomonas aeruginosa,* which is known to carry genetic determinants conferring resistance to a vast list of antibiotics, have been found in drinking water.

  To overcome the environmental contamination of chemical and biological pollutants, some wastewater plants have been updated with advanced treatment processes, namely Advanced Oxidation Technologies (AOTs). These aggressive oxidative treatments, such as ozonation, are effective in the removal of most of the chemical pollutants, including medicines. Simultaneously, these AOTs are efficient at disinfecting the wastewaters. Indeed, drinking water treatment relies also upon the utilization of AOTs. However, due to the lack of competition, a few surviving organisms, sometimes multi-resistant, may become predominant in the treated (waste)water. While efficient disinfection processes are needed to assure the reduction of dangerous microorganisms in drinking or wastewater, collateral reduction of microbial diversity may cause health problems, due to the eventual dominance of ubiquitous resistant bacteria. In addition, the absence of a diverse microbial community may contribute to the impoverishment of the immunologic library of children, with adverse consequences for health.
- **Goal 12. Ensure sustainable consumption and production patterns** (*achieve sustainable production and use/consumption practices, reduce waste production/pollutant release into the environment, attain zero waste lifecycles, inform people about sustainable development practices*). The whole manufacturing process of medicines, from the synthesis of active pharmaceutical ingredients to the formulation process, involves numerous production steps involving major inputs of toxic chemical and energy resources. Efforts have been made by research institutes and companies to overcome this issue by implementing the principles of green chemistry. Another environmental problem results from the significant wastage of medicines caused by unnecessary dispensing and excess supply of

medicines. This often leads to improper disposal in landfills and/or discharge into sewage, reaching in both cases the aquatic environment. Many countries have adopted policies to reduce unnecessary medicine wastage and incorrect disposal, by informing healthcare staff, optimizing the supply chains, educating the patients, and creating recycling programs.

- **Goal 13. Take urgent action to combat climate change and its impacts** (*reduce greenhouse gas emissions, mitigate consequences of global warming, develop early warning systems for global warming consequences, improve education about greenhouse gas production and global warming*)**.** Greenhouse gas emissions are pervasive in industry and pharmaceutical manufacturers are not an exception. Moreover, many natural pharmaceutical ingredients are extracted from biological materials by polluting chemicals, such as solvents.
- **Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development** (*reduce pollution of marine systems by toxic chemicals/agricultural nutrients/wastes like plastics, develop mitigation measures for acidification, enhance sustainable use of oceans and their resources*)**.** A great number of cities and industrial areas are located near the coast. Consequently, industrial, hospital and urban wastewaters may run into marine systems. In fact, although at very low concentrations due to the dilution effect, pharmaceutical residues (e.g., tramadol, clarithromycin, and fluoxetine) have been found in the marine environment. The presence of these pollutants in sediments and water may contribute to the contamination of wildlife and food-webs, reduce biodiversity, and disturb microbial functions.
- **Goal 15. Conserve life on land** (*micro-organisms and invertebrates are key to ecosystem services, but their contributions are still poorly known and rarely acknowledged*)**.** The land ecosystems need to be equilibrated. Improper disposal of unused medicines and/or animal excreta contaminates the soil with medication residues, high loads of faecal microbiota and antibiotic resistant bacteria. These contaminants may alter the natural microbiome, eventually leading to an imbalance that can hinder the productivity of such soils. Moreover, plants can adsorb and/or uptake pharmaceuticals and antibiotic resistant bacteria. Consumption of contaminated edible plants may lead to the contamination of the food-web, where **bioaccumulation** of pharmaceuticals may occur. Wildlife and human contamination with medicines and antibiotic resistant may occur, particularly through the consumption of raw produce such as lettuce or carrots.

Box 3: Benefits and threats of medicines and related microbial aspects for the SDGs treated in this chapter

MEDICINES	SUSTAINABLE DEVELOPMENT GOALS	MICROBIOLOGICAL ASPECTS
• Increase food production • Reduce food costs • Risks of contamination of food products	**2: End hunger**	♣ Reduce food-born intoxications/ infections ♣ Reduction of microbial diversity ♣ Promotion of **antimicrobial resistance** dissemination
• Access to health and wellness care • Longer life expectancy	**3: Healthy lives**	♣ Improved infection prevention ♣ Reduced infection rates

• Successful modern medicine interventions • Inequality to medical care, depending on socioeconomic factors		♣ Infectious agents mobilization in a global world ♣ Emerging infectious threats
• High loads of drugs in industrial effluents, hospital and domestic sewage • Accumulation of contaminants in (drinking)water • Need of increased technological sophistication and costs to clean waters • Export of highly polluting pharmaceutical industries to world regions with low cost of man-force and permissive environmental restrictions	**6: Clean water**	♣ Emergence and dissemination of antimicrobial resistant organisms ♣ Reduction of microbial diversity in the environment with loss of **ecosystem services** (recycling of S, N, P, etc)
• Development of new molecules, materials and systems for drug industry • New prevention, treatment and diagnosis systems being launched regularly • Incentive for research and cooperation between industry and academia • Increase of qualified employment • Improvement of life quality	**8: Jobs for all**	♣ New vaccines ♣ New drugs ♣ New microbial-based cell factors ♣ Stimulus for creating new concepts of clean spaces in **Biotechnology** companies
• Need for implementing fair global production and trade principles for pharmaceutical industries • Promote equal access to medications at a global scale	**10: Equality**	♣ Urgent need to attenuate the infectious disease burden contrasts around the globe and socioeconomic sectors
• Need for adequate disposal of unused medicines • Need of implementing dedicated sewage treatment for health care facilities • Promote lifestyles that reduce the need for medicines	**11: Healthy Environments**	♣ Risks of excess of hygiene and lowering of numbers of "good" microbes ♣ Progressive impoverishment of the immunologic libraries of children
• Risk of excessive prescription and use of medications • Excretion and improper discharge of pharmaceuticals • Antibiotic stewardship	**12: Eliminate pollution**	♣ Risks of reduction of microbial diversity ♣ Increased spread of antimicrobial resistance forms ♣ Drug **biodegradation**
• Contamination of water and sediments • Aquatic wildlife threatened due to adverse effects of medicines • Contamination of the aquatic food-web • Biodiversity loss	**14: Sea conservation**	♣ Reduction of microbial diversity in the environment with loss of ecosystem services (light harvesting, CO_2 fixation, recycling of S, N, P, etc)
• Contamination of soils and wildlife • Wildlife threatened due to adverse effects of medicines • Contamination of the food-web • Biodiversity loss	**15: Land conservation**	♣ Reduction of microbial diversity in soils ♣ Impairment of the interactions host-microbe with impacts in biodiversity and agriculture production
• Encourage the interaction and dialogue among the society, the academy, governments and industry	**17: Partnerships**	♣ Highlight and explore the immense and powerful capacity of microbes to promote health and wellbeing!

Potential Implications for Decisions

1. ***Individual***
   a. Consequences of incorrect disposal of medications (sink, garbage).
   b. Consequences of incorrect consumption of medicines.

2. ***Community policies***
   a. Local environmental consequences (pollution of local water bodies and soils with medicines and antibiotic resistant bacteria), provision of clean drinking water
   b. Health costs associated with antibiotic resistant bacteria or toxicity of chemical micropollutants
   c. Non-microbial parameters: support of local businesses and research – pharmaceutical industries, pharmacies, research institutes

3. ***National policies relating to medications in the environment***
   a. Environmental pollution
   b. Ensuring safe food and drinking water supplies
   c. Combatting the antibiotic resistance crisis
   d. Non-microbial parameters: policies relating to education on the correct usage and disposal of medications

Pupil Participation

1. ***Class discussion of the issues associated with medications***

2. ***Pupil stakeholder awareness***
   a. Medications have positive and negative consequences for the SDGs. Which of these are most important to you personally/as a class?
   b. Can you think of anything that might be done to reduce the negative consequences?
   c. Can you think of anything you might personally do to reduce the environmental footprint of medicines?

3. ***Exercises (could be made at any level, but these are probably secondary education level)***
   a. Frequently, hospital effluents are discharged into the urban sewer and are treated in municipal wastewater treatment plants. In addition, in some countries pharmaceutical industries are not efficiently treating their effluents before disposal in natural waterbodies. What other alternatives to disposing heath care /industrial wastewaters can you envision?
   b. Intensive animal farming relies on massive consumption of medicines (**prophylaxis**/metaphylaxis/ **growth promoters**). What other alternatives of animal farming can you envision to reduce the environmental negative impacts?
   c. Many medications do not need a medical prescription and are freely available in pharmacies, on-line shops or even in supermarkets. Can you envision some recommendations to the consumers of these drugs?
   d. Looking at the SDGs, how can we change our approach to the disposal of medicines to bring them into line with sustainable development? What are the challenges and A child-centric microbiology education framework opportunities?

The Evidence Base, Further Reading and Teaching Aids

Kahoots: see teaching aid provided

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Glossary

Activated carbon: is a special kind of coal, with a very high number of tiny pores.
Activated carbon adsorption technology: procedures designed to remove organic (and sometimes also inorganic) compounds from water or wastewater through adsorption onto the surface of the tiny pores of activated carbon. *Clue: It is widely used to purify water at home!*
Advanced oxidation technologies: procedures designed to remove organic (and sometimes also inorganic) compounds from water or wastewater through chemical oxidation reactions. *Clue: It may have been used to clean the tap water you use!*
Antibiotic: a natural or synthetic compound that inhibits the growth or even kill bacteria. *Clue: the doctor can prescribe it when you have an infection caused by bacteria…*

Antimicrobial compound: inorganic or organic compounds that cause injury or even destroy microbial cells. They can be natural (i.e., biosynthetic), semi-synthetic (upon chemical modification of a natural compound) or totally synthetic (i.e., produced by chemical reactions designed and executed by humans). Clue: *Bleach or ethanol used for cleaning or wound disinfection are good examples!*
Antimicrobial resistance: capacity of some microbial cells to resist the hazardous effects of a given antimicrobial compound. Clue: *Take care! It may result from an excessive or inadequate use of antimicrobials!*
Aquifer recharge: a process where adsorption of micropollutants onto the soil particles and/or biodegradation occurs when water moves from the surface into the groundwater aquifer.
Bacteriophage: a virus that infects bacteria. It is also called phage. Clue: *The bacterial glutton!*
Bioaccumulation: is the accumulation of a substance in an organism. It occurs when that substance is stuck or held in the organism, being accumulated over time.
Bioactive compound: A substance that has an effect on a cell. Clue: *when you take vitamins, you are ingesting bioactive compounds!*
Biodegradation: capacity of a cell to break a given organic molecule. Some cells may have only the capacity to break the organic molecule, so, they accumulate the resultant metabolites. Some other cells may have the capacity to further degrade the metabolites, and transform them into inorganic compounds, such as carbon dioxide, and ammonium. Clue: *Biodegradation is cleansing the environment from the pollutants we produce, but sometimes microbes are not enough to handle all the pollution* **humans produce. Think about that!**
Biomagnification: is the bioaccumulation of a chemical compound through the food chain, eventually reaching toxic levels in the organism of the top of the chain. Clue: *Pesticides are used in agriculture to produce more and more appetizing food crops, but it will contaminate the soil and it will reach our plate… and or body!*
Biosphere: is the sum of all ecosystems. It integrates all living organisms and their interactions with the surrounding environment. Clue: *Humans are a tiny part of the biosphere!*
Biosynthesis: synthesis of organic compounds occurring within a cell, or with the help of biological catalysts, i.e., enzymes.
Biotechnology: Any process carried out with the help of living organisms or thereof enzymes. Clue: *It seems rocket science but it is practiced since humans started to brew milk, bread or beer, back in the antiquity, more than 10 thousand years ago!*
Constructed wetland: artificial wetland, which includes a sequence of processes to treat wastewater. In this process, adsorption, biodegradation by soil microorganisms and/or plant uptake of pollutants occurs.
Endospore: a special type of spore produced by some bacteria. Endospores are the most resistant dormant forms in nature. Clue: *Spores are like seeds, think about beans! They will germinate when the conditions are favourable, but endospores are really special, they can stand boiling water and the Egyptologists use them for dating the antiquities they discover!*
Ecosystem service: all the goods and functions provided by the ecosystems that benefit humans. Clue: *Think about the biosphere as an unstoppable, invisible and giant factory that make of Earth an inhabitable place!*
Gene: a sequence of DNA base pairs encoding for a macromolecule necessary for a cell to live, such as a protein. Clue: *In your body you have about 25 thousand genes giving instructions to build and maintain your body!*
Growth promoter: any substance added to feed and/or water of farm animals to stimulate their growth, it can be antibiotics. When antibiotics are used, at low concentration (sub-therapeutical

doses) they improve the efficiency by which feed is transformed, and hence, the animal grows faster. *Clue: But these antibiotics may be selecting for antibiotic resistant bacteria…*
Membrane based technology: any process carried out with membranes. The membranes have pores by which water can pass from one compartment into another. Depending on the diameter of the pores, membranes are able to retain microbial cells or even molecules.
Metabolites: organic molecules produced by cells upon modification of their nutrients or any other molecule that the cells can transform with their own enzymatic machinery.
Mutation: alteration in the sequence of DNA nucleotides. When occurring within a protein encoding gene, may change its function. *Clue: Mutations make live beings evolve, they were the basis of the natural selection process that Darwin proposed more than 150 years ago!*
Microbiome: all microorganisms and genetic information inhabiting and living in a given site. *Clue: In your body you will find as many microbiome cells as human cells!*
Micropollutant: a pollutant that is present at very low concentration (from ng/L up to µg/L, i.e., 1,000,000,000 to 1,000,000 fold lower than 1 g per liter).
Mineralization: total oxidation of an organic compound, producing inorganic products such as carbon dioxide, water and ammonium.
Pathogen: a microorganism that when in contact with a multicellular organism (named host) causes illness. *Clue: The bubonic plague pandemic that just in the 14th century killed more than 50 million people in Europe, Asia and Africa, and the current COVID-19 pandemic, are examples of the regular and continuous exposure of humans to pathogens!*
Plasmid: an extrachromosomal DNA molecule.
Pollutant: a natural or synthetic compound that when present in a given site causes adverse conditions to the inhabiting living beings. *Clue: While the condition of Earth is deteriorating, pollution is everywhere, water, air, soil, wildlife – You can help stop this. Think about it!*
Prophylaxis/metaphylaxis: procedures taken for infectious disease prevention. The best prophylaxis practices involve isolation of infected individuals or vaccination. But these measures are not always possible, hence in animal (meat or fish) production, antibiotics are often used in the feed and/or water of all the animals to prevent infections. *Clue: These practices may affect the environment as well as the fish or meat quality and both will affect human health. It's all connected!*
Riverbank **filtration**: a water treatment process, by which water if filtered through the banks of a water body (river, lake). During this process, the pollutants are adsorbed and/or chemically or biologically degraded.
Spore: a cellular structure produced by some microorganisms when the environmental conditions became adverse to the cell. The spore out-layers protect the DNA of cell, which dies, releasing the spore into the environment, where it may stay for very long time. Because during this period the spore does not carry out any metabolic reaction, it is called a dormant form. When the environmental conditions become favourable, the spore germinates giving rise to a cell identical to the original one. *Clue: Did you ever notice the green powder growing on bread or the blue powder that grows on lemons? This powder is comprised of millions of fungal spores! Microbial spores are not visible at naked eye, but if you take a bean you can consider it is a giant spore – pay attention how its cover repels water, it is said to be hydrophobic and this is to avoid that chemical reactions take place inside the bean. If you open the bean, protected from the outside, you will find the genetic material that will give rise to a germinated plant and the nutrients necessary for germination.*
Wastewater treatment plant: "A facility designed to treat wastewater through a series of biological and physical-chemical processes. Pollutants such as organic substances and nutrients are removed to make wastewater dischargeable into natural water bodies". *Clue: Did you ever think what will happen after you flush the toilet? It will be a long journey through which the water we use at home for cleaning and bathing and all other activities will be pumped into these important urban facilities where*

water will be cleaned before it is returned to nature. Did you know that in many world regions these facilities are not available and sewage directly enters rivers and lakes?