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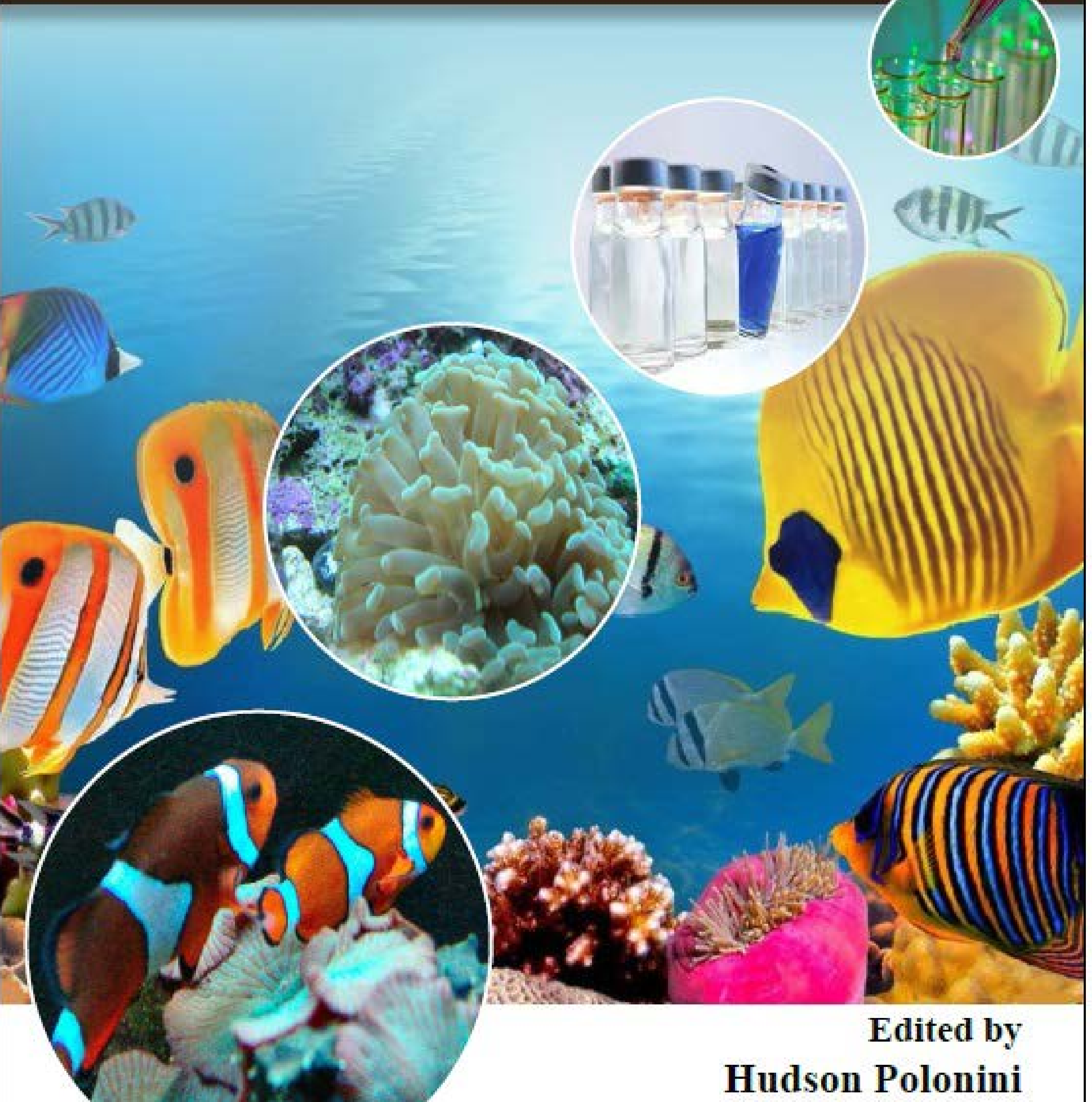
Aquatic Toxicology



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Aquatic Toxicology

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Preface

The rapid development and dissemination of nanomaterials can put them in contact with living organisms in situations not yet fully understood. Relatively new researches indicate that nanomaterials may have different toxicity profiles when compared to large particles, due to their small size and high reactivity.

The ecotoxicology is a science whose core is to study the contaminants and their effects on the biosphere components, including humans. The ecotoxicological research developed rapidly due to pollution of the environment induced by the rapid industrial development by that time, pervaded by serious industrial accidents. Policies have been developed since then, and ecotoxicology has become a significant part of the environmental and ecological risk assessment required by the new legislations to come.

With this book, we hope researchers can find a source of information on the main topics of toxicology on aquatic environment, on the grounds that this is one of the major sources of nanomaterials entry in our environment.

Signature

About Editor

Dr H. Polonini (30 yrs) has completed his PhD at 2014 from Federal University of Juiz de Fora. He works in a pharmaceutical company (Ortofarma) and gives classes for two Pharmacy Colleges (Suprema and Unipac). He has published more than 40 articles. His major interests are analysis and control of medicines and related products, and pharmaceutical and cosmetic technology.

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Aquatic Toxicology As A Tool To Dimension The Impact Of Nanomaterials In The Environment

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Abstract

The rapid development and dissemination of nanomaterials can put them in contact with living organisms in situations not yet fully understood. Relatively new researches indicate that nanomaterials may have different toxicity profiles when compared to large particles, due to their small size and high reactivity [1].

Humans can be exposed directly or indirectly to these materials [2]. Some of the possible routes of exposure that may occur vary from their synthesis and production of derivatives (occupational exposure) until the end use thereof (consumer exposure), also including their removal and subsequent accumulation in environment (environmental exposure) [3]. Among these, the air route is considered more significant. In fact, epidemiological studies have shown that ultrafine particles are associated with respiratory and cardiovascular disease, which has resulted in morbidity and mortality in susceptible segments of the population [4,5].

The main entry routes of nanomaterials in the human body are the skin, the gastrointestinal tract and the respiratory tract, whether by the use of topical creams or oral medications, either by contact with contaminated water, air and soil [6,7].

Some studies suggest that nanomaterials, for their small size, may have a higher permeability through the skin, mucous membranes and cell membranes, and may have their toxic effect magnified, since they have a higher reactivity, due primarily to increased surface area. A classic example is gold, which is a practically inert metal, but in the form of nanoparticles becomes highly reactive [8].

An existing classification for nanomaterials can be made according to the fact that the nanostructure is or not immobilized in a large size material (composites, functionalized nanoparticles, etc.); for example, as part of a surface of a micronised material. The opposite to this case would be free nanoparticles capable of mobility in the environment and in the human body [9]. It is the latter that poses more threat from a toxicological point of view, and it is the interaction of these particles with living organisms that is not yet fully known. The complexity comes from the ability that the nanoparticles possess to bind and interact with biological material and changing its surface characteristics depending on the environment in which they are. For instance: lysosomes, Golgi complex, NDA and mitochondrias can be physically damaged. The recent scientific knowledge on cell interaction mechanisms and nanoparticles have indicated that many cells readily internalize the nanoparticles, either by active or passive mechanisms. Intracellularly, however, the mechanisms and pathways are more difficult to understand. Even particles of a same material may have completely different behavior due to, for example, small differences in the coating surface, or in its loading capability. This is one of the main distinctions between nanotoxicology and classical toxicology. Furthermore, bioassays involving nanomaterials is still under development and, in general, have not yet been internationally validated [10].

In addition to that, there are many more variables to consider when working with nanomaterials, and these include: size, shape, surface charge, dispersion state (agglomeration *versus* aggregation), concentration and the media they are in. Otherwise, the possible toxic effects could not be easily assigned to a specific quality of the nanomaterial or even to the very nanomaterial itself because, for example, impurities and other components may be held responsible [11].

The importance of establishing the nanomaterial dispersion state lies in the fact that certain particles are extremely reactive in an aqueous medium, which changes its size and shape, compared to the dry powder. The dried nanomaterials may take two forms: aggregates (strong links between primary particles) and agglomerates/pellets (controlled by the weaker forces such as van der Waals). The state of the nanoparticles, may be controlled during synthesis [12,13]). After dispersing the nanomaterial into a solution/suspension, they may remain as singlets or form agglomerates, or remain as aggregates, surrounded by a double layer. Typically, when agglomerated nanoparticles are added to a liquid they can be separated by overcoming the weak attractive forces by various methodologies, such as the use of ultrasound. For their turn, e aggregated nanoparticles cannot be separated.

This is the point where the concept of ecotoxicology gains in importance. Exposure to nanomaterials in different environmental compartments (water, soil and air) may result in a greater bioavailability and accumulation along the food chain, i.e., nanomaterials are likely to interact with other living beings, causing effects hitherto unknown in its entirety. The three base elements for screening nanomaterials toxicity profile, according Oberdörster et al. (2005a)[14], are: (i) physical and chemical characteristics (size, surface area, shape, solubility, aggregation); (ii) elucidation of the biological effects from *in vitro* studies; (iii) confirmation of the effects in *in vivo* studies.

These three pillars were formulated in view of the potential effects of nanomaterials in humans. But when an entire ecosystem is taken into account, the question becomes broader and more complex. Despite an increasing volume of information about the toxic effects of nanomaterials on humans by direct or indirect exposure, studies of environmental impacts of these were just at its beginning in 2010, according to Kahru and Dubourguier (2010)[15]. The trend, however, is that this evaluation is increasingly a global concern, but they should be required by regulatory agencies in order to become mandatory worldwide.

The ecotoxicology is a science whose core is to study the contaminants and their effects on the biosphere components, including humans [16]. It was René Truhaut in 1969 who first mentioned the term "ecotoxicology", defining it as the branch of concerned toxicology study of toxic effects caused by natural or synthetic pollutants for the components of animal ecosystems (including humans) vegetable and microbial, in a full context [17]. The ecotoxicological research developed rapidly due to pollution of the environment induced by the rapid industrial development by that time, pervaded by serious industrial accidents. Policies have been developed since then, and ecotoxicology has become a significant part of the environmental and ecological risk assessment required by the new legislations to come.

Ecotoxicological tests have greatly developed to aquatic environments. In this context, Blaise (1998) classified the development stages for aquatic toxicity tests by decades: (i) "dark ages", until 1950; (ii) the decade of fish assays in 1960; (iii) the regulatory decade, 1970; (iv) the ecotoxicological decade in 1980; and (v) the decade of microbiotests in 1990. More recently, Kahru and Dubourguier (2010) designated that the 2010-2020 period can be defined as the “era of (eco) toxicogenomics and eco-toxicology.”

Despite the growing understanding that synthetic nanomaterials should be assessed for their environmental potential danger prior to use in products and their inevitable release into the environment, there are currently few data in this regard. The first few research began in the 1990s, mainly by assessing lung impact of ultrafine particles [18]. There was a gap of 10 years, until the number of nanotoxicology researches began to increase exponentially, most focused on human health [3]. In relation to environmental impact, the number of searches is still small and does not reflect the substantial number of new applications developed for these materials.

In this regard, the aquatic toxicity tests are widely used because these ecosystems are the main receptors of contaminants, whether they are coming from direct release into water bodies through wastewater discharges, or issued in the air or deposited in soils [19].

The aquatic environment is complex and diverse. It comprises various types of ecosystems, among which are rivers, lakes, estuaries, oceans and seas. They are still open and dynamic systems capable of undergoing continual changes in its chemical composition. For example, in freshwater, calcium, magnesium and carbonate ions are the more abundant but compounds of sodium, potassium, phosphorus, iron, sulfur and silicon are also present. There are also non-conserved components, which include dissolved gases such as O_2, CO_2 and N_2; nutrients such as phosphate and nitrate; dissolved organic compounds such as amino acids and humic substances; trace elements such as copper, zinc, chromium, molybdenum, vanadium, manganese, tin, iron, nickel, cobalt and selenium, and particulate materials such as sand, clay, colloids, non-living tissues, and excreta.

Among the biochemical and physiological effects caused by toxic agents on aquatic organisms, we can mention: change in the permeability of cell membranes; interference in ATP production; reversible or irreversible enzyme inhibition; disturbances in the metabolism of carbohydrates and disorders in the respiratory process by inhibiting electron transport and oxidative phosphorylation; disturbances in lipid metabolism, which may result in liver disorders; change in the structure or activity of enzymes that participate in regulatory processes, affecting the synthesis and release of hormones [20]. It is also recommended that the toxic effect of a sample should be evaluated for more than one representative species of aquatic biota, for example, an alga (*Chlorella vulgaris*) am *Euglenophyta* (*Euglena gracilis*) and a cyanobacterium (*Anabanea flos-aquae*), minimally.

The use of primary producers as biological indicators is important because they are located at the base of the food chain and any change in the dynamics of their communities can affect higher trophic levels of the ecosystem. Usually, they are also quite sensitive to changes in the environment and its life cycle is relatively short, which allows the observation of toxic effects in several generations [20]. The resulting inhibition effect on the population of algae and cyanobacteria, after a predetermined time interval (usually 3 or 4 days, in the present study, 4 days) is determined by comparing the growth observed in the presence of toxic agent and the “normal” growth, observed in an agent-free system, which is called a control.

Still, toxicity tests can be classified into acute and chronic, depending on the length and final responses that are measured. Acute toxicity tests are used to measure the effects of toxic agents to aquatic species for a short period of time relative to the life of the test organism. They have to estimate the dose or concentration of a toxic agent that would be able to produce a measurable response in a test to a specific organism or population in a relatively short period of time, typically 24 to 96 h. The toxic effects measured in acute toxicity tests include any response displayed by a test organism or population resulting from a chemical stimulus. Typically, the measured effect in acute toxicity studies with aquatic organisms is lethality or some other manifestation of the body which precedes, for example, the resting state [20].

As for the chronic toxicity tests, they are performed to measure the effects of chemicals on aquatic species for a period which may cover all or part of the life cycle of the test organism. The fact that a chemical does not produce toxic effects on aquatic organisms in acute toxicity tests does not indicate that it is not toxic to them. Chronic toxicity tests allow to evaluate the potential toxic effects of chemicals under conditions of prolonged exposure to sub-lethal concentrations, i.e., concentrations that allow the survival of organisms but which affect their biological functions, such as reproduction, development of eggs, growth and maturation, among others [19,21].

Toxicity tests can be further classified into static, semi-static and dynamic, in accordance with the method of addition of the test solutions. Static assays are performed without renewal of the test-solutions and are recommended for samples that do not cause oxygen depletion, which are not volatile and are stable in aqueous media. On the other hand, unstable or volatile toxic substances have their concentrations reduced throughout the test, contributing to its result be underestimated. In such cases it is recommended semi-static tests, in which test-solutions are renewed periodically. Chronic toxicity, long-term tests are usually performed in dynamic mode, with test-solutions continuously renewed [22-24].

Given that, it is highlighted the important role that (nano)ecotoxicology tests play in the current estate of nanomaterials development, mainly in aquatic systems. I hope the next chapters can elucidate more on that, contributing to a better understanding of the mechanisms involved. In this case, new and more complete studies can be performed gradually each day.

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Eco-toxicological Impact of Pharmaceuticals for Human Use in Aquatic Systems

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Abstract

Current society consumption patterns have increased the production of chemical residues causing negative impacts on environment. Pharmaceutical waste is among the environmental contaminants that receive great prominence and international attention, and it is causing impact especially in aquatic environments. Interaction happening between different classes of drugs present in environment in unknown proportions and amounts may produce higher toxicity compounds, whose effects have not been studied. Moreover, many drugs are not metabolized in the human organism and thus they are excreted with no changes in its chemical properties, making them persistent contaminants in the environment. Pharmaceuticals and Personal Care Products (PPCP) such as cosmetics, hormones, antidepressants, and antibiotics are well known about the effects of their residues and are characterized as environmental pollutants. Some cosmetics and especially hormones can be potent endocrine disruptors for non-target organisms while antibiotics can select extremely resistant bacteria, leading to serious problem for human health. All these medicines usually contaminate aquatic systems through improper disposal, and they are often present in urban wastewater. Faced with the potential of pharmaceuticals to reach the environment and to affect the exposed biota, this chapter aims to provide information on the occurrence of major pharmaceuticals in the environment, to present the main effects that such agents can cause on the different non-target organisms, and to correlate the impacts caused by different types of pharmaceuticals on environmental health.

Key-words: Antibiotics; Anti-Inflammatory; Anticancer; Cosmetics; Hormones; Neuroactive Compounds

Introduction

Pharmaceuticals for human and veterinary use are substances belonging to the group of contaminants of emergent concern and have recently received much attention for its ability to contaminate water bodies and water supplies [1]. These substances have been continuously discarded in the environment, without pre-established criteria of safety, and according to STAMM et al., [2] some of them can be are potentially toxic to the environment and to humans, even at low concentrations. Several drugs have been found in marine water systems and freshwater throughout the world, which makes them one of the most important current emerging environmental pollutants [3-5].

These compounds constitute a highly heterogeneous group of chemicals, because they have complex chemical structures with physicochemical properties and diverse biological effects. They are known

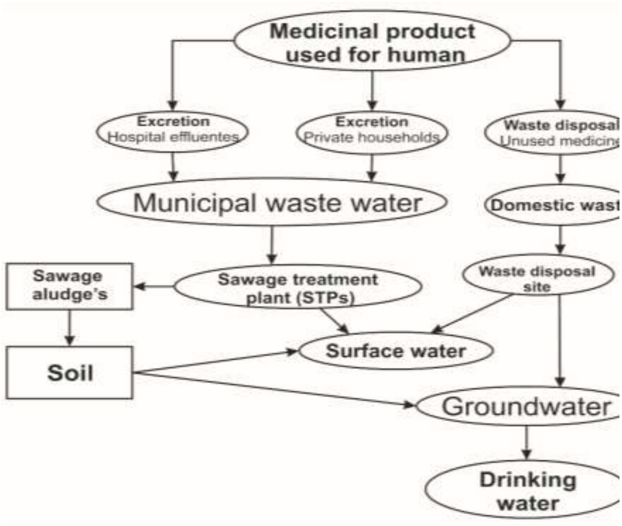

Figure 1: Scheme of aquatic environment contamination by different types of pharmaceuticals. Adaptated from Heberer.

for their specific action mechanisms, and many of them have persistence in organisms [6,2]. Sampling programs of surface water in the UK, continental Europe and North America have shown

the presence of different classes of pharmaceuticals, some of which are known to be environmentally persistent and have high polarity and low volatility [7]. Faced with such characteristics, there is increasing concern about the potential effects of these products on the aquatic fauna and flora, since the organisms may undergo chronic exposure, representing the main and most important targets of pharmaceutical waste [1].

The main form of introducing pharmaceuticals into the aquatic environment is through municipal sewer systems, and the incomplete removal of these chemicals in Wastewater Treatment Plants (WTPs). After ingestion, most of these substances are excreted in its original form, or as secondary metabolites, along with feces and urine [2]. According to Ueda et al., [8], about 50% and 90% of the ingested drug dose is excreted without modification in the body and the same persistence behavior is also observed in the environment. Other forms of introduction into the environment occurs by wastewater from hospitals and pharmaceutical industries, and through sanitary landfills leachate that may contain significant concentrations of these products [9,10]. Research has shown that sewage sludge used in agricultural areas is loaded with medical waste that contaminates the soil and may drain into surface water by leaching [11]. Thus, pharmaceutical traces have been detected in hospital effluents, sewage effluent, surface water, and groundwater. Figure 1 summarizes the main ways through which the drugs reach the aquatic environment.

The major environmental concern with these products is not only the quantity in which they are introduced into the environment, but mainly in their environmental persistence and their interference with important biological functions, such as reproduction [1]. Several studies show that about 15 to 20% of medicinal products for human use have potential for bioaccumulation, which indicates a priority in studies of these compounds in aquatic systems [12].

Some studies claim that many drugs are not an imminent risk to public health, when found in low concentrations in the environment [6]. However, ecotoxicological investigations demonstrate chronic toxicity to aquatic organisms, due to continuous exposure to small drug doses. In this context, it is also important to consider that different pharmaceuticals are available on the environment and they can interact to form complex mixtures totally unknown about chemical structure and biological action. In fact, several investigations have shown that toxicity of pharmaceuticals for non-target organisms may be occurring in environmentally relevant concentrations due to interactions and synergy between the different drugs [1, 6, 13-16].

Currently, the evidence of high environmental contamination associated with the presence of contaminants of emergent concern reinforces that studies for development are still necessary on parameters of investigation to better understand the environmental problem, especially with the pollution by drugs. It is important to identify pharmaceuticals and their metabolites and determine their concentration in the environment; understand their patterns of organic metabolism and excretion; evaluate the efficiency of wastewater treatments for removing these pollutants; understand the dispersion, mobility and persistence of these chemicals in biotic and abiotic environmental conditions; and evaluate its degradability and absorption, as well as, the effects on non-target organisms [17].

According to Kostopoulou and Nikolaou [18], new analytical methods that allow quantify small concentrations of drugs are being developed. However, the complexity of environmental samples and the lack of high-resolution techniques able to quantify these concentrations, still represent a great difficulty in performing such analyzes [19].

Europe and the United States, limits in the use of some components in cosmetic formulations are already being established. The European Union Directive 76/768/EEC [20], for example, establishes a maximum triclosan concentration on the order of 0.30% (m/m) in hygiene and personal care products. Additionally it is determined that the labels of pharmaceutical products must specify the amount of the active compounds present in chemical formulations.

Research into the presence of pharmaceuticals in aquatic environments also offers several challenges. In order to achieve a better evaluation of the presence and action of drugs on the environment and biota, establish interdisciplinary cooperation is necessary, and this will allow a fully understanding of physical and biological impacts that such waste can have on water resources [21].

Hormones

Hormones belong to a group of chemicals called endocrine disruptors, a term used to define a substance capable of interfering with the natural function of the animal endocrine system, including humans. Chemicals suspected of causing changes in the endocrine system are potentially associated with diseases such as cancer in testes, breast, and prostate, decrease in sperm counts, deformities in the reproductive organs, thyroid dysfunction, and changes in the neurological system [22].The exogenous estrogen ethinyl estradiol is a derivative of 17-β estradiol, which is the main endogenous estrogen in humans, and a bioactive compound used in many formulations combined with progesterone of oral contraceptive pills. Women use contraceptives for long, then they are considered of continuous use. After its introduction in the body, this hormone is metabolized by the hepatic system and part of it is excreted as xenoestrogen, along with urine and feces [23]. Environmental Agency of England and Wales conceded enough evidence to include ethinyl estradiol in compounds risk list in 2004, and the European Commission recognized it as priority substance in the Directive for protection and preservation of European water bodies [24]. However, since the objective of its Directive was the control of other microcontaminants of emergent concern the decision was postponed to 2016 in order to seek further corroborative studies that could certify the toxicity of this product [25].

The release of hormonally active substances in water bodies, even at low concentrations, can promote serious impact on the dynamics and structure of aquatic populations [26]. Studies in Canada were the first indicating that 17α-ethinyl estradiol estrogen concentrations similar to those found near effluent releases (5-6 ng/L^{-1}), led fish populations to collapse in experimental lakes [27]. Exposing the fish *Pimephales promelas* to a concentration of 3.2 ng/L^{-1} 17α- ethinyl estradiol for a year induced vitellogenin [28]. In a study conducted in Malaysia, Al Odaini et al.,[29] monitored the occurrence of four synthetic hormones (17α-ethinyl estradiol, norethindrone, levonorgestrel and cyproterone acetate) in five treatment plant effluents, and on Langat river water. The study results confirm literature data about low efficiency removal of these compounds in treatment plants, and its presence in the monitored river water. Synthetic sex hormones present in low concentrations in surface water can induce, in long-term, the feminization of fish. These compounds promote the reduction of testosterone concentration, because they work as endocrine disruptors, i.e, acting on the development and growth of animal gonads. This effect is not limited only to fish, and may even occur in humans, according to Mimeault et al., [30] and Viglino et al., [31].

The effects triggered by hormones in the environment reaches from micro invertebrates to large vertebrates, being widely reported in the scientific literature and therefore considered as a matter of global scope [32]. According to Manahan [33], the main ecotoxicological concern with these substances is its evident ability to affect species reproduction and interfere with the healthy development of the offspring. It is particularly important the stage of development regarding the organism exposure to the hormone. If exposure occurs in the embryo-larval stage, which is critical for aquatic species, the induced damage will be more severe, impairing various organs and systems, and might be permanent [34].

Antibiotics

Antibiotics are natural or synthetic compounds able to inhibit growth or cause the death of fungi or bacteria. They can be classified as fungicides when they cause the death of fungi and yeasts, bactericides when they cause the death of bacteria, or bacteriostatic when they promote inhibition of microbial growth [35]. Antibiotics are used worldwide in large quantities, but few studies assess the impacts of their direct disposal in the environment or of their possible metabolites derived from the degradation. They are compounds that exhibit complex form and that interfere with the physiology of innumerable living beings. Studies indicate that global consumption of antibiotics is estimated at between 100.000 to 200.000 tons/year. In contrast to the expected pharmacological effects of antibiotics, its chemical structure cause deleterious effects on non-target organisms and the environment [36].

As a result of extensive use of antibiotics, their residues are found in urban sewage, surface water, water bodies, sediments, and biota from around the world territory [37-42]. Antibiotic metabolism varies according to their metabolic class. This variation is related to its interaction with the organism enzyme system, which influences directly in excreted products. Such metabolites may have variation in the toxicological profile, and they can, therefore, be more or less toxic to the environment, when compared to the original molecule [43,44].

Kümmerer [45] evidenced in his study, the presence of several classes of antibiotics in the water supply, sewerage, drinking water, surface water, hospital waste, sewage sludge, sediments and marine environments. Several chemical classes were found, among them penicillins, macrolides, aminoglycosides, quinolones, sulfonamides, and other.

Although antibiotic concentrations found in the environment are considered low, its continuing release and its potential for persistence, bioaccumulation, bioactivity and resistance to biodegradation can result in serious environmental problems [46,47]. Living organisms exposed to antibiotics and their metabolites present in aquatic systems can suffer multiple effects. The toxic potential of antibiotics on aquatic invertebrates is still poorly understood and often controversial [48].

In bivalve organisms, studies report many kinds of changes including effects at the molecular level as in DNA and enzyme systems of organisms [49-53]. Lacaze et al., [48] evaluated the genotoxicity and immunotoxicity of antibiotics by exposing *Mytilus edulis* bivalves to sulfamethoxazole, trimethoprim, and erythromycin. Since all evaluated antibiotics have induced genotoxicity and immunotoxicity, this result highlighted the need for further studies to evaluate the impact of the pharmaceutical products disposal. Although the results obtained with these organisms cannot be extrapolated directly to mammals, they seem to be highly relevant to assess the pharmaceuticals toxic action mode in the aquatic environment [54].

Tetracyclines comprise a class of antibiotic with broad spectrum of biological action and chemical structure formed by four organic rings. Zhang [55] reported that tetracycline induced impairment in development and molecular response of zebrafish (*Danio rerio*) embryos, interfering, for example, in the gas bladder formation. These results indicate risk for environmental contamination with tetracyclines at concentrations of 10–1000 mg/mL. Ma and collaborators [56] evaluated the distribution of 20 different types of antibiotics, including the tetracyclines, fluoroquinolones, macrolides, and sulfonamides in northern China groundwater. Antibiotics were found in 34 % of the samples, among them sulphas were the most prevalent.

Tong [57] showed that large concentrations of antibiotics found in groundwater were proportional to the recorded concentrations for the same antibiotics in surface waters. However, the authors found that antibiotics in groundwater had already suffered deterioration because they presented changes in their chemical structures. According to the authors, these changes should be further evaluated, to allow the understanding of the chemical processes involved in the degradation and the impact of these modifications. Burkina, Zlabek and Zamaratskaia [58] also pointed out the need to evaluate the action of the metabolites derived from antibiotics degradation. According to the authors, the lack of studies with these compounds prevents the understanding of the effect of such drugs on aquatic species. In mammals and fish, antibiotic drugs are metabolized primarily by cytochrome enzymes P450 (CYP450). Thus, CYP450 activity would be an important factor to be investigated to determine the detoxification capacity of organisms exposed to these substances.

Triclosan is an antimicrobial widely used in personal care products and in some cosmetics destined for the anti-acne treatment. As the use of this product has been widely spread, there has been an exponential growth in the presence of this compound in the environment. Ramaswamya [59] evaluated by gas chromatography and mass spectroscopy water and sediment from Kaveri, Vellar, and Tamiraparani rivers, and also from Pichavaram mangrove in India. In all environments, the presence of triclosan was detected, regardless of the effluent type received by the river. These data call attention to the fact that limits for triclosan disposal should be set for both urban and industrial sewage.

Despite the relatively high number of works addressing the impact of antibiotics in aquatic environment, more ecotoxicological studies are needed to confirm the risk that these compounds promote in biota, so that they can assist regulators in developing new standards to be applied in legislation, and including the drugs on the list of priority pollutants.

Neuroactive Compounds (Antiepileptics, Antidepressants)

Neuroactive compounds are among the active substances most widely prescribed in the world. These drugs, used for problems related to the nervous system, have great importance in behavioral regulation and act directly on the central nervous system by changing the neuroendocrine signaling [17]. As noted in the other classes of pharmaceuticals, psychoactive medications are also not completely metabolized by the human body, being excreted unchanged, as metabolites or conjugates (glucuronides) [11].

Benzodiazepines are drugs indicated for the treatment of anxiety, emotional and sleep disorders, and epileptic seizures. They have also been used as centrally acting muscle relaxants and as analgesia-inducing [60]. Diazepam (DZ) is the best-known drug in this therapeutic class and also the most widely studied as an environmental contaminant. The presence of DZ has been detected in hospitals wastewater and also in effluent from municipal Wastewater Treatment Plants (WTP). This product has also been found in drinking water in concentrations of 23.5 ng L^{-1} [61]. Studies conducted in WTP of Germany detected concentrations up to 0.04 mg/L [62], and according to Van der Hoeven [63], the WTP are capable of removing about 93% of DZ from wastewater.

According to Nunes et al., [64] DZ has the ability to change cellular redox systems leading to conditions of oxidative stress. A study conducted by Pascoe et al., [65] demonstrated that exposing *Hydra vulgaris* to DZ concentration of 10 μg/L^{-1} resulted in inhibition of regeneration ability of dissected body parts.

Fluoxetine is a Selective Serotonin Reuptake Inhibitor (SSRI). SSRIs are drugs prescribed for the treatment of depression, but they are also indicated for the treatment of compulsive, eating, and personality disorders [66]. In view of the variety and nature of functions covered by serotonin, SSRIs present in the environment may change appetite, immune and reproductive systems, as well as, the behavioral functions of exposed animals [6,67].

The presence of fluoxetine in water bodies and municipal effluents has been described by various authors. According to Chu, Metcalfe [68], some pharmaceutical products can undergo accumulation in fish biological fluids and tissues. According to Brooks et al., [69], fish collected in urban water bodies, wastewater receivers in North Texas, USA showed fluoxetine and sertraline values greater than 0.1 ng/g in all examined tissues.

Fluoxetine concentrations around 12 ng/L^{-1} have been found in surface water [3], but Weston et al. [70] indicated that fluoxetine concentrations in effluent water could be larger than 0.540 mg/L^{-1}. Both fluoxetine and paroxetine were detected in concentrations of ng/L in untreated sewage effluent in Norway [71] while in Canada they were found in concentrations of 142 ng/L^{-1} in sewage from WTPs [72].Researches about the influence of fluoxetine in the reproductive system of aquatic organisms have been developed. According to Flaherty et al., [73], *Daphnia magna* exposed to 36 g/L^{-1} of fluoxetine for 30 days had a significant increase in reproductive rates, and according to Fong [74], SSRIs can enhance spawning and oocyte maturation in bivalves and crustaceans.

Japanese Medakas exposed to different concentrations of fluoxetine (0.1, 0.5, 1.0 and 5.0 mg/L^{-1}) during four weeks, were evaluated for fertilization rate, hatching eggs, development of embryos and endocrine function, including plasma vitellogenin and steroids. Adult fish exposed to fluoxetine had affected their fertility, and the developing embryos showed several abnormalities such as edema, curved spine, and incomplete development (no pectoral fins, reduced eyes). The number of changes in the development of these fish was 4-5 times higher out than in control group [75].

Van der Ven et al., [76] used molecular markers to demonstrate estrogenic activity of mianserin, a tetracyclic antidepressant, which works as an endocrine disruptor in zebrafish.

The Carbamazepine (CBZ) is among the most common pharmaceutical residues detected in water bodies. The CBZ is an antiepileptic drug used to control seizures. Approximately 72% of orally administered drug is absorbed while 28% is not metabolized and is excreted unchanged in the feces [77]. The metabolites 10,11-dihydro- 10,11-expoxycarbamazepine (CBZ-epoxide) and trans-10,11-dihydro-10,11- dihydroxy carbamazepine (CBZ-diol) are as active as the original molecule and they are present in water bodies at similar amount and concentration to the parent drug [78].

Ferrari et al., [79] evaluated acute and chronic effects of three different drugs (carbamazepine, clofibric acid, and diclofenac) by bioassays conducted on bacteria, algae, micro-crustaceans, and fish. Effluent samples were collected after treatment in sewage treatment plants in four European countries (France, Greece, Italy, and Sweden). Only the carbamazepine was identified in all samples. Toxicity tests, performed with different test organisms, enable the classification of the drugs found in the evaluated waste as their toxicity: Diclofenac> carbamazepine> clofibric acid. Carbamazepine was the most frequent and the second largest toxic potential tested.

The effects of CBZ were tested in vitro regarding quality parameters and oxidative stress in sperm of common carp (*Cyprinos carpio* L.). Sperm was incubated for two hours with different concentrations of CBZ (0.2, 2.0 and 20 mg L^{-1}). The results revealed that the number of mobile spermatozoids and its movement speed significantly decreased when they were exposed to CBZ higher concentrations. The activities of antioxidant enzymes Superoxide Dismutase (SOD), Glutathione Reductase (GR) and Glutathione Peroxidase (GPx) decreased significantly demonstrating the ability of this substance to induce oxidative stress [80].

Antineoplastic

Currently, the higher incidence of cancers in the population increased the concern for the presence of anti-cancer compounds in the environment. Cancers are treated with chemotherapy, which are cytotoxic or cytostatic drugs, and which have different action mechanisms on cells [10].

These drugs comprise a heterogeneous group of chemicals belonging to five different classes, according to the Anatomical Therapeutic Classification (ATC): alkylating agents; antimetabolites; plant alkaloids and other natural products; cytotoxic antibiotics and related substances, and other antineoplastic agents [81]. According to Lleweiiyn et al., [82], cytotoxic drugs used in chemotherapy are designed to stop or prevent cell proliferation and to interfere with DNA synthesis. Because of their pharmacological potential, their genotoxic, mutagenic, and teratogenic effects, they would be among the most dangerous contaminants in aquatic systems [83].

Antineoplastic compounds are often excreted unchanged or partially metabolized in the urine and feces of patients under medical treatment [84]. Hospital wastewater usually reaches the municipal sewer system after single disinfecting. Thus, hospitals can be considered as the most important point sources of cytostatic drugs in the aqueous environment. The occurrence of these drugs in hospital effluent may serve as a starting point to monitor their destination in the environment [85].

Environmental monitoring studies in hospital effluent water detected residues of several cytostatic drugs (5-fluorouracil, cyclophosphamide, ifosfamide, methotrexate, doxorubicin, epirubicin, and daunorubicin) at concentrations less than μg/L^{-1} [86,87]. However, in countries such as Austria and Spain, 5-fluorouracil concentrations ranging from 4 to 150 mg/ L^{-1} have been found in wastewater from hospital treatment plants, showing that this drug has low biodegradability in the processing stations. Among the cytostatic drug, 5-fluorouracil is the most used worldwide [84, 88].

Other cytostatic drugs like Cyclophosphamide (CP) and ifosfamide (IF) are widely used in cancer treatments, autoimmune diseases and as an immunosuppressant after organ transplantation. According to Buerge [89], these two compounds are not completely metabolized in the body, reaching a rate of renal excretion of 13 to 15 %. The action mechanisms of these drugs involve metabolic activation and nonspecific alkylation of nucleophilic compounds, which are responsible for genotoxic and carcinogenic effects described in the literature. So, they are substances that have aroused great environmental concern [90].

Biodegradability studies of CP and IF, carried out in laboratories, showed that these products were not degraded both in high concentrations (up to 750 mg/L^{-1}), as in low concentrations (approximately 100 ng/ L^{-1} and 1 mg/ L^{-1}), which are similar amounts to those found in wastewater from Water Treatment Plants (WTP). The results show that these compounds have a high persistence in sewage treatment plants, demonstrating its difficult deterioration [89]. There are no studies on the effects of chronic exposure on aquatic organisms, hindering the risk assessment of these compounds. However, according to the powerful action mechanism displayed by them, their presence in the aquatic environment should not be ignored [85].

Drugs belonging to anthracycline family are used for the treatment of hematologic malignancies (acute leukemia) and certain solid tumors like bladder and breast cancers [84]. According to International Agency for Research on Cancer (IARC), drugs such as Doxorubicin (DOX) and Daunorubicin (DAUN) are classified respectively as probably carcinogenic and possibly carcinogenic [91].

Caminada et al., [92] investigated the cytotoxicity of 34 different pharmaceutical therapeutic classes, including the Hydrochloride Doxorubicin (99%) by the thiazolyl blue tetrazolium bromide (3- (4,5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide - MTT) assay and the neutral red assay (NR) in two fish cell lines (*Poeciliopsis lucida* hepatoma cell - PLHC-1; and rainbow trout gonad cell line - RTG-2) and correlated results with toxicity acute in vivo in *Daphnia magna*. The most toxic compound found in this study was doxorubicin, whose EC50-value observed was 21 μM (~14.1 mg/L^{-1}) determined by the MTT test in PLHC-1 strain. Despite the metabolic differences of both cytotoxicity tests (MTT and NR), the results were very similar, with a correlation of 98% with tests of acute toxicity on *Daphnia*, suggesting that the toxicity mechanism is the same both *in vitro* and *in vivo*. Poeggeler et al., [93] also tested the doxorubicin hydrochloride at a concentration of 100 μM (54.3 mg/L^{-1}), for a period of 24 hours in Rotifera species *Philodina acuticornis odious*. The tested concentrations were lethal showing survival rate of 1.0 ± 0.5%. Belyaeva et al., [94] studied the toxicity of doxorubicin in zebrafish embryos (*Danio rerio*). The authors compared the normal development of zebrafish embryos with this same fish embryos exposed to doxorubicin concentrations between 0.08 – 0.2 mg/L^{-1}. All tested concentrations caused tail flexure; 0.11 mg/L^{-1} caused tail flexure head and cardiac edema, and 0.2 mg/L^{-1} caused strong cardiac and yolk sac edema, and impaired locomotor activity. The increase in abnormalities in zebrafish embryos were observed according to the concentration of doxorubicin, revealing a dose-response effect.

Nonsteroidal Anti-Inflammatory Drugs (Nsaids)

Nonsteroidal anti-inflammatories (NSAIDs) are a special group of drugs that exhibit three major effects: reducing the inflammatory response, inflammatory pain (analgesic effect), and fever (antipyretic effect) [95].Santos et al., [96] conducted a review on 134 scientific papers, published in the period of 1997 to 2009, and demonstrated that NSAIDs are the class of drugs most frequently detected in the aquatic environment. These compounds are weak acids that act by reversible or irreversible inhibition of cyclooxygenase enzymes (COX-1 and COX-2), involved with prostaglandin synthesis from arachidonic acid. They are commonly used for the treatment of inflammation, alleviation of pain and fever, and sometimes they are used for the treatment of chronic rheumatic diseases [97].

Diclofenac represents the class of NSAIDs constantly prescribed for the treatment of musculoskeletal disorders, such as rheumatoid arthritis, osteoarthritis, and spondylitis [98]. This NSAID is the most consumed drug worldwide each year, with an average consumption of about 940 tons [78]. A study conducted by Islas-Flores et al., [99] identified diclofenac in urban effluents from several European countries, in concentrations ranging from 10 to 2200 ng/L^{-1}, while in Mexico in urban effluents, its concentrations were even higher reaching 0.25 to 0.50 mg/L^{-1}. In Brazil, diclofenac concentrations observed in water bodies were always in the order of ng/L to few µg/L. In Curitiba City (Paraná), concentrations of 0.285 µg/L^{-1} of diclofenac were detected in water from rivers Atuba, Barigui, Bethlehem, Iguaçu and Iraí [100].

Hoeger et al. [101] identified chronic pathological effects on kidneys and gills of rainbow trout (*Oncorhynchus mykiss*), after 28 days of exposure to 5 µg/L^{-1} of diclofenac. In addition, tests with rainbow trout exposed to different concentrations of the same drug have demonstrated accumulation of this compound in muscle, gills, kidneys and liver of animals [102]. Chronic toxicity assays conducted with this same organism (*O. mykiss*) showed cytological changes in the liver, kidney and gills, after only 28 days of exposure to 1 g/L^{-1} of diclofenac. At a 5 g/L^{-1} of diclofenac renal lesions and bioaccumulation in the liver, kidney, muscle, and gills were observed [103, 104], confirming the studies of Hoeger et al., [101].

Weigel et al., [105] studied the effects of 0.5 g/L^{-1} of diclofenac in brown trout (*Salmo trutta* f. *Fario*). After 21 days of exposure, the authors found cytological damage similar to those observed in *O. mykiss* by Schwaiger et al. [102], and a significant reduction of hematocrit values. Toxicological studies conducted by Saravanan et al., [106], showed hematological and biochemical changes in carps exposed to concentrations of 1, 10, and 100 mg/L^{-1} of diclofenac.

A research has shown that methods used by treatment plants are capable of removing only 20% of diclofenac present in raw sewage [107], and this substance is minimally adsorbed by sediments [108], which contributes to its availability in surface water. Phytotoxicity of diclofenac and its photodegradation products were evaluated in algae species *Scenedesmus vacuolatus*. Research results showed that not only the parent compound can be dangerous for non-target species, but also its transformation products can pose a potential risk for the aquatic environment [109].

Ibuprofen is another NSAID which has a potential for chronic toxicity. Tests with female Japanese Medaka (Japanese Killies and *Oryzias latipes*), exposed concentrations of 2.5 to 25 ug/L^{-1} of ibuprofen during more than six weeks, showed a dramatic increase in animal liver weight, as well as an increase in egg production, and a reduction in the number of weekly spawning events [110].

Drugs of Continuous Use

Antihypertensive

The high consumption of antihypertensive drugs worldwide reflects the frequent detection of this product in aquatic environments [111, 112]. The main reason for the contamination of the aquatic environments by antihypertensives is the incomplete removal of these drugs by the current methods used in conventional sewage treatment plants [62,112]. Antihypertensive drugs more frequently detected in the environment are the beta- blockers [96]. This group belongs to the class of endocrine disrupters, which can disturb testosterone levels of male organisms. Within this class, there are the drugs atenolol, metoprolol, propranolol and sotalol, widely used in human medicine [113]. The degradation of atenolol is incomplete due to its low rates of biodegradation [114]. This chemical has been found in Portugal hospital waste at concentrations up to 8.0 µg/L^{-1} [115]. In Brazil, the antihypertensive detected in larger amounts in hospital waste is metoprolol at concentrations up to 9.9 µg/L^{-1} [116].

Besides beta-blockers, inhibitors of Angiotensin Converting Enzyme (ACE), inhibitors such as captopril, enalapril, lisinopril, ramipril, antagonists of the angiotensin II receptor (losartan, valsartan), and calcium channel blockers (nifedipine, diltiazem, verapamil) are also antihypertensive drugs. Metoprolol and losartan, at concentrations of 800 to 950 μg/ L^{-1} and 2400 to 2500 μg/ L^{-1}, respectively, were observed in effluent from the pharmaceutical industry in Patancheru, India [117]. Sotalol was detected in concentrations up to 6.7 μg/ L^{-1} in hospital waste in Italy [118]. The authors state that effluents from pharmaceutical industries are the great responsible for high drug concentrations in the aquatic environment. Villegas-Navarro et al., [119] determined toxicity to four cardioactive drugs through tests performed with *Daphnia magna*, for periods of 24 and 48 hours exposure. All tested drugs, verapamil- 7.04 mg/L^{-1}; metaproterenol - 32.45 mg/L^{-1} and metoprolol - 76.21 mg/L^{-1}, were lethal to *D. magna* for 48 hour exposure. The observed effects after 24 hours of exposure to metaproterenol, and metoprolol compounds were similar to the observed effects in the human heart. These results show that aquatic organisms can suffer from induced side effects similar to those recorded in mammals.

According to the review of Godoy et al., [120] 34 kinds of anti-hypertensive molecules were detected in treated water, but only 16 of these molecules have been studied regarding their ecotoxicological effects.

Anti-glycemic

Diabetes mellitus is a chronic disease that affects approximately 200 million people worldwide. The use of anti-glycemic drugs have been widely adopted for the treatment of this disease and, therefore, are continually being released into the aquatic environment via sewage [121].

Among anti-glycemic drugs, metformin is highly relevant, because it is the most widely prescribed drug for the treatment of diabetes mellitus type II. This drug has the characteristic of being excreted unchanged in the urine [122] and it is among the pharmaceuticals most commonly found in aquatic environments [123].The high consumption of metformin, combined with its low degree of metabolism by the human body [124], leads to high levels of this medicine in wastewater. This fact associated with its incomplete removal during wastewater treatment [125,126], represent a highly dangerous situation for the environment.

A screening of samples from a natural region of Rhone - The Alps in France revealed the presence of metformin in average levels of approximately 100 ng/L^{-1} in surface waters and 10 ng/L^{-1} in the soil. This drug showed a consumption growth in regions such as the Netherlands and Western Europe, in the order of 26% between the years 2008 and 2012, indicating an increase of future use [127].

Metformin prescriptions in Germany reached 601 million daily doses in 2011 [128], corresponding to more than 1200 tons/ year, considering one dose as 2 g of metformin per day on average. However, despite their high prescription rates and volumes of consumption, there are few studies evaluating the effects of antidiabetic drugs in water bodies and urban effluents [121].Chronic effects of metformin were studied by exposing Fathead minnow fishes (*Pimephales promelas*) to a concentration of 40 μg/L^{-1} during 4 weeks [129]. The authors analyzed the genetic mechanisms related to the metabolism and endocrine function and the endpoints related to reproduction. Metformin treatment led to significant increase in expression of encoding gene to vitellogenin egg protein in males. These experimental results obtained for environmentally relevant concentrations (similar concentrations to the average found in WTP effluent, Milwaukee, Wisconsin, USA), highlighted the need for further studies about endocrine effects of metformin on aquatic organisms.Researches have revealed a close link between insulin signaling and steroidogenesis, indicating that antidiabetic drugs can act as endocrine disruptors. This hypothesis is supported by the prevalence of intersex fish in locations that receive WTP effluent, where a mixture of endocrine disruptors is common, and metformin is the most commonly drug found at the site [129, 130].

Anticholesterolemic

Anticholesterolemics are drugs that act as lipid regulators and which have been detected in domestic waste, surface, and drinking water, both in North America and Europe. Within this class of drugs, fibrates are chemicals able to induce the proliferation and the increase in the size of liver peroxisomes [131].

Some active compounds as atorvastatin, fluvastatin, gemfibrozil, pravastatin, rosuvastatin, simvastatin, fenofibrate, and bezafibrate, were prioritized in environmental risk assessment carried out by Roos et al., [132]. The gemfibrozil, representative of the fibrate, is capable of reducing levels of serum triglycerides and of density lipoproteins while it increases the level of high-density lipoproteins [133]. This drug is metabolized by the liver, originating four major metabolites. About 70% of the delivered content is excreted in the urine, mainly as glucuronide conjugate [134].

The gemfibrozil has a photodegradation half-life in the aquatic environment of 15 hours [135]. The highest concentrations of gemfibrozil found in surface water in North America and Europe was 3 and 6 nM, respectively [136]. However, this same drug has been identified in concentrations of 2.1 µg/L-1 in sewage treatment plants effluent, and concentrations of 0.5 ug/L-1 in surface water [3, 72, 137].

Methodologies for Waste Treatment

Quality water is essential for human health and the balance of all ecosystems. However, because of the numerous possibilities for contamination, several studies seek to firstly evaluate techniques for identification and quantification of microcontaminants such as drugs and their metabolites present in water bodies.Many pharmaceuticals are contaminants of water environment that are not easily removed during conventional water treatment, due to their physicochemical properties which give them high persistence in the environment. These compounds have a high potential for bioaccumulation and low biodegradability. Therefore, the currently conventional wastewater treatment processes are not able to completely remove those more persistent pharmaceuticals from water [138].

Conventional methods currently available for water treatment, are unable to completely remove contaminants derived from the use of drugs and cosmetics. Facing this panorama, it is extremely important to carry out studies to develop technologies for a major removal of these compounds from environment compartments. Methods able to determine with accuracy these substances in low concentrations on the range of µg/L and ng/L in complex environmental matrices, such as water resources, soil, sediment, biological sludge, and WTP effluent, are still challenges for many researchers in the environmental area [139]. Because of the increasing incidence of these compounds in the environment, it is necessary to improve the analytical techniques for the identification and quantification of these compounds at very few concentrations, as well as to improve the methods of drugs removing from drinking water and wastewater, in order to achieve a more satisfactory elimination or at least a reduction of the (eco)toxicity caused by them. Among the main techniques of identification and quantification of pharmaceutical residues in aquatic environments the liquid chromatography integrated with mass spectroscopy is of great importance and it is widely reported in the literature. Mass spectrometry has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. The use of these techniques is essential for understanding the compound present in the environment and can ensure the proper use of the drug removal techniques [45].

Nowadays, due to the large presence of drugs into waterways, the removal of these substances from the environment can guarantee the quality of water bodies, reducing or eliminating the impact on the fragile balance of this ecosystem. Other possible analytical technique for identification of drug residues in aquatic matrices is the fluorescence emission cavity-enhanced spectroscopy, reported by Bixlera and contributors. This technique has the advantage of applying condensed phase media and biological systems, having excellent reproducibility and efficiency [140].

The removal of the micropollutants may be done by biodegradation with the use of micro-organisms [141], or by physicochemical techniques such as sorption, photolysis, hydrolysis and redox reactions [142] in order to reduce the toxicity of contaminants. In these degradation processes it is of great importance to evaluate and standardize the environmental characteristics, such as temperature, pH, and humidity, which may interfere in several steps of removal, both in biological as physicochemical techniques.

The elimination of pollutants from water by phytodegradation process, such as constructed wetlands for pollutants conventional treating has been well documented. However, available research studies about pharmaceuticals removal systems based on aquatic plants are limited [143].Several technologies have been evaluated for removal of pharmaceutical compounds, including ozonization [144], reverse osmosis [145] and advanced oxidation processes [146]. In these techniques, factors such as time and temperature, must be assessed to permit the process optimization in order to increase the removal efficiency [147] or to reduce the level of pharmaceutical in water. However, these processes are not currently widely used due to their high costs [1].

When choosing the technique to be used for the removal of pharmaceutical contaminants from environmental matrices, it is appropriate to evaluate the physicochemical properties of the drug or its metabolite and its concentration. Only after this step, it is possible to define the most appropriate technique for removing the pollutant.

Studies assessing the efficiency in the removal of pharmaceuticals from aquatic environments present a considerable increase on the efficiency of contamination removal. Such studies infer that the removal efficiency could reach 99% [7,62,148], however, the main difficulty pointed out is to extrapolate, at affordable costs, the removal techniques for macro environment.

All of these studies presented underlie the need for a new regulation on the quality of water, sediment and biota, including limits and monitoring by inserting the drugs on this list of priorities.

Conclusion

The presented data showed different sources of contamination, destination and occurrence of pharmaceuticals in the aquatic environment. Despite extensive review about the effects of different classes of drugs and personal care products on the aquatic environment, it is possible to see a deficiency of studies that evaluate the effects of chronic exposure to drugs and their metabolites. The environmental impairment promoted by drugs requires more effective biological models for studies, and also need assessments of the effects of drug metabolites present in water, as these compounds have, in general, higher solubility and distinct toxicity when compared to the original molecule. Another important point is the need for improvements in conventional waste treatments, which often are unable to withdraw embedded waste from water courses. Many steps remain to be taken in relation to environmental impact promoted by pharmaceuticals, especially in relation to the degradation forms of these substances into the aquatic environment. This has been the major bottleneck of the whole environmental issue since several studies highlight the low efficiency of removal of pharmaceutical residues during wastewater treatment pointing out the need to develop alternative treatments more effective.

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Water Reuse: Safety and Applications

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Abstract

World water scarcity is due to the rising use of this resource in urban, industrial and agricultural purposes, and the damage that the water bodies has been suffering as a result of the reception of various contaminants effluents. Water reuse has been considered as a solution for water scarcity. However, one of the major challenges related to this alternative is the establishment of adjustable standards for water quality, aiming at providing an appropriate security level to its applicability. In order to attend all activities that do not require potability, the water needs physicochemical and/or biological treatments. To acquire potability, more advanced processes are requested to ensure the quality of the obtained water, however, in general, the public acceptance is still an issue. The recycled water may have three different applications: 1) cover great water demands (urban, agricultural and industrial); 2) provide environmental enhancement; 3) enhance or recover degraded natural resources. As water reuse may present some contaminants and pathogens, it is necessary to perform tests to guarantee the water quality. The most used tests to assess the water reuse quality are those related to decreasing the risk of infection or toxicity. Furthermore, there is a need to regulate the reuse of water, establishing parameters to assure quality and safety. In this chapter we will discuss the general concepts of water reuse, the most suitable treatments to specific purposes, methods for assessment of water quality and the situation of global directives.

Keywords: Environmental Impacts; Human Health; Reclaimed Water; Reuse Water Regulations; Water Treatments

Introduction - Why Recycle Water ?

Of all water found on the planet, 3% is in the form of freshwater, and of those, only 1% is related to groundwater reservoirs, rivers and lakes, which can be used for human consumption [1]. The natural water cycle happens by evapotranspiration, which comprehends the transpiration of all living beings and the evaporation of liquid water (mainly from oceans). The steam present in the atmosphere condenses and precipitates, returning to the terrestrial environment by the rain. However, this cycle has been changed by human activities, like water extraction from rivers and aquifers and the discharge of wastewater, treated or not, in the hydric system [2]. The increasing of water extraction from hydric systems, for urban, industrial and agricultural uses, and the discharge of several effluents, including domestic and industrial, have led to the degradation of water sources [3]. Therefore, water reuse has been recognized as a viable solution to solve the problems related to low water availability needed to supply the human population demands [4,5].

There is an estimation that global industrial water requirements would increase from 800 billion m^3, registered in 2009, to 1500 billion m^3 in 2030, as stated by the 2030 Water Resources Group [6]. This fact, combined to the increasing water demand in agriculture and urban environments, to the deterioration of water bodies and to the alteration of aquatic systems due to high evaporation caused by climate changes, shows the need for developing strategies that could solve the problem related to water scarcity in urban areas, mainly in developing countries [7], such as the urgent implementation of reuse water techniques.

According to Garcia and Pargament [5], the wastewater without treatment, poorly treated or treated by advanced technologies, can be reused for a large variety of purposes. According to the review work made by these authors, an example of use for untreated or barely treated wastewater could be the direct application for irrigation of specific types of crops. However, to avoid risks for the environmental and human health, there are guidelines that must be followed.

The industries and agricultural activities, besides the large volume of water needed to perform their activities, which require increasingly restrictive grants due the limited water resources, also faces severe environmental regulations related to the discharge of their effluents [8]. Due to the high costs associated to the treatment of effluents, these sectors have been encouraged to perform projects that allow the reuse of water. The domestic wastewater can be classified in two major categories: greywater - derived from all residential uses, except from toilets, comprehending 60-70% of water consumption; and blackwater - derived from the toilets, which comprehends 30-40% of water residential consumption [9].

The reuse of greywater is typically directed to irrigation or on toilets of urban areas, being considered as good strategy in water conservation and used in both, inside and outside of commercial and residential establishments [10]. Reuses that do not require potability have been well accepted by several communities, since it meets the existent demands without raising risks for the population. When water reuse is destined to potable consumption, *e.g.* directly delivering the treated wastewater or making a blend with the potable water system, there is a mistrust of the population, since there is a lack of convincing explanations related to the advantages and disadvantages of this practice [11].

Hence, there are several studies related to methods for treating wastewater, in which the researchers try to gather as much information as possible about its applicability, efficiency, need of combination of technologies, costs and safety.

Methods and Alternatives to Treat Wastewater

The effluent sources and the reuse of treated water may vary among the countries, *e.g.*, whereas in Europe around 70% of reuse water is used in agriculture, in Australia the use is prevalent urban (40%) [12]. In general, the potential of water reuse in Europe is very high, existing differences in its use according to the different regions. In the south, the prevalent use is on agricultural irrigation and environmental and urban applications, while in the north, the main use is industrial [13]. Due to such variability of uses, one of the most difficulties in using wastewater is the selection of the most appropriate treatment, according to its purpose [14,15].

The selection between one treatment or a combination of technologies for treating wastewater depends on the standard of the required water quality and on the most efficient treatment with the lowest cost. Regarding the selection of the most suitable treatment, Oller et al., [16] listed the main factors to be considered: 1) the condition and composition of the original wastewater; 2) which major contaminants must be removed; 3) possible treatment by conventional technologies; 4) resilience of treatment; 5) capacity of the decontamination facilities; 6) efficacy of the treatment system related to the final wastewater quality; 7) evaluation of costs; 8) life cycle assessment to determine the environmental compatibility of the treatment technology; 9) potential application of treated water.

Water treatments directed to non-potable reuse can be performed in three levels: primary, secondary and tertiary. Among these levels, several methods such as flocculation, sand and grit filtration, disinfection by ultraviolet radiation or chlorination and other techniques, are applied. Thus, the water treated by these three levels can be used in irrigation (plantation, gardens, sport fields, and others), in industries, during fire controls and other purposes [1,9]. In order to make the treated water potable, more advanced processes are required, such as ultrafiltration and nanofiltration, which are performed by means of specific membranes, and the use of activated carbon to absorb chemical and biological contaminants, removing phosphorus and nitrogen, organic carbonaceous compounds, dissolved organic and inorganic compounds and pathogens [1]. However, according to this same author, the addition of this treated water to the public water supply is not recommended, since part of the population may reject this water.

According to Cristóvão et al., [17] integrated treatment systems consist in a combination of physicochemical and biological methods. The physicochemical processes (sedimentation, flotation and pH adjustment) are primary treatments, whereas biological processes (aerobic and anaerobic) are considered as secondary treatments, with a better performance in organic compounds removal. When the water treatment is performed, there is a significant decrease on the amount of pathogenic microorganisms and on the concentrations of nutrients, trace elements and heavy metals. However, to remove hardest contaminants, *e.g.* salts and ions, more advanced and expensive technologies, such as filtration by reverse osmosis membranes, are necessary. These latest procedures can be considered as tertiary treatments [13,18].

One of the challenges related to water reuse is raise the possibilities of application of the water obtained by this process and reduce the possible risks to human and environmental health. However, the key question is the association of the challenges already cited with the selection of the best treatment technology, among the several existents, which could attend to a water quality standard related to the purpose of use, but with low costs [15].

Thus, it is not possible to establish an ideal treatment to water reuse, because this must be done according to the source of this water, the intention of its use and the cost of its application and maintenance. Usually, the employment of only one type of treatment to obtain water for reuse does not reach all quality and safety parameters needed for several human activities. Therefore, hereinafter, different methods and available technologies to treat an effluent, aiming its reuse, will be presented.

Physicochemical Methods

Physicochemical processes are the most traditional among water treatments, and can be applied on every steps of the treatment, including on the composition of more advanced and efficient methods.

The natural recharging of groundwater happens slowly. During this process of restitution, many substances are adsorbed by soil particles or are trapped between these particles. On the other hand, the artificial recharge of aquifers consists in a very used practice, mainly to replace or decrease the decline of groundwater levels and to store water from excessive rains or from water reuse treatments. A recharge can be done by water dispersal on soil surface or by direct injection on the aquifer, wherein each of these methods has their own advantages and disadvantages. In general, the biggest concern related to the replenishment of the aquifers is its possible contamination. Therefore, the water to be used in this process must undergo previous treatments, where more advanced treatments should be used, before direct injection on the aquifer. There is also a concern about the impregnation of certain contaminants in the soil, which may cause its impermeabilization [19].

The soil aquifer treatment is based on the treated water dispersion, so that this “clean” water infiltrates the soil and reaches the aquifer. As this process filters the water, this could be removed from the aquifer after a period of 6-12 months to be reused. But, as some organic compounds can persist in the water, even after it pass through the soil and stay in the aquifer, it becomes necessary the use of complementary methods, *e.g.* biofilter and ozonation [20].

Among the methods for treating water, the adsorption of contaminants is part of a series of used technologies. It presents many advantages: ease of application, applicability at low concentration, ease to recover and reuse the adsorbent, which reduces the its cost [21].

The process of adsorption by activated carbon is frequently used in water treatment to remove natural and synthetic organic compounds, turning the treated water in potable water. The high adsorption potential of activated carbon is due to the high contact surface, to its porosity and to the physicochemical properties of its surface [22].

Filters composed by activated carbon can remove ammonia, biodegradable organic compounds, toxins produced by algae and several organic trace elements, including those who cause bad smell and flavour. However, these filters cannot assure the total removal of pathogenic agents, especially in cold regions and, for this reason, require other techniques to guarantee the water quality [23].

As an alternative to activated carbon, the biochar is also a carbon-based porous material, derived from the thermal conversion of residual biomass under limited conditions of oxygen or even anaerobic. This material has been used in water treatment mostly in the removal of persistent organic pollutants, being even more effective than common activated carbon [24]. Furthermore, both biochar and activated carbon can be used to remove some residues from initial treatments, as those from chlorination [24].

Ultraviolet irradiation (UV) is a well-known method and is used on the elimination of microorganisms. The UV induces the formation of pyrimidine adducts on the genome, leading to errors during the replication and transcription, making it capable of eliminating several microorganisms resistant to chemical disinfectants. However, some bacterial spores and virus may still resist to this irradiation [25]. Despite this activity, UV also can degrade organic compounds by direct photolysis or by processes assisted by the addition of oxidant agents or photosensitizers, as humic substances. The direct UV irradiation and its use in advanced oxidative processes have been studied in the last years [26].

There is a series of micropollutants that can be degraded by the direct use of UV, both on water and effluent treatments. However, depending on the circumstances in which this treatment is performed, for example, the type of UV lamp used, the pH of the water or effluent, the temperature and the presence of other substances in water may benefit or harm the process. In general, the UV lamp used and the pH of the water must be suitable to the chemical composition of the water to be treated. The temperature appears to have a direct relationship with the degradation by UV, once the higher the temperature better will be the yield of the treatment. Regarding the presence of other substances, *e.g.*, organic matter, this could be used to produce a greater amount of free radicals, derived from photolysis, which could enhance the degradation promoted by UV [26].

Another physicochemical process that is well studied is based on the use of filtration/ separation membranes. The treatment system of membrane works by exclusion and adsorption of contaminants, depending on the size of the membrane pores. Microfiltration uses membranes with pores varying from 0.1 to 10 μm, which is enough to block efficiently protozoan parasites and bacteria, due to the size of the cells of these organisms, but it also can retain some virus by adsorption. Even so, some microorganisms have the capability to modify and reduce their cell volume, and pass through this type of membrane [25,27].

However, the ultrafiltration system is based on membranes with smaller pores, which vary from 5 to 100 nm, and for this reason this system is considered more efficient even for virus removal. Nanofiltration uses membranes with pores of ca. 1 nm, while reverse osmosis system uses layers of membranes and a high-pressure system, with even smaller pores, measuring 0.6 nm of diameter. These more advanced types of filtration are considered, theoretically, effective on the total removal of pathogens [25,28]. The reverse osmosis system is based on the exclusion of molecules dissolved in the water, by their size and electrical charge, and by the physicochemical interactions between the solutes, the solvent and the membrane. Despite its efficacy being dependent on the operational parameters, the membrane itself and the properties of the water, in general, the treatment with reverse osmosis can be used to treat several effluents (petrochemical, mining, agricultural, textile, pharmaceutical, and others) [29].

Although it is proved, in theory, the effectiveness of membrane systems, in practice it is not possible to guarantee the total retention of microorganisms, due to the presence of imperfections on the structure of the membrane [30]. Besides, the water treatment performed only with simplest filtration systems is not capable of removing determined contaminants, such as pharmaceuticals and cosmetics, endocrine disruptors and pesticides, which can affect the physical environment, its biota and the human health [31,32].

Therefore, one of the major concerns about using the water derived from ultrafiltration systems is the probability of many compounds being present, simultaneously, in this water and their capability to induce synergistic effects, such as some physiological alterations already evidenced in fishes [31,32]. Because of these issues regarding the passage of microorganisms through membranes, micro and ultrafiltration are not recommended to disinfect water without other specific treatment.

Besides the physical treatments, there are several technologies based on chemical agents. During the primary treatment, chemical substances can be inserted in the treatment process to remove mainly suspended solids, by coagulation and/or flotation. Coagulation, for example, can be performed by using alum, starch, activated silica and aluminium salts, which are natural coagulants, or by using polymers, which are synthetic coagulants that imply higher costs. Even this method being dependent on pH, temperature and exposure time of the coagulant, it has the ability to remove microorganisms while treating effluents [33]. Flotation system consists in the capture of contaminants by specific chemical substances (like alum and activated silica) that, by an aeration system, induce the flotation of clusters, making easy to remove it mechanically from water [33].

Water treatment can also be performed using chemical disinfectants (*e.g.,* ozone, chlorine, hydrogen peroxide and peracetic acid) that are able to oxidize cellular components. Their efficacy depends on their oxireduction potential over the targeted cellular structures, their solubility in the medium and their decomposition rates. According to these parameters, usually the resistance of the microorganisms varies, from less resistant (gram-negative bacteria) to more resistant (bacteria spores and protozoan parasites) [25].

Chlorination is the most applied treatment to disinfect water and it is used in many countries due to the low cost, the good efficiency against bacteria and the applicability in any scale [25,34]. Nonetheless, there are several disadvantages related to chlorine use, as the formation of by-products in the treated water, the persistency of chlorine residues that impacts the aquatic biota, the inefficiency against protozoan parasites and some virus and the production of toxic gases when it is used in high concentrations [25,35]. Due to the disadvantages listed above, some countries substitute the use of chlorine by peracetic acid to disinfect water. This compound is efficient against bacteria, viruses, bacterial spores and protozoan cysts, without producing great amounts of harmful by-products. Unlike chlorine, in general, the peracetic acid is decomposed to non-toxic and non-mutagenic by-products, being safer. However, a treatment based on peracetic acid has two main problems: the increase of organic content in the effluent, due to acetic acid that remains as a product peracetic acid decomposition, and the high cost to apply this compound to treat wastewater [36].

Ozonation is a type of treatment considered effective to reduce the smell and the flavour of the water, promote disinfection, decrease the formation of trihalomethanes and haloacetic acids and oxidize several organic trace elements, beyond increasing the degradability of dissolved organic compounds [23,37].

The Advanced Oxidation Processes (AOPs) involve the generation of free radical highly oxidants, especially hydroxyl radicals, which are known by degrading recalcitrant contaminants and by oxidize and mineralize almost all organic contaminants [38]. However, due to the high costs of AOPs, the use of this method is still scarce. Therefore, there is an effort to reduce the costs of its application, mainly in relation to the alternative use of renewable sources of energy, since it is hard to reduce the amount of reagents needed [39].

Among the existent AOPs, the Fenton process is very promising, because it is characterized as a low cost alternative to treat industrial effluents (*e.g.,* textile). This method seems to be very effective in degradation of toxic and/or non-biodegradable compounds, such as aliphatic compounds, azodyes, phenols, etc. [40]. In this context, the use of solar irradiation may be an interesting alternative of energy source that could be used to reduce the costs of AOPs, like the treatment by photo-Fenton degradation, and to maintain the good yield of treatment [39].

As it is impossible removing all water contaminants by physicochemical treatments, the biological treatments are also a viable and efficient process used to remove most of carbon-based pollutants present in wastewater.

Biological Methods

One of the most used treatment processes to eliminate pollutants is the biological degradation. This method is based on the metabolic activity of microorganisms, such as bacteria and fungi, naturally found in water bodies and soil, but which can also be added in the activated sludge [16]. According to Gupta et al., [33], the secondary treatment can be performed by microorganisms, which use their metabolic pathways to remove, mainly, organic pollutants.

Usually, fungi and bacteria convert organic matter into water, carbon dioxide and gas ammonia, but some alcohols, nitrates and glucose can also arises as result of this degradation. The aerobic processes are performed when air or oxygen is provided for aerobic or facultative bacteria degrading the organic matter. Although this method presents effectiveness of ca. 90% in removing organic matter, a large amount of biosolid residues may be formed, which need to be removed and well disposed [33]. The anaerobic processes consist in the generation of an environment without the presence of oxygen, in which anaerobic or facultative bacteria decompose complex organic matter into simpler organic compounds, consisted by nitrogen, carbon and sulphur, many of those in gas form [33].

The processes that use these organisms have not always led to satisfactory results, mainly in relation to industrial effluent treatment, which can be toxic or resistant to the activity of these microorganisms [16]. Textile industry, for example, produces effluents with non-biodegradable and/or toxic organic matter, which makes the biological treatment ineffective [40].

Besides the possible toxicity or recalcitrance of the contaminants, some effluents can present other limiting factors to the existing microorganisms, such as a high ratio between the Chemical Oxygen Demand (COD) and the Biochemical Oxygen Demand (BOD), in addition to the deficiency of nutrients. For this reason, most studies using conventional bioassays evaluates the biodegradation rate by the correlation between the COD and BOD, to ensure the survival and effectiveness of the microorganisms [16]. There are other limiting factors for the microorganisms, but these are related to the operational part of the treatment process, like the sludge production, corrected disposal of residues and the generation of foam, which may compromise the treatment efficiency [41].

According to Bunani et al., [42], saline effluents are conventionally treated by physicochemical processes, because biological treatments do not seems to be effective to remove the contaminants, due to the inhibitory action of the salts on the microorganisms' metabolism. Therefore, water with high content of salt significantly reduces the yield of the process based on activated sludge, with anaerobic organisms and with mechanisms of nitrification and denitrification.

Another characteristic that alters the efficiency of biological treatment is the presence of personal care products and pharmaceuticals, mainly antibiotics. These products, when found in some effluents, can interfere on the microbiota, causing selection and inducing the resistance of bacteria that, depending on the chemical action, may restrain the treatment effectiveness [39].

Due to the possible toxicity of the effluent, generally, conventional chemical and physical treatments are used first to reduce the amount of toxic substances present in this effluent, to further perform a biological treatment. It is important that, before the effluent already pre-treated be submitted to the biological treatment, it passes through specific toxicity tests, in order to assess the toxic potential of the samples to the microbiota, and consequently avoid their elimination, once they will make biodegradation feasible. Moreover, the analytical parameters of the effluent should also be considered, to allow optimal conditions for microorganisms perform their metabolic activity [43].

A biological treatment used worldwide, especially in developing countries (located in tropical zones), is the stabilization pond based on microorganisms, due to its low costs of installation and maintenance, and the favourable climate conditions of these regions. When well projected and well operated, stabilization ponds can obtain a removal of almost all helminths (99.99%), bacteria and virus (99%), resulting in an inodorous effluent, attractive to irrigation. However, some nutrient residues may persist on the effluent, requiring complementary treatments. These residues are discharged in the environment and may contaminate the water bodies, making it unsafe for human consumption [44].

In addition to stabilization ponds, the wetlands are also environments that promote the water purification. According to Salati [45], wetlands are natural ecosystems (floodplain of streams, swamps and lacustrine formations) or constructed (simulation of natural wetlands), both with shallow waters, which can be partial or totally flooded during the year.

Wetlands are ecosystems which comprehends a transition zone between aquatic and terrestrial environments, composed of soaked soils, plants adapted to a thin layer of water and a water table capable to keep a determined water depth. Three types of wetlands can be identified according to the predominant plants: 1) swamps, as flooded areas dominated by woody plants and water tolerant trees; 2) marshes, dominated by soft stem plants; and 3) bogs, dominated by moss and acidophilic plants [44].

As the wetlands are limited to some regions, the construction of this environment becomes an alternative to treat effluents. The constructed wetlands consist in a low cost alternative to conventional treatments of domestic effluents, being recommended for small communities. The combined use of several constructed wetlands has been employed successfully to gain better yields to treat domestic effluents, mainly to remove nitrogen compounds, but being confirmed by also removing some pharmaceuticals [46]. These authors suggest that the main factors responsible for the treatment success is the presence of biotic and abiotic microenvironments, associated with solar photodegradation and with the adsorption by the matrix of grit and organic matter.

Another biological process routinely used as a secondary treatment for industrial and domestic effluents is based on the use of activated sludge. This technique consists on biodegradation by microorganisms present in tanks with controlled oxygen supplement, followed by the separation of the biomass present in the water, by gravity [47]. If a lower content of solid is required, the treatment with activated sludge is limited by the difficulty on separate the suspended solids, which could be solved by associating this treatment with membrane separation process. This association is known as Membrane Bioreactors (MBR),that uses advanced membrane modules (micro and ultrafiltration) instead of the traditional gravity separators, allowing a better water quality as it eliminates even bacteria and some types of viruses [48].

Valderrama et al., [49] tested the efficacy of MBRs. These researchers evaluated the treatment of an effluent from a Spanish winery using this technology and concluded that after treatment, the water could be used in the agriculture, in the cities and even for recreation, respecting the criteria established by Spain's legislation and other international recommendations. Jraou et al., [48] also confirmed the applicability of MBRs to treat domestic effluent, which satisfactorily reduced the COD and eliminated the pathogens, making the water good enough to be used in agricultural irrigation. However, the application of MBRs to treat domestic wastewater is too expensive and, in most cases, not feasible.

Similarly to physicochemical treatments, biological treatments also have advantages and disadvantages that must be considered in wastewater recycling, aiming its reuse. Until now, the combination of conventional methods with more advanced ones have been studied in order to obtain a treatment more effective with a more viable cost.

Combination and Advanced Methods

The most conventional water treatment system throughout the world is based on the combination of clarification, filtration and chlorination. However, when it comes to treating wastewater, usually loaded with a wide variety of contaminants, other technologies are required, *e.g.* biological treatments combined with other technologies.

Besides the standard techniques already established to water disinfection, other methods to disinfect water are still being studied, so they can turn into new and effective systems to treat water. Among them are electrochemical disinfection, ultrasonication and heterogeneous photocatalysis, which are novelty and already showed interesting results [25]. Nevertheless, these technologies still are expensive and must be more studied to achieve better yields with higher cost-benefit rates.

The treatment by electrochemical disinfection consists in the inactivation of microorganisms by an electric current. This electric current is provided by electrodes that, in contact to the water, induce the generation of free chlorine (electrochlorination) and/or of other oxidative agents, depending on the material used in the electrodes and the solutes present in the water. Due to these characteristics, this technology have been considered more effective than traditional chlorination, justly because it produces additional oxidant compounds [25], although some by-products can persist in treated water and offer risk to environmental health. The alteration of the material used in the electrode can affect the final result, by excluding the need to apply additional reagents or by inhibiting the production of high concentrations of hazardous compounds [35].

In the process of water disinfection by ultrasonication occurs a formation and an explosion of bubbles, when in high temperature and pressure, and a formation of oxidants by pyrolysis. However, the application of this technique demands elevated costs, while the disinfection rate is still low. Thus, its cost/benefit is not attractive [25]. Acoustic waves with frequencies higher than 16 kHz have energy enough to break the chemical bonds and produce hydroxyl radicals, which can oxidize the substrate. In spite of this technique be effective to degrade organic compounds, it is too hard to achieve a full mineralization of the contaminants. To obtain a major efficacy with this technology, the association of complementary techniques would be necessary, such as the application of ultrafiltration membranes [50].

Until now, the heterogeneous photocatalysis did not reach enough results to be applied and commercialized to disinfect wastewater. This technology is based on the induction of series of oxidoreduction reactions, by the photons energy. The combination of UV with titanium dioxide has been shown as effective to decrease the amount of microorganisms. Nonetheless, the total removal of catalytic particles of TiO_2, after water treatment, is still not possible; which makes this water unsuitable for consumption [25,51].

The use of nanotechnology to treat certain wastewater is advancing, but many studies are still being carried out to assure better results. The Nanosized Metal Oxides (NMOs) are considered promising tools to remove heavy metals, during water treatment [52]. According to these authors, NMOs have a great potential to adsorb heavy metals, showing high capacity and selectiveness to remove toxic compounds from water and, consequently, meet the requirements of current legislation. As these compounds can easily agglomerate and lose the adsorption capacity, as already described by Chong et al., [51] about TiO2, they can't be used freely in treatments, needing to be impregnated in porous supports. Several technologies to remove these particles are still being tested, to enable its use in water treatment [52].

There are magnetic NMOs, which can be easily removed from the water by a magnetic field, reducing the cost of application and enhancing the yield of this technology [52,53].

Recent studies showed a development in the engineering of biochar, resulting in a combination of this material with nanoparticles and other chemicals, or in its improvement by biological transformations. This development supported the advent of a new class of hybrid-chars, which possess a huge potential to treat a wide variety of organic contaminants [54].

Some advanced technologies, especially used for the reuse of textile effluents, include membrane processes such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis, as well as advanced oxidation processes, electrochemical processes, adsorption and ion exchange. These technologies have been more efficient for remove colour and COD from textile wastewaters when compared to conventional methods [55].

Singh et al., [32], assessed, chemically and biologically, the water reuse quality from a wastewater treatment station of Canada (by fish exposure). In this station is performed a pre-treatment based on screens and grit tanks, a primary treatment with sedimentation, a secondary treatment with bioreactors and, finally, a tertiary treatment with a membrane filtration. Chemical analysis registered the presence of pesticides, pharmaceuticals and stimulant substances in the water samples, which affected the immune system of the exposed fishes. However, after treating these samples with UV and H_2O_2 systems, these authors recorded a decrease in the concentration of these substances, inferring a partial efficacy of these techniques. Furthermore, the researchers recommended the use of activated carbon, as it could remove these residuals substances from water.

Blanco et al., [40] reported the efficacy of a combination of biological treatment with advanced oxidation processes, reducing up to 75% of total organic carbon from textile effluent. Sirtori et al., [39] demonstrated that the combination of the photo-Fenton treatment (supplied by solar irradiation) with biological treatment (biomass reactor of activated sludge) obtained efficiency higher than 95% in the removal of pharmaceuticals from the tested effluent.

Based on these and on other examples, several studies have shown that the sequential combination of diverse technologies, i.e. treatment trains, offers multiple barriers to almost all pathogenic and chemical contaminants, which do not reach the treated water at the end of the process [56].

Uses and Applications for Recycled Water

The water reuse have become one of the most viable alternatives both for rising the water supply and for decreasing the pollution caused by wastewater that are discharged in the environment [57].

Comparing the standard treatment to obtain water reuse with the standard treatment for water capture in conventional sources, Hochstrat et al.,[12] defined three types of different applications: 1) reuse in activities that already requires a major water demand, (*e.g.* agricultural or industrial purposes); 2) reuse to additional or new aims, generally not supported by freshwater coverage (*e.g.,* environmental enhancement); 3) reuse to recover or enhance natural resources (*e.g.* to rise the flow rate of aquatics environments and recharge of groundwater).

To obtain water reuse to most variable purposes, a treatment system realized directly in wastewater can be used. This treatment consists on the recuperation of an effluent using water for urban and agricultural purposes, thus supplying a determined demand of great requirement. This resource is able to provide a trustable water supply for many years, due to its constant production, benefiting the users who suffer with water scarcity [5,58].

Uses in Agriculture and Livestock

As the wastewaters can have significantly concentrations of organic and inorganic nutrients, as nitrogen and phosphorus, there is also the possibility of these nutrients remain in the recycled water. This way, the water reuse in agriculture can be characterized in a beneficial action for both, the agriculture and the environment, because this water could be a potentially source of nitrogen and other nutrients for plants and, thus, could decrease the necessity of using fertilizers in the agricultural area. The water reuse in agriculture can also act as a dilution agent for other pollutants present in the effluent, elapsing in another benefit to the environment [18,59].

As the water is of great importance to many agricultural practices, for example in irrigation, pesticides application, washing and post-harvest transport, it may also be a vector for many pathogens for plants and cultures [60]. In several countries, many bacteria contamination by *Salmonella* sp. and *Escherichia coli*, for example, and also contamination by norovirus were related to the use of contaminated water, before and after harvesting fruits and vegetables, showing the importance of using water of good quality for food safety [25].

Furthermore, it is necessary to attempt to the high levels of disinfectant chemicals in the water reuse, such as chlorine and its by-products, which can negatively interfere on the plant growth, induce damages on the leafs and roots, affect plants colours and change the taste of agricultural products and nutritional components [25].

According to Tal [61], Israel was the first country to establish a set of standards for water reuse. Currently, 91% of domestic sludge of this country is treated, and of those, 73% is recycled. The secondary treatment has an additional removal of nitrogen. Then, the water is injected into groundwater aquifers, to recharge the same and, after filtration and seasonal storage, to be provided for agricultural use. In 1992 was established a new standard for secondary treatment of wastewater that would be used in agriculture, based on determined parameters, such as BOD (20 mg/L) and total suspended solids (TSS – 30 mg/L). However, according to Tal [61], these values were inadequate once most of the cultures exhibit sensitivity to the pathogens presented in the effluent; vegetables and fruits, which are ingested *in natura* cannot be irrigated with water treated by this method; the salinity of sewage may be transferred to soils and hydric resources; boron compounds common in detergents are not efficiently removed and may accumulate on reuse water, which may change soils structure. Studies developed by Muszkat et al., [62] showed that organic solvents (benzene and toluene) used by Israelite industries during 1980s began to appear in water samples from wells installed in rural areas, due to the inadequate sewage treatment and to the extensive use of the effluent in irrigation.

Cirelli et al., [63] demonstrated that it is possible to reuse water in vegetable cultures, if the municipal wastewater passes through a tertiary treatment. In the same study, the authors observed that the contact of a fruit with the soil irrigated with reclaimed wastewater may led to a contamination by the microorganisms presented in this water. However, this contamination can be reduced to acceptable levels if there are post-treatment measures of control, such as the removal of pathogens between the natural microorganisms or the cleaning and disinfection of the production.

The reuse of water in plant irrigations should be done cautiously because some substances, such as drugs and personal hygiene products, may persist on the water, even after treatment. Jin et al., [64] reported that some studies detected residues of these substances in vegetables irrigated with reclaimed water, both in roots and aerial parts. In addition to the presence of organic pollutants, metals can also be found in irrigated vegetables with reuse water.

Amin et al., [65] verified the presence of several metals in high concentrations in the vegetable's edible parts. The authors alert that if these vegetables were consumed in excess, they might induce health problems. In this same research, these authors evaluated the irrigation with domestic and industrial effluents without treatment in Pakistan, and observed that this practice offers great risks to population and to the environment.

Livestock also generate large amounts of effluents that, in addition to containing organic matter and minerals, there are many micropollutants, germs and pathogens, which can contaminate the soil [66]. Although no case of direct use of water reuse in livestock has been found in the literature, Carretier et al.,[66] showed that the treatment of livestock effluents by associating ultrafiltration, nanofiltration and reverse osmosis technologies is very efficient on the removal of pollutants, suspended solids, pathogens and organic matter, and on the reduction of salt concentrations, being a viable alternative for the use of this water in agriculture and, posteriorly, to animal feeding.

Uses in Industry

Many industries use water in their production processes, *e.g.* for cooling equipment, to cleaning or even cooking, and this practice, besides high level of water consumption, also produces high amount of wastewater that is constantly discharged in water bodies without a proper treatment, harming the environment. Thereby, the water reuse would be an interesting and important alternative to water consumption and to the reduction of industrial effluents generation.

In general, food industries consume high amount of water, using it as ingredient, for dewetting, in washing, rinse, heating, pasteurization, vapour production, sanitation and food disinfection. Despite reports of reuse water derived from food industries on irrigation, the most effective use of this kind of water would be in the industry itself, due to the complexity of the generated mixture after the industrial process [67].

According to Vourch et al. [68], the dairy industries, for example, is one of most polluting industries, if compared to other food industries, due to its high consume of water and its wastewater production. Furthermore, Cristóvão et al.,[17] showed that the sedimentation, flocculation, aerobic biological degradation, filtration, reverse osmosis and ultraviolet irradiation disinfection processes, in sequence, consists in a very efficient treatment on water used by canning industries. The reutilization processes of these waters were described only for cleaning and equipment cooling purposes, or even, the reutilization of condensed water from dairy industries evaporators for washing or cooling, on butter production [67].

However, the reutilization of water in food industries has to deal with many obstacles. The implementation of treatment systems is complex, due to a lack of guidance for water treatment processes. The reuse water needs to be tested and have documentation certifying the quality and safety of this water, and accessibility to different methods of quality evaluation of this water [67].

In oil refineries, water is extensively used, especially for equipment cooling, with an estimated spending of 245 to 340 litres of water per oil barrel produced [69,70]. The reuse water resulting from reverse osmosis treatment has been highly used for these purposes, despite the fact that this technology has a reduced flux rate due to the organic matter deposition, which limits its application [70].

The textile industry is another obvious example of an industry demanding high amounts of water for production and generating large volumes of wastewater. In textile practices, wastewaters have a huge amount of dyes and high concentrations of organic and inorganic dissolved solids, once the dyes may not be removed by conventional treatments, ending up diluted and discharged on the sewage systems [71]. The processes of dyeing, discoloration, printing and washing generate complex residues with low degradability, which difficult the treatments [6]. The implementation of combined treatments (MBR and nanofiltration or oxidation and ultrafiltration advanced processes) generates sufficient reuse water to decrease the consumption of freshwater by the industries, in about 40% [6].

Urban Uses and Potability

The urban development induces a significant impact on water cycle for reducing rivers flow rate and returning to water bodies highly polluted waters, which frequently compromise the receptor bodies quality [2]. Thus, the development of an integrated system would be able to promote a more efficient wastewater treatment and, thus, supply potable water for human uses, decreasing the extraction from water sources [72].

As several urban uses do not require potability, there is an increase on the incentive of politics of structures installation for stocking and capitation of both wastewater and rainwater, to be used in landscaping, for streets cleaning, in commercial buildings and homes [73].

Furthermore, it is possible to reuse greywaters from sinks and showers/tubs, using a system that captures and redirects this water for using in toilets flush, for example. However, in case of many commercial buildings (*e.g.* malls), which do not have showers on toilets, the water discharged from sinks are not enough to supply the demand of toilet bowls [7]. Unlike the previous examples, where the water does not require potability, obtaining potable reuse water for human use, which meets or exceeds the standard quality, can be a cheaper alternative in comparison to desalination processes or even to water importation [74]. With the advanced methodologies for treating water, there are two ways of reusing water with high quality: indirect, where the water effluents are highly treated and returned to the reservoirs supplying potable water, such as superficial or groundwater; or direct, in which the water effluent is highly treated and mixed directly on public water supply system of potable water [11].

MBR systems have been largely studied and combined with other technologies, such as nanofiltration and reverse osmosis, to obtain a high quality water, because these processes leave just a few trace compounds and act like a barrier for chemical contaminants on waters of indirect use [75].

Although there is a high possibility of removing contaminants, there is still a lack of reliable assessments to ensure the quality of this water, and a need to achieve greater clarity between the indicators used in evaluations and human health [67]. Due to this lack of information, there is low public acceptance for reusing water for consumption purposes. In this context, some studies showed that, to try to achieve public acceptance, it is necessary to effectively divulgate the technical information that are interesting to the population, to present the social, environmental and institutional costs/benefits of the water reutilization, to compare risks related to the implemented method to those already described in other technologies of treatments, to elaborate specific regulatory systems for water reuse, and to analyse others alternative solutions [76].

According to Harris-Lovett et al., [74], a study in California affirmed that, to sensitize the population and explain the benefits of water reuse, it is necessary to first elaborate a project with a legit structure for obtaining potable water reuse, associated to the understanding of the history and values of local residents. Furthermore, according to them, the project needs to present real benefits to the users, increasing the public involvement to both, the planning and the decision-making, to incorporate managers procedures of risks and organizational reputation of high quality, have procedures of risk management and emergency innervations and have services managers which aware the population and create a relation between water recycling in social practices.

Assessing the Quality of Water Reuse

The acceptability of water reuse for any purpose depends on its physical, chemical and microbiological qualities. Factors that affect the quality of water reuse include basically the quality of the source, the used treatment of wastewater and the operation of distribution systems [14]. Many chemical constituents are well understood for non-potable uses of the recovered water, where quality limits are more easily determined. However, the health risks due to pathogenic microbiological agents are more difficult to establish and to evaluate [77].

Reclaimed water can present several pathogens, dissolved solids, heavy metals, pesticides, and other substances which may cause damage to ecosystems, crops or human beings [78]. The presence of pathogenic microorganisms and potentially toxic chemicals in water reuse may pose serious risks to environment and human health, so, it is crucial to establish quality standards for water reuse, allowing this resource to be safely applied for different purposes. In this respect, some assays have been conducted to assess and ensure the quality of these waters.

According to Salgot et al., [14], the most used tests for assessing the water reuse quality are those related to minimizing exposure to the infection or toxicity risks of such water resource. These authors still proposed quality categories for different reuses of the water, and they had compiled microbial risk estimations and chemical limits for each category. Although the biological parameters regulated and used in several countries are coliforms indicators of the occurrence of fecal contamination and the possible presence of all pathogens which occur in warm-blooded animal faeces, these authors consider that biological parameters of reclaimed water derived from sewage must indicate all potential pathogenic organisms (viruses, bacteria and parasites from different sources) causing diseases in all living beings. The large number of possible chemical parameters in relation to the recycled water has to be adapted with regard to the origin of the sewage, the treatment process and the intended use, since they must cover a broad spectrum of toxicological and ecological risks.

The required degree of water reuse quality may significantly vary according to its application. The International Reference Center on Water Reuse (CIRRA) [79] points out that the level of water quality required for a determined use, nowadays, may be different from what has been standard in the past or of what will be in the future, because technology development, as well as problems associated with scarcity of resources and pollution, may cause restrictions on the level of quality considered appropriate until now.

Whereas the detection and quantification of *E. coli* is an insufficient tool for precisely evaluating the quality of reuse water, conventional or advanced wastewater treatment processes have usually been determined, with other organisms/methods, such as: bacteriophage detection (somatic, F-specific and Bacteroides fragilis HSP40 and RYC2056 phages), amount of DNA/RNA of heterotrophic and aerobic bacteria, detection and analysis of the viability of nematode and helminths eggs, direct detection and analysis of the viability of *Giardia lamblia* cysts, direct detection and analysis of the viability of *Cryptosporidium parvum* oocysts. Furthermore, other molecular biological methods have been used for enabling a faster determination of specific microorganisms [80,81].

There are many physical and chemical parameters that can be established in relation to wastewater reclamation and reuse, *e.g.* pH, Electrical Conductivity (EC), BOD, endocrine disruptors. Some simple parameters which are already included in existing guidelines for all final uses, such as salinity, turbidity, TSS, organic matter, Dissolved Organic Carbon (DOC), nitrogen and phosphorous related, can give useful information about the quality and success of the treatment process and thereby indicate the elimination rate of pollutants that are difficult to remove, such as organic pollutants. Besides, assays that determinate the concentration of metals in wastewater effluent, mainly from industrial regions, are indicated to be performed at regular time intervals [14].

The water reuse assessment, especially for recharge of groundwater for drinking water purposes and for agricultural use, must consider, beyond the rigorous control of physicochemical and microbiological quality, the presence of several pollutants, such as pharmaceuticals and pesticides, to ensure that there is no damage to the environment and population health. For example, the endocrine activity of some substances may be measured by bioassays, such as E-screen [82].

A study performed by Christou et al., [83] assessed the impacts of the reuse of wastewater effluents for the irrigation of a tomato crop. Prior to being applied to the irrigation, the water was analysed by physicochemical (pH, EC, BOD, COD, SS, total N and P, chloride and metal content) and microbiological parameters (*E. coli* and helminthic eggs content), based on FAO's water quality for agriculture report [84]. Christou et al., [83] stated that the irrigation with wastewater did not affect the soil pH and the organic content, as well as the crop productivity; the metal content of the fruits was found to be below the maximum permissible levels set for fruit safety; and no microbiological contamination (faecal coliform, *E. coli, Salmonella* sp., *Listeria* sp.) was observed in the tomato fruits irrigated with water reuse. These results suggest that the effluents submitted to the tertiary treatment (advanced treatment of good quality) might safely be reused for vegetable irrigation and dry areas.

Lutterbeck et al., [85] assessed the toxic effects of 4 pharmaceuticals used in anti-cancer treatment (cyclophosphamide, methotrexate, 5-fluorouracil and imatinib) and confirmed the phytotoxicity of all these chemical compounds to *Lactuca sativa*. Cytotoxicity was also observed for the *Allium cepa* meristematic cells exposed to cyclophosphamide, methotrexate and 5-fluorouracil (registered by the significant differences in the mitotic indexes compared to the negative control), genotoxicity to cyclophosphamide and 5-fluorouracil (recorded by high levels of chromosome aberrations) and mutagenicity for the 4 tested drugs (registered by the significant frequencies of micronuclei). Since these compounds may affect the growth and development of plants and knowing that they may not be completely removed by conventional oxidative and biological treatment, these authors inferred that the phytotoxicity assays with *L. sativa* and that the cytotoxicity, genotoxicity and mutagenicity assays with *A. cepa* meristematic cells could be important tools for a rapid screening of environmental contamination, especially for the evaluation of wastewater to be used for irrigation purposes.

Zhang et al., [86] evaluated the safety of reclaimed water reuse in an artificial groundwater recharge system, by means of different bioassays (acute toxicity to *Daphnia*, estrogenicity using yeast tests and genotoxicity using the SOS/umu test based on *Salmonella typhimurium* TA1535 without S9). Based on the assays with *Daphnia*, the authors showed that the ozonation and the treatment of soil aquifer removed the pollutants responsible for acute toxicity. Regarding the tests with yeast, these authors registered a low estrogenic activity of wastewater recovery system, since the inhibition indices of yeasts were lower than 10%. By the umu test, they found that both the ozonation and soil aquifer treatment were able to reduce the genotoxicity of the tested samples at 99.8%. This way, the bioassays used in this study were effective in demonstrating that the combination treatments (such as soil aquifer treatment associated to ozonation) may provide new sources of water with low toxicity, similar to that observed in conventional natural sources of potable water. Using these same bioassays, Aguayo et al., [87] and Escher et al., [88] identified different organic pollutants in samples of effluents from sewage treatment plants. The authors reported acute toxicity, estrogenicity and genotoxicity for the water reuse tested in their assays.

As stated before, the main negative impacts induced by textile industries on the environment are related to intensive water consumption and wastewater discharge, characterised by greater amounts of organic chemicals and colouring agents, low biodegradability, and high salinity [6]. The determination of physicochemical parameters (COD, pH, alkalinity, total hardness, EC, turbidity, colour, odour, nitrate, nitrite, chloride, sulphate, aluminium, copper, chromium, iron, manganese, zinc, organic load) considered relevant to the required quality of water, should be required for wastewater from textile industrial intended for reuse [6,89]. Ribeiro [89],recommends the implementation of microbiological and toxicological assays to investigate the risks of handling and subsequent disposal of these effluents into water bodies, especially considering the possibility of formation of more toxic substances.

Worldwide Regulations to Reuse Water Safely

Water reuse represents an alternative source to satisfy part of the growing water demand in cities and reduce the problem of water scarcity [90]. Each purpose of reuse requires different levels of water quality, not only to protect health and the environment, but also to fulfil the requirements of each reuse [91]. However, as previously mentioned in another topic of the present chapter, one of the major challenges of the implementation of water reuse is the public acceptance, and this aspect has been widely discussed worldwide [92]. Thus, for the safe use of this water, and in order to get popular acceptance, it is essential that legal specifications related to its quality be determined by public authorities (international, national, regional or local) [90].Therefore, it is necessary to create standards or guidelines governing the reuse of water, stipulating the use of parameters to examine the quality of these waters and establishing a specific legal framework for water reuse, which allows its safe use without compromising human health [93]. It is also necessary that reuse programs are constantly reviewed and monitored throughout their development [4]. These patterns may vary according to the needs of each locality [91].

According to the Mediterranean Wastewater Reuse Working Group [94], several countries are developing their legislative framework for the safe use of wastewater. This framework can be divided into directives and regulations (or standards), where the directives can be defined as guidelines to ensure safe use of treated wastewater at acceptable levels of risk [94,95]. The regulations, on the other hand, are legal requirements promulgated by laws, rules or technical positions [4] mandatory and enforceable [94], established by authorities at national level, adapted to the local priorities and technical, economic and social limitations. The regulations are always established based on general risks/benefits criteria associated with health-related characteristics, the environment and the economy and social reality, which may be amended or supplemented, according to scientific recommendations, technology and national trends [4]. The directives may vary between countries or regions within a country, enabling distinct flexibility of legislation implementation, according to the specific local conditions and characteristics of the implemented programs. This is the case of directives presented by organizations such as the World Health Organization (WHO), the US Environmental Protection Agency (EPA) and the Australian organization, which may be used by states that have limited regulations or guidelines, or which do not have any established rule [94].

In 1980, the EPA organized a directive guideline and a planning program for the reuse of wastewater, entitled “Guidelines for Water Reuse” [55,96]. This document aimed at assisting the regulations and guidelines developed by the states, societies and other North American authorities, once in USA, the use of wastewater is a responsibility of state and local agencies. Since then, this document has been updated (1992 [97], 2004 [98] and 2012 [56]), in order to guide the North American society concerning the possibilities for reuse of wastewater, in an attempt to ensure the quality of these waters, after proper treatment. The latest version of this report, published in 2012 [56], provides updated directives, which are used not only in the USA but also worldwide, as a reference for the establishment and/or adaptation of local directives.

In 1989, based on epidemiological studies, the WHO prepared a wastewater reuse guideline entitled “Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture”, which presented new criteria for water reuse in agriculture and aquaculture [99].

The Australian government created, in 2006, its own guidelines for reuse of wastewater, establishing the need for environmental and health risks management, developing a risk framework for the beneficial and sustainable management of water reuse systems, before the release of this water to the various activities related to it. Although Australian guidelines have no formal legal status and allow a flexibility of responses to different regional and local situations, all Australian states and territories are encouraged to adopt this directive framework, applying the measurements according to their own jurisdictions, *i.e.* according to the established management for local wastewater treatment. Australian guidelines are one of the most appropriate and useful for the reuse of wastewater in the world [94].

According to a review performed by the Mediterranean Wastewater Reuse Working Group [94], the guidelines presented by the EPA, the WHO and the Australian proposals are well structured and present information from different aspects including: 1) applications of treated effluents in agriculture, in landscape, in dams, in industrial uses, in aquifer recharges for potable or non-potable purposes, in environmental improvement and others; 2) reuse methods of treated wastewater, such as agricultural, surface, sprinkler, drip and subsurface irrigations; 3) treatments performed to obtain water reuse as secondary treatment (activated sludge process, biological filters, rotating biological contactors or biodiscs, stabilization ponds), filtration (transfer of treated wastewater for natural soils not degraded, wetlands, sand, anthracite, filter cloth, micro-filters or other membrane processes) and advanced wastewater treatment (tertiary chemical treatment, carbon adsorption, reverse osmosis and other membrane processes, air stripping, ultrafiltration, ion exchange); 4) microbiological constituents including bacteria, protozoa, helminths, viruses; 5) chemical constituents such as biodegradable organic compounds, Total Organic Carbon (TOC), nitrates, metals, pH, trace substances, by-products of disinfection procedures, total dissolved solids; 6) physical properties such as suspended solids, turbidity, temperature; 7) monitoring the water obtained in relation to pH, BOD, COD, TSS, coliforms, chlorine, turbidity, baseline; 8) validation, operation and verification of the processes used and the water quality obtained in these processes.

According to Peasey et al., [100] and Almeida [93], in Latin American countries, such as Brazil, Argentina and Chile, there are no specific standards for the system of wastewater reuse. Due to this lack of standardization, these countries often adopt international standards such as the EPA guidelines or technical guidelines prepared by private national institutions [101].

The European Union has attempted to implement wide and national provisions to ensure a method of sustainable water management, and an important result of these actions is the Water Framework Directive (WFD) [102]. The WDF established a legal guideline that guarantee a sufficient amount of good quality water across Europe, necessary for the different uses and environmental quality. Among the WFD aims, are: to ensure the protection of all kinds of water, including inland and coastal surface waters and groundwater; to combine emission limit values with environmental quality standards; to ensure water tariffs that provide appropriate incentives for efficient use of water resources; to ensure better involvement of citizens and simplify legislation [94].

In 2014, the European Commission held a public consultation on policy strategies to optimize water reuse in the European Union, and to assess the most appropriate instruments to implement the reuse of water, to ensure the environmental health and trade of food products. The results of this consultation may guide the possible application of water reuse in agriculture, urban and industrial activities, such as recreational uses, groundwater recharge, etc. Data obtained with this study are presented in the report "Optimizing water reuse in the EU Public consultation analysis report", prepared by the group BIO by Deloitte [103].

Also according to the European Commission [104], although the reclaimed water reuse is an accepted practice in many European countries that suffer with water scarcity (Cyprus, France, Greece, Italy, Malta, Portugal and Spain), and where water reuse is already considered essential for the management of water resources, only a small portion of the reconstituted water production is being used in these countries. Facing this reality, it is necessary to develop efficient strategies to solve problems related to the safe reuse of water and to set goals that encourage the production and use of this water, since, despite the advantages and efficient treatment potential of wastewater, this reuse not a widely implemented, even in EU member countries.

The WFD [102] emphasized that to obtain more efficient programs of water reuse, it is necessary to first deal with some obstacles, such as: 1) the inappropriate pricing of reuse water, where, often the water reuse tariffs are far superior to the fresh water tariffs, limiting the economic attractiveness of reusing projects; 2) the inefficient monitoring of freshwater distribution, which is observed mainly in the agricultural sector, with many examples of free and illegal distribution or granting distribution licenses that go beyond the available resources; 3) uncertainties conferred by the different quality standards established by regulatory agencies, which lead to different levels of security for different uses of water - there is a lack of regulatory clearness, a lack of confidence in the health and environmental safety of reuse practices, and a lack of public awareness and acceptance related to the benefits of water reuse; 4) elevated cost for practicing treatments, elevated number of water quality parameters to be monitored and high sampling frequency required, which increases the costs of monitoring; 5) technical obstacles and scientific uncertainties, that safely guide specificities for the reuse of wastewater, such as removal techniques of metallic micro-pollutants, pharmaceutical and pharmaceutical metabolites, as well as household chemicals and others; 6) the non-recognition of water reuse as a component of the integrated approaches to water management, and the lack of communication and cooperation among stakeholders involved in the entire water cycle, in particular between the water supply and sanitation.

The California state, in U.S., is a pioneer when the water reuse is the subject. About the public acceptance, Hartley [105] have pointed some aspects that can raise the acceptance of the U.S. public: 1) minimal human contact on water processing; 2) clarity about the protection of public health; 3) clarity about the benefit of water reuse and the environmental protection; 4) high relationship between water conservation as a result of water reuse; 5) reasonability about the cost of treatment and distribution; 6) minimal perception of wastewater as the source of reclaimed water; 7) disclosure about the high demand of water and the real situation about the community water supply; 8) what is the importance and the participation of reclaimed water in order to maintain the water supply of the community; 9) elucidation about the high quality of the reclaimed water and its safety; 10) trustworthiness in local management responsible for the wastewater treatment.

Final considerations

The water reuse is a viable, and in some cases, affordable alternative to increasing water supply in agriculture, industries and cities, and also to contribute to the reduction of the pollution caused by contaminated effluents that are discharged into the environment. Besides being able to supply the demand for the development of human activities, the water reuse may ensure the supply of a considerable amount of water, for many years, once it is a continuous production process.

To be widely used in agriculture, it is very important that the water reuse passes through tertiary treatment, to remove organic and inorganic chemical contaminants and pathogens, considering that many agricultural products are consumed *in natura*, or used in livestock.

Just like in agriculture, industries require large volumes of water for subsistence or for the development of their production activities, for equipment cooling, washing and sanitizing the surroundings. A complicating factor for obtaining water reuse is to establish discerning environmental regulations, instituted to minimize the impacts of contaminants present in the effluents, which may increase the costs of treatments. It is expected that, with a major public acceptance and the consequent use of water obtained by wastewater treatment, it would be possible to reduce this high consumption, as well as this waste generation, with an advantageous cost/benefit.

In the cities, many activities, such as landscaping, environmental enhancement and street cleaning, do not require potable water. The reuse of water, after passing through primary and secondary treatments, may serve for these purposes, which would be easily accepted by the population. However, when the purpose is to use this water for food production or drinking, there is a certain prejudice by the population. Although some studies have shown the effectiveness of treatments in removing pollutants and pathogens, there is a lack of public insights and awareness regarding this use, as well as a lack of establishment of regulatory measurements on the level of water quality.

Generally, researchers attempt only to the effectiveness of certain types of water treatments for reuse, not considering the wastes generated during treatments, where little is known about their fate. The sludge formed during biological treatment, for example, may contain a wide variety of pathogens and contaminants; the membranes used in filtration processes may contain various emerging contaminants. Few studies have shown that there must be concerns related to the disposal of these wastes, as these may also function as new sources of contamination and may even disperse contaminants to another location.

Studies aiming at seeking alternative sources of water must be constantly encouraged, to expand the knowledge related to treatments with better cost/benefit ratios, appropriated to the purpose of the water reuse and that can ensure public and environmental safety.

As water reuse may present a varied amount of chemicals and several pathogens, it is extremely necessary to perform chemical, physical and biological tests, in order to guarantee the quality and safety of its use.

In addition, for safe practice of reuse, directives and regulations should be established and constantly reassessed, for a better adjustment of the requirements for safety use and updating of technologies applied to this end. Studies should also be encouraged to provide lower cost treatments and to increase efficiency in removal of contaminants from wastewater. It is also important to always inform the population about the risks and benefits of water reuse, in order to increase public acceptance of the recycling water practice.

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Ecotoxicological Impact of Nanomaterials in Aquatic Systems

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Abstract

Various Engineered Nanomaterials (ENMs) are currently in use in the industrial, agricultural, pharmaceutical and medical fields. However, while ENMs can provide great benefits, we know very little about their potential negative effects on aquatic environments. ENMs can be more ecotoxic than bulk materials of the same composition, mostly because of increased surface area and high reactivity of their atoms and molecules. This high reactivity of ENMs can cause adverse effects in the biological componentes of organisms. We describe some ecotoxicity effects of ENMs in aquatic organisms (phytoplankton, bacteria, zooplankton, zoobenthos and fishes) and consider the means by which these ENMs enter aquatic environments, their aggregation status and their bioaccumulation in the aquatic environment. However, research on establishing appropriate ecotoxicity-test strategies and methods should first define realistic conditions for aquatic systems. Thus, we can assess the potential risks posed to aquatic environments by nanotechnology.

Keywords: Bioindicator; Interaction ;Nanotechnology; Nanotoxicity

Introduction

In recent years, many newly Engineered Nanomaterials (ENMs) have been developed by this fast-growing area of nanotechnology. The global market for ENM applications was estimated to be half a million tons in 2020, it is almost certain that ENMs will be released into the environment [1]. The great advantage of ENMs is the possibility of enhancement of their mechanical, optical, magnetic and chemical properties, enabling the creation of improved materials, devices, and systems that exploit these new properties. However, these same characteristics that confer their unique properties to ENMs may also be responsible for their negative effects on the aquatic environment.

The development of nanotechnologies may lead to dissemination of ENMs which are potentially toxic in the environment and lead to the contact of these materials with live organisms. The increased presence of ENMs in commercial products such as cosmetics and sunscreens, dental fillings, photovoltaic cells, and water filtration and catalytic systems has resulted in a growing public debate on the environmental effects of these materials [2]. In the context of ecotoxicity, some studies have investigated the impact of ENMs on species of phyoplankton [3], zooplankton [4] and fishes [5] considered environmental quality bioindicators.

However, some ENMs such as carbon black and Multiwall Carbon Nanotubes (MWCNT) currently are not known to pose a serious risk to the aquatic environment [6]. Thus, the development of nanotechnology industry is dependent on research in the ecotoxicology area to ensure the safe production and use of these materials. A better understanding of the interaction between ENM and organism can mitigate environmental impact before this technology fully develops.

What is An Engineered Nanomaterials ?

The prefix "nano" is derived from the Greek word for "dwarf" and means one billionth (10^{9}) of a meter. The initial concept of nanotechnology was introduced by physicist Richard Feynman in a talk titled "There's Plenty of Room at the Bottom" in 1959 [7].

An ENM can be considered as a small particle with at least one dimension between about 1nm and 100 nm. The number of dimensions of these elements determines its various forms and properties: (a) three dimensions less than 100 nm (nanoparticles); (b) two dimensions less than 100 nm (nanotubes and nanofibers); (c) one dimension less than 100 nm (nanofilms) [8]. Figure 1 shows some possible ENM shapes. The ENM may have a natural or anthropogenic origin, and the first group includes, for example, those produced and released during fires and volcanic emissions, while the second includes those produced as a result of human activities, such as the refining process, welding, food production or car combustion [9]. The manufactured ENMs are those produced by man intentionally, with physical and chemical properties related to the final application of the product [10].

Classification of ENMs for commercial purposes includes metal nanoparticles, metal-oxide nanopowders, semiconductors and alloys, carbon-based nanomaterials (e.g., fullerenes) and nanorods (carbon nanotube- CNT and nanowires) [11].

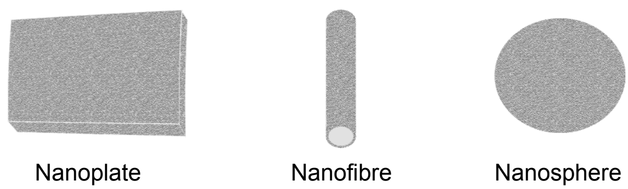

Figure 1: Schematic diagrams showing some shapes for nanomaterials.

Entry of Engineered Nanomaterials into the Aquatic Environment

The release of ENMs into the environment might occur throughout the whole chain of industrial production. The ENMs that are released into the environment interact with the air, soil and water, and may cause still unknown effects. Thus, the development of the nanotechnology industry is dependent on studies in the ecotoxicology area to ensure safe production and use of these materials.

In the aquatic environment, the toxic potential of ENMs is dependent on factors such as solubility, stability, mobility of colloidal suspensions or the tendency to aggregate into large particles, deposition and accumulation in this environment [12]. Therefore, determination of the capacity for agglomeration and aggregation is essential for nanoparticle characterization.

Baveye and Laba [13] suggested that aggregation can have impacts on toxicity, resulting in very different biological activity from that seen in the dispersed materials. For example, the binding of algae *Pseudokirchneriella subcapitata* to aggregates of Titanium Dioxide Nanoparticles (TiO_2) had a greater role in the toxicity of this ENM type [14]. Pereira et al., [3] *reported* that the proximity of algal cells clogged inside CNT agglomerates leads to different growth conditions. Such behavior can disrupt the supply of sufficient nutrients, which is a crucial factor to microalgae growth [15]. Photosynthetic organisms play an integral role in the ecological system, producing the biomass that forms the basic nourishment for food webs and much of the oxygen humans breathe. Thus large alga population changes due to ENM toxicity will have negative effects on the entire environment [16].

Since significant ENM sedimentation in aggregates is expected in aquatic systems, the aquatic sediments must be considered as important sinks of these particles released into the aquatic environment [17] and aquatic organisms as key receptors for ENMs (Figure 2). A number of studies have investigated the transport and interactions of ENMs in water systems [5,14,18]. Given that the exposure of aquatic organisms to ENMs is probably long-term, judgment on the overall effects of ENMs must consider their uptake and accumulation [19].

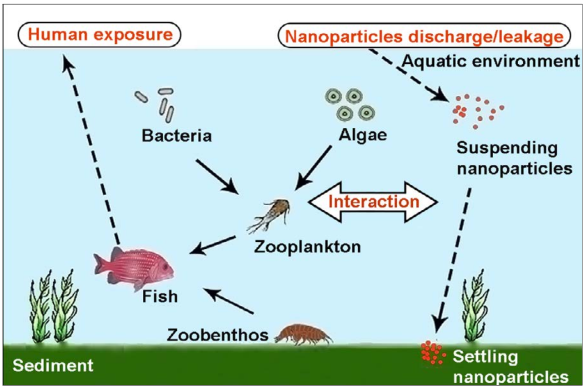

Figure 2: Generalized aquatic food web. In aquatic environments, nanoparticles can react with various organisms.

Given the potential entry of ENMs into the environment, their bioaccumulation throughout the food chain should be regarded with great concern. Despite the reports of the toxic potential of ENMs there is an evident lack of available scientific literature concerning their toxicity in the environment and a significant knowledge gap persists regarding the trophic transfer of ENMs in the food chain [20]. The transfer of ENMs through the food chain can lead to bioaccumulation and biomagnification resulting in a long term negative impact on ecosystem functions. Few studies have been conducted on effects of the ENMs' shape on their toxicity and bioaccumulation in deposits within aquatic organisms. Yeo and Nam [21] observed a high level of transfer of TiO_2 nanoparticles from the water in dropwort root to nematodes. Ferry et al., [22] showed that gold nanoparticles can pass from the water column to the marine food web in three laboratory-constructed estuarine mesocosms containing sea water, sediment, sea grass, microbes, biofilms, snails, clams, shrimp and fish. In a recent study, Pakrashi et al., [20] showed bioaccumulation of the aluminium oxide nanoparticles in the primary consumer *Ceriodaphnia dubia,* after this invertebrate was exposed to an algal suspension with nanoparticles. For humans, Handy and Shaw [23] reviewed the risks to human health and identified a number of exposure routes including the discharge of ENMs to water. However, many adverse biological effects in humans may not yet be known because the ENMs have been developed only recently, and the relevant experiments have not yet been done [24].

Interactions of Engineered Nanomaterials With Aquatic Biological Systems

Physicochemical Properties of ENM

The physicochemical properties of ENM determine their interaction with living organisms [25]. The physico-chemical properties of EMNs are due to their small size (surface area and size distribution), chemical composition (purity, crystallinity, electronic properties) surface structure (reactive surface groups, and inorganic or organic coating) solubility, shape, aggregation and load [26,27]. At the nanoscale there are forces and phenomena that do not occur at the macroscale. With the reduction of the dimensions of the bodies, the frictional forces, and gravitational combustion become less important. On the other hand new forces, such as electrostatic, van der Waals, Brownian, quantum mechanics increase the intensity [28].

Some studies suggest that the smaller the size of the ENMs the greater are its toxic effects [29]. Thus, the properties that provide technological applications of ENMs also determine their possible adverse effects [30]. The increase in surface area determines the number of potential reactive groups on the particle surface. With decreasing particle size the surface area increases exponentially and a large proportion of atoms or molecules become potentially reactive. This great reactivity of ENMs can cause greater interaction with biological components, increasing the activities that may be desirable (antioxidant action, entrainment capacity of therapeutic molecules, penetration of cellular barriers) or cause undesirable effects (toxicity, induction of oxidative stress and cellular dysfunction) [30].

The ability of agglomeration and dispersion is also linked to the size of ENMs, which tend to come together due to the action of Van der Waals forces [31]. The aggregation process may start during the synthesis of ENMs, but can become greater when in contact with water components. The aggregation of ENMs depends on particle concentrations, pH, zeta potential and the characteristics of the aqueous media. Clément et al*.,* [32] showed that aggregates of silica nanoparticles reduce the fluorescence of algal cells, affecting toxicity. On the other hand, Dhawan et al., [28] reported that dispersed CNTs were less cytotoxic compared to those aggregates.

Additionally, ENM characteristics such as the presence or absence of particle surface coating as well as varying environmental conditions (i.e. pH and dissolved organic matter) considerably influence the ecotoxicological potential, particularly of silver nanoparticles [33]

Engineered Nanomaterials-Induced Changes to Biological Target Sites

Due to the physicochemical properties of ENMs these materials are able to enter inside organisms by ingestion, respiration or skin penetration. Once inside, the bodies of these materials have the potential to interact with intracellular structures and macromolecules over long periods [34]. ENMs smaller than 40 nm may enter the cell nucleus, whereas those less than 35 nm may potentially cross protective barriers such as the blood-brain [35,36]. The incorporation of ENMs in living systems is influenced by features of the ENM and the organism. Inside the organism, the ENMs may remain structurally unchanged, be modified or metabolized.

Several studies have been developed to better understand the mechanisms of ENMs entry into cells, such as endocytosis, cellular uptake and particle transformation efficiency in the endocytic pathway [30] as well as the physiological response, distribution and elimination of these materials [37]. However, these mechanisms vary depending on the cell type tested and the nanomaterial.

The contact of ENMs with organisms can cause adverse biological effects not observed by the same material in a bulk form. The known toxic effects include mechanical injury or lipid peroxidation of biological membranes, organelles, intracellular damage to DNA [38] and impairment of mitochondria function [3,39].

Figure 3 shows adsorption of both M*ulti*-Walled Carbon Nanotubes (MWCNT) and Cellulose Nanofiber (CNF) to the cell surface of microalgae *Chorella vulgaris*. This may result in the disruption of the cell wall and membrane. Such loss in cell membrane integrity may lead to cell death.

Another factor that determines the toxicity of ENMs is the loading surface that influences the adsorption of ions and biomolecules, altering cellular responses or organisms exposed [34]. The surface charge is the major determinant of colloidal behavior, which may influence the biological response, according to the change in size or shape of EMNs due to the formation of aggregates or agglomerates. In general, it is believed that the cationic surfaces are more toxic than the anionic in interacting with the phospholipids; more groups or more negatively charged proteins are present in the plasma membrane [40].

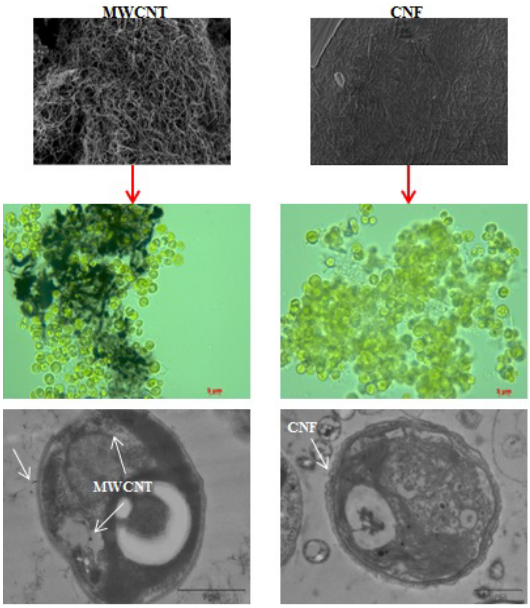

Figure 3: Optical and Transmission Electron Microscopy (TEM) micrographs of *Chlorella vulgaris* exposed to *Multi-Walled Carbon Nanotubes (MWCNT)* or Cellulose Nanofiber (CNF). *C. vulgaris* cells in close contact to MWCNT and CNF aggregates. White arrows indicate MWCNTs or cotton CNF.

Biological Models for Nanotoxicology Studies

Phytoplankton

Phytoplanktons are dominant producers in the aquatic ecosystems and they comprise a substantial component of biogeochemical cycles [41]. These primary producers are important as biological indicators because they are situated at the base of the food chain and any change in the dynamics of their communities can affect higher trophic levels of the aquatic systems.

Pereira et al., [3] provide a direct comparison of the impact of MWCNTs and cotton CNFs on microalgae *C. vulgaris*. Exposure to MWCNT and cotton CNF led to reductions of algal growth and cell viability. Exposure to both ENMs induced *Reactive Oxygen Species* (ROS) production and a decrease of intracellular ATP levels. The same study showed that MWCNTs penetrate the cell membrane and individual MWCNTs are seen in the cytoplasm while no evidence of cotton CNFs was found inside the cells. However, quantum dot nanoparticles did not cause oxidative stress in *Dunaliella tertiolecta [42].* Yet, CNT surface functionalization may alter the toxicity response and lead to mitigation of MWCNT nanotoxicity [19].

Bacteria

Bacteria are usually amongst the most sensitive species, because they can sorb, and sometimes internalize, many types of ENMs [43]. The ENMs might become problematic to the environment, as their bactericidal effects might have negative consequences for ecosystem health, impairing critical bacteria-driven nutrient cycles and biodegradation of organic matter [44].

The major cause of bacterial death is the impact on membrane integrity when ENMs are adsorbed onto the bacterial surface and internalized in periplasm [45]. Nano-CeO_2 caused morphological damage to the bacterium *Nitrosomonas europea [46] and was* toxic to a Gram-negative bacterium (*Escherichia coli*) [47] and as well as a Gram-positive bacterium (*Bacillus subtilis*) [48].

Surface properties of bacteria could play an important role in influencing the net toxicity of ENMs. For example, Joshi et al., [49] showed that bacteria covered with Extracellular Polymeric Substance (EPS) showed a lower toxicity in the presence of silver nanoparticles. The EPS can alter the interaction with the *nanoparticle and* avoid its internalization.

Zooplankton

Zooplankton are the food and energy link between the primary producers (algae) and secondary consumers (fish and fish larvae). They consume settled organic materials either as particles, bacteria, or algae, or they eat fragments of the leaves [17]. Particularly, research has shown that ENMs can be harmful to the freshwater zooplankton *Daphnia magna* [4,50]. Zhu et al., [51] showed that chronic exposure to TiO_2 nanoparticles for twenty days induced significant inhibition of growth and reproduction, and even caused mortality. In addition, *D. magna* can accumulate TiO_2 nanoparticles from the ambient environment and their elimination is difficult. *Additionally, D. magna* change their behavior when a contaminant is present in a body of water. These changes in behavior may make them more likely to be preyed upon by fish and could affect the food web [52].

On the other hand, Feswick et al., [53] demonstrated that quantum dots nanoparticles were taken up to a greater extent by *Daphnia*. But, the implications of quantum dot nanoparticles retention within the tissue of *Daphnia* have unclear consequences for either toxicity or trophic transfer of ENMs.

Zoobenthos

The aquatic sediment aggregates nanoparticles, hence sediment feeders can accumulate high contaminant concentrations. Filter-feeding invertebrates present in marine as well as freshwater bodies (e.g. *Elliption complanata*), may be contaminanted with ENMs passing from the water to the blood [17]. Musee et al., [54] showed that differents ENMs induced observable developmental deformities of the freshwater snail *Physa acuta* embryos. These authors suggest that long-term exposure of aquatic organisms to ENMs – potentially can alter certain ecological populations at different trophic levels – and may compromise the entire aquatic ecological functionality. However, more studies are needed using other representatives of this trophic level.

Fishs

The zebrafish *Danio rerio* is a well-known model specie used in standard toxicological studies and ecological risk assessments [55]. This organism provides a simple model for food chain transfer. In fish, ENMs could cause oxidative damage in the brain [56], affect the locomotion of rainbow trout or the ability of fish to compete for social status [5,57]. Gill and digestion tract were considered as the major uptake sites of ENMs in fish [10] because ENMs can be transferred through the aquatic food chain from algae, through zooplankton to fish. On the other hand, it must also be considered that some works show low toxicity levels of fullerenes in fish [58, 59].

Methods For Assessing Ecotoxicity of Engineered Nanomaterials

Several studies have been developed for better understanding of the mechanisms of interaction between ENMs and organisms. The main methods used in studies of ENM toxicity in aquatic environments are described.

Microscopy Analysis

Usually when ENMs come in contact with cells the nanomaterials can cause morphological and ultrastructural changes in cells. To evaluate the morphology, cellular ultrastructure changes and interaction between ENMs and cells, optical microscocopy, Scanning Electron Microscopy (SEM) and Scanning Electron Microscopy (TEM), respectively, are used. Thus, with these techniques we can evaluate the c*ellular* mechanisms involved in *nanoparticle uptake.* The elucidation of the affinity of the cells to ENMs is important since close interaction between cells and nanomaterials can cause particle adhesion and may lead to physical effects, such as the disruption of the cell membrane, or to a reduced cellular uptake of nutrients.

Photosynthetic Activity

The photosynthetic activity of microalgae after contact with ENMs can be measured using a PAM fluorometer. This method uses the saturation pulse method, in which a phytoplankton sample is subjected to a short beam of light that saturates the PSII reaction centers of the active chlorophyll molecules. This process suppresses photochemical quenching, which might otherwise reduce the maximum fluorescence yield. A ratio of variable-over-maximal fluorescence (Fv/Fm) can then be calculated which approximates the potential quantum yield of PSII. ENM toxicity after contact with *photosynthetic organisms* is also exhibited by reductions in the photochemical efficiency of the PSII. A decrease in the photosynthetic activity may be caused by a defect in the quantum yield of PSII itself, such as non-photochemical quenching. Pereira et al., [3] suggested that the accumulation of MWCNT on the surface of *C. vulgaris* cell walls may inhibit photosynthetic activity because of shading effects, i.e., reduced light availability.

Activity of the Antioxidant Enzyme

The ENMs are known to stimulate the cells' ability to produce toxic Reactive Oxygen Species (ROS) because of their large surface area [60]. ROS production may result in physiological changes, which can be divided into indirect effect mechanisms, caused by changes through perturbation of the redox homeostasis, or direct effects caused by direct damage to key cellular molecules such as lipid peroxidation, protein damage, and membrane destruction [61]. Thus, ROS creates a toxic environment in the cells leading to oxidative stress and cell death. In this context, Superoxide Dismutase (SOD) is one of the most important antioxidative enzymes, which catalyzes the superoxide dismutation (O_2^-) into oxygen and hydrogen peroxide. It plays an important role in the protection of cells against ROS by lowering the steady state of superoxide anions. The activity of SOD in cells after contact with ENMs can be used to study the possible cytotoxic effects of nanomaterials.

ATP Production in Cells

Mitochondria are responsible for an efficient coupling of cellular respiration to ATP production. ATP is a universal energy unit in all living cells, and a decline in ATP levels is indicative of loss in mitochondrial function [62]. Thus, the decline in ATP content may reflect a decrease in mitochondrial activity, which implies that ENMs can lead to disturbances in the energy metabolism of cells. Moreover, mitochondrial dysfunction can contribute to cell death by increasing ROS production and releasing regulatory death. Measurement of ATP is therefore fundamental to the study of living processes.

Among existing methods for ATP determination, the most successful technique is the bioluminescent method, because of its sensitivity and wide dynamic range.

Conclusions

From the data generated through literature it is evident that certain tested ENMs have the potential to negatively affect survival and behavior of various aquatic organisms; and alter the aquatic environment and the food web. However, these studies are still at an initial stage of development. Research on establishing appropriate ecotoxicity-test strategies and methods should first define realistic conditions for the aquatic enviroment. The influence on aggregation and deposition processes of ENMs in aquatic systems need to be considered.

Additional systematic research focusing on the safe production and use these ENMs, is required to assess the potential risks posed to aquatic systems by nanotechnology.

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Eco-Genotoxicity in Aquatic Systems

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Abstract

Contamination of aquatic systems has led to their degradation and, consequently to a threat to all the biota associated to these environments. Most of these impacts occur due to the release of chemical substances derived from human activities. Due to the significant impacts that these pollutants have caused to the environment, several assays have been developed in order to evaluate, efficiently, the impairment that these contaminants can cause on aquatic organisms and associated biota. Eco-genotoxic studies consist in assessing the occurrence of damages in the DNA of somatic or germ cells induced by environmental contaminants, making use of different endpoints, organisms or parts of them (bioindicators). Bioindicators are considered useful tools for eco-genotoxicity studies of rivers and sediments, since allow to assess, by several parameters, the action of xenobionts on the biological environment and the risks that the exposed organisms might be subjected. To perform these studies there are excellent biomarkers to evaluate the quality and degradation of the aquatic systems, being even recommended and included in decision-making such as, for example, determination of the limit concentrations of certain pollutants, as well as risk assessment. This chapter aims to present a review on the eco-genotoxicity studies performed with water and sediments of different hydric systems, using different biomarkers and endpoints. The assays that will be discussed will allow highlighting the relationship between the aquatic contamination and a variety of genotoxic effects observed in vivo, in vitro and in situ.

Key-words: Bioindicators; Comet assay; *In vitro* assay; *In vivo* assay; *In situ* assay; Micronucleus; nuclear abnormalities

Introduction

Human development and economic growth, related with the use of natural resources and production of several residues continuously released into the environment, interfere in the ecosystems homeostasis and, consequently, in the environmental health. The disposal of contaminants derived from anthropic activities tends to increase even more, both by the constant alteration of the lifestyle and by the continuous human population growth [1].

All organisms depend directly or indirectly on the environment where they live. Water is one of the most important natural resources for life, since it is characterized as essential element for the survival of all living beings. The aquatic ecosystem, which covers great part of the planet Earth, has many animal and plant species that exploit different ecological niches of this environment, from which; several of them are even human food sources [2]. Water is also one of the main components of human well-being and is a key factor for the social-economic development. However, this resource has been under constant threats due to impacts resulted from anthropic activities [3,4]. According to Frenzilli et al., [5] waters of lakes, rivers and marine coastal areas directly receive large amounts of residues derived from industries, agriculture and urban centers and indirectly from sedimentation of substances transported by the air. Thus, aquatic ecosystems are always in contact with stressor agents such as organic and inorganic anthropic pollutants, geomorphological alterations, and use of the land, water catchment, invasive species and pathogens [6].

Due to the great impact that the hydric resources receive, the sediments of these aquatic environments end up being deposits of physical and biological debris and act as the main sink for a range of organic and inorganic chemical contaminants [7,8]. Toxic compounds can be accumulated as an inert form in the sediments, but can, at any time be re-introduced in the water column by re-suspension and trophic transference. Sediments are also subjected to processes of transformation and activation, which can lead them to trigger adverse effects on the aquatic ecosystems, representing a long-term source of pollution [9,10]. Therefore, the environmental impacts in the hydric resources, besides having different origins, can also have different classes of contaminants.

Substances present in polluted waters can cause biological modifications that can affect the exposed populations and, consequently, entire ecosystems [11]. Great part of these pollutants can present carcinogenic and genotoxic potential [12], influencing the integrity of the DNA molecules of the organisms, reflecting, negatively in the individuals, populations and community [13].

Eco-genotoxicology

Substances released into the aquatic ecosystems present a complex chemical nature, which makes the conventional chemical analysis limited for the characterization of the genotoxic and carcinogenic potential of the chemicals present in chemical mixtures [12]. A simple chemical characterization of complex mixtures does not assure their effects on different biological systems, since a mixture of chemical compounds can alter the toxicity of isolated substances due to possible interactions that can occur between these substances. Substances can present different behaviours when isolated or in mixtures, such as additive, synergistic or antagonistic effects [14,15], i.e., present a sum of the toxicity of the isolated compounds; a potentiation of the toxic charge of the contaminants when mixed; or a decrease of the toxic effect of one of the substances involved in the mixture, respectively.

Several genetic disorders are resulted from the interaction between genotoxic pollutants and the DNA, resulting in irreversible damages, which can be transmitted to future generations [2]. For the evaluation of the direct or indirect effects of the contaminants on the genetic material, a branch of the toxicology that studies the interaction of the contaminants with the DNA was created and it is called eco-genotoxicology [2].

Genotoxicology studies aim to assess the effects of an agent on the DNA such as, for example, the formation of adducts, lesions in the DNA strand, unscheduled DNA synthesis and sister chromatid exchanges. Such effects can induce transitory damages in the cell since they can be repaired [16]. Damages in the DNA molecule can occur due to exposure of the cells to chemical products. When the exposure happens at very low levels but for a long period of time it can result in severe consequences to the population [14], since it compromises fertilization, embryonic life, development/growth and survival of the organisms [17,18]. According to Frenzilli et al. [5], the analysis of DNA alterations in aquatic organisms has showed to be an adequate technique to evaluate the genotoxic contamination of the environments, with the advantage of detecting and quantifying the genotoxic damages without the detailed knowledge of the physical and chemical properties of the contaminants present in the environment. Thus, the detection of the genotoxic effects is of paramount importance in the assessment of environmental risks and conducting bioassays in important to show the real impact of the pollutants in the ecosystems [19].

As the physiological alterations of the organisms may have a genetic basis, a comprehension of the changes at the genetic level (DNA) can help to determine the modification in the ecosystems. Therefore, molecular biology techniques can help to describe and understand, in advance, alterations that the DNA may be exposed [20].

Use of Bio-indicators to Assess Impacted Environments

Bioassays evaluate the effects of chemical compounds, individually or in a mixture, on organisms [21]. Eco-toxicological assays are performed with organisms that present little ecological tolerance, when exposed to certain contaminants. These organisms respond to the toxicity with physiological, morphological, biochemical, genetic or behavioral modifications [22,23].Thus, bioassays with indicator organisms are fundamental for the monitoring and evaluation of the quality of water and sediments.

Many species have been used worldwide in toxicity tests, producing important information in the assessment and characterization of acute and chronic effects of several toxic agents. The most used groups in laboratory assays are microalgae, microcrustaceans, echinoids, polychaetes, oligochaetes, fishes and bacteria, representing the diversity of ecosystems and trophic levels [23].

Biomarkers are biological parameters used as instruments of environmental evaluation that allow to observe alterations resulted from exposure to xenobionts both in cellular and in biochemical levels, molecular and/or physiological levels, which can be measured in different structures of an organism (cells, body fluids, tissues or organs) [24]. Several methodologies are applied to analyze the results of the interactions of xenobiotics with the DNA. Among the methods used for this purpose, we can cite the comet assay, micronucleus test and analyses of chromosome and cytogenetic aberrations.

This chapter aims to present the most recent studies that evaluate the eco-genotoxicity of waters and sediments of different aquatic systems, carried out in *in vivo, in vitro* and *in situ* tests. In the following sections it will be addressed the main test organisms used in the eco-genotoxicity evaluations of the several hydric resources and sediments.

Eco-Genotoxicity Using Cell Culture

In vitro experiments using fish and human cell lines have been used in the eco-genotoxic assessment of aquatic environments. To evaluate the genotoxic risk of samples collected in aquatic environments, the comet assay stands out and it is considered the most used tool in this area [25]. This test is considered an interesting alternative to estimate the DNA damage that can be repaired. The original protocol of the comet assay, published by Singh et al., [26] quickly evolved and has been used by the main research groups that aim to monitor the DNA primary damages.

Rigonato et al., [27] investigate the water quality of several sites of the Cambé Stream-Brazil, located in an area that receives intensive charge of industrial and domestic effluents. In this study, the authors evaluated the genotoxic potential of the samples using the comet assay with Chinese hamster ovary cells (CHO-K1). The results showed the presence of contaminants with genotoxic potential in all the samples collected, evidenced by the significant increase of the frequency of cells with DNA fragmentation. Sinos River, also located in Brazil, is considered the final destination of several types of pollutants. Thus, it was assessed, by the comet assay and Micronucleus (MN) test with Chinese hamster lung cell (V79), the genotoxicity of samples collected along this river. The comet assay showed a significant increase in the frequency of DNA damages in the six samples studied and only two samples presented increased frequency of MN when compared to the negative control. The authors concluded that these effects could be related with the discharge of domestic and industrial contaminants in this river [28].

Manzano et al., [29] determined the genotoxic potential of pollutants present in the waters of Ribeirão Tatu (São Paulo State-Brazil) by the comet assay in mammal Hepatoma Tissue Culture (HTC). The authors correlated the seasonality with the amount of pollutants potentially genotoxic present in the waters of this river. The seasonality in the region determines hydric deficits in the winter and floods in the summer and the pollutant charges from different sources (domestic, industrial and agrochemical effluents) are relatively constant throughout the year, the impacts in this hydric system are different between the seasons. The authors observed that the samples collected during the period of heavy rains (February) were genotoxic, possibly due to the entrainment of contaminants into the bed of the stream promoted by the outflow of rainwaters.

Vincent-Hubert et al., [30] investigated the main genotoxic contamination sources in the Seine River estuary, located in a region impacted by different sources (dye industry, petrochemistry, paper and pulp industry). The genotoxicity was analyzed by the comet assay with human hepatoma cells (HepG2), after exposure to the water and sediments extracts collected in the estuary. The authors recorded that 12 of the 14 samples presented genotoxic potential when compared to the negative control, indicating that the micro-pollutants retained in the extract have genotoxic characteristics in human hepatocytes.

Ye et al., [31] studied the genotoxicity of 16 water sources in China, by the SOS/*umu* test (*Salmonella typhimurium*), which estimates damages in the DNA and by the MN test (HepG2 cells) by flow cytometry, which estimates damages in the chromosomes. The authors concluded that the combination of these assays could be effective to the analysis of the genotoxicity of complex mixtures. Zeng et al., [32] also compared the genotoxic potential using these two assays (SOS/*umu* test and MN test) in extracts of chlorinated drinking water. The authors report that all the samples presented at least one type of damage and highlighted the importance of combining different bioassays to assess the genotoxicity. Kolkman et al., [33] collected water samples of the Lekkanaal at Nieuwegein (hydrologically connected to the Rhine River) to evaluate the genotoxic potential of compounds present in these samples by the comet assay in HepG2 cells. The authors carried out a comparison between the exposure period (3 and 24 hours) and the genotoxic effect and observed that the DNA damages were lower after the 24 hours exposure. The authors suggested that the cell responses were due to the direct genotoxic action of the contaminants in the period of 3 hours and the possible repair of DNA damage, observed after 24 hours.

Llorente et al., [14] collected effluent samples of seven Sewage Treatment Stations (STS) that receive pollutants of different origins. The micronucleus test with RTG2 cells (rainbow trout gonad cells) was carried out to evaluate the genotoxicity of organic extracts obtained from the samples. The authors compared the genotoxic effects of two different concentrations of 11 samples collected in the seven effluents, totalling 22 extracts. The results showed that only two samples presented genotoxic effects for both concentrations; one presented higher genotoxicity index for the highest concentration (5 g); and the 8 remaining for the lowest concentration (2 g). The difference in the responses of the extracts could be explained by the chemical composition and extraction procedure.

Water quality of some rivers has improved over the years; however, the sediments still reflect the historical contamination by organic pollutants and metals that these water bodies suffered along the years. Sediments are considered final receptors of several chemical products discharged into the environment by anthropic activities [34,35]. However, the evaluation of the toxic potential of sediments by biological assays is difficult to be performed since the complex mixtures can present different responses according to the association of the substances present.

Boettcher et al., [36] evaluated the genotoxic potential of sediment samples of 10 sites in the Danube River by DNA damages analyses observed by the comet assay and micronucleus test in RTL-W1 cells. The authors observed that 8 of the 10 samples studies presented a significative increase of DNA fragmentation (comet assay), indicating the presence of genotoxic contaminants in the samples. Moreover, all samples tested presented a significant difference in the MN frequency, when compared with the negative control.

Lagos lagoon, in Nigeria, is located in a region that receives intense pollution load of urban and industrial origins. AMAEZE et al., [37] assessed the genotoxicity of 11 sites of this lagoon by the conventional comet assay and a modified comet assay with Formamidopyrimidine DNA Glycosylase (FPG) and endonuclease III enzymes with RTGW1 cells (rainbow trout) maintained in culture. The compounds present in the sediments collected in this lake were extracted with organic solvents. Two concentrations for each collections site were tested (eQsed 7 mg/mL and 3.5 mg/L). The comet assay showed that all extracts at the concentration of 7 mg/L induced significant DNA damages. Furthermore, it was possible to observe a significant reduction in the DNA damages in 5 extracts. The results obtained in the DNA oxidative damage assay, assessed by the modified comet assay, showed toxicity results similar to the conventional comet assay.

Perovic et al., [38] studied by the comet assay in rainbow trout liver cells (RTL-W1), the possible genotoxic effects of the sediments of Skandar Lake. The authors tested five concentrations of each extract obtained from the sediment samples and observed a positive dose-response. From the results found, the authors concluded that the comet assay is a sensitive method to detect the genotoxic potential of the samples studied.

Berre Lagoon is located in the most industrialized region of France. The Palun marshes (Berre lagoon, France) suffer impacts of industrial, rural and urban activities on two runnels. Di Giorgio et al., [39] performed the MN test in CHOK1 cell with and without S9 mix to evaluate the genotoxic effects of sediment samples from this location. All the sediments pointed to a genotoxic effect with and without S9 mix, with exception of one of the samples collected that did not present significant response for the MN test without S9 mix. The authors concluded that several organic compounds confer a worrying genotoxic risk contamination to this region. Another study conducted in the Berre Lagoon compared the genotoxic properties of 4 sediment samples, obtained by different extraction methods, by the comet assay and micronucleus test with CHOK1 cells with and without the S9 mix. The non-polar extracts of three sediment samples induced significative DNA damages, exclusively in the presence of S9 mix. All polar extracts indicated an increase in the DNA damage and one of the samples presented contaminants dependent on metabolization and three samples had contaminants with direct action and dependent on metabolization. Thus, the authors stressed the importance of using appropriate solvents to assess, with reliability, the genotoxic danger of aquatic sediments [40]. Rigaud et al., [41] also assessed the genotoxic risk of Berre Lagoon in four sites with different contamination levels. The authors investigate the presence of trace metals with the induction of DNA damages. The results obtained by the comet assay and MN test in CHO-K1 cells did not show correlation between the content of metals and genotoxic potential, suggesting that the genotoxicity of the samples may be related with other compounds that were not detected by chemical analyses.

Marine sediments accumulate numerous anthropogenic contaminants, which can affect the quality of these ecosystems and, consequently, the organisms exposed, such as, for example benthonic organisms. Yang et al., [42] evaluated the genotoxicity of marine sediments of three coastal zones of Qingdao (China), which present different contamination levels. The comet assay in cells derived from gills of *Paralichthys olivaceus* (FG cells) indicated that all raw extracts caused DNA damage in the cells exposed. The authors believe that this genotoxicity of the samples is related with the Polycyclic Aromatic Hydrocarbons (PAH) concentrations present in them. Marine sediment samples from the Kvarner Bay (Croatia), studied by the comet assay in PLHC1 cell (hepatocellular carcinoma of *Poeciliopsis lucida*), induced dose-response genotoxic effects and the location considered the most contaminated presented a high index of DNA damage [8].

Schnell et al., [35] studied a coastal region in the North of Spain that is influenced by portuary, industrial and urban activities. Samples of 10 sites of the estuary and coastal areas were collected to estimate the genotoxic potential of this region. The PLHC-1 cell line was exposed to the sediment extracts to evaluate their genotoxicity by the MN test. The authors concluded that the samples affected by industries, close to the port and to urban discharge presented significant MN indices and suggested that the lack of treatment of the products discharged into the environment jeopardize the environmental health.

Pinto et al., [43] studied, using the comet assay and MN test, the contamination of estuarine sediments (Sado, SW Portugal) in HepG2 cells exposed to samples collected in two areas contaminated with mixtures of organic and inorganic substances. The sediment extracts collected in the industrialized region induced MN and DNA fragmentation in the cells, however the samples collected in the rural area presented the highest rates of DNA oxidative damages. The authors classified the estuary as moderately contaminated but considered the sediments of the industrial area as carriers of contaminants capable of inducing permanent damages and potentially mutagenic. Another evaluation performed by the same authors compared the genotoxic effects of sediment extracts of Mira Estuary (SW Portugal) using polar and non-polar solvents. The results indicated a higher genotoxicity in the cells treated with non-polar solvent extracts. Thus, they concluded that extracts with different solvents allow identifying which is the most dangerous set of contaminants in complex environmental mixtures [44].

Sado Estuary belongs to a basin of high ecological and socio-economic importance in SW Portugal. The effects of the contaminants present in five sediment extracts with different concentrations (0 to 200 SEQ mg/mL) were assessed in HepG2 cells. The results obtained by the modified comet assay (FPG) showed significant induction of oxidative damages in the DNA both for the raw extract and for its dilutions [45].

Mining activities generates a considerable amount of residues contaminated by metals. These residues end up reaching the aquatic environment and causing serious environmental problems. Therefore, Ternjej et al., [46] investigated the genotoxicity of water and sediment samples of a region contaminated by residues from a gypsum mining by the comet assay In Channel Catfish Ovary cells (CCO). The results obtained showed a reduction in the cell viability and increase in the DNA damage. However, water and sediment collected in the river spring did not interfere in the DNA integrity of the cells.

Eco-Genotoxicity Using Fish

Fish are considered excellent bioindicators of aquatic contamination since they are organisms that explore the aquatic environment during their whole life cycle, are capable of accumulating the pollutants present in the water and respond to chemical substances similarly to higher vertebrates. As fishes can be maintained in laboratory, they are widely used in eco-genotoxicity of aquatic environments.

Ameur et al., [47] evaluated the genotoxicity of Bizerte Lagoon waters (Tunisia), located in an important economic region but with environmental pollution problems due to intense fishing activity and local agriculture. The authors collected mullet (*Mugil cephalus*) and European sea bass (*Dicentratchus labrax*) in the Bizerte Lagoon and Mediterranean Sea (region considered not polluted), whose livers were used to perform genotoxicity tests by the comet assay. Both fish species collected in the lagoon presented high levels of DNA damage when compared to the reference population (Mediterranean Sea). However, it was not observed statistically significant difference of DNA damages between the species studied. The authors correlated the increase in the DNA damage with the metabolization of contaminants in the liver.

Yazici and Şişman [48] studied the Karasu River in Turkey, which receives agricultural and industrial effluents and domestic sludge disposal. The researchers evaluated the genotoxic effects of metals potentially toxic present in that river. For this evaluation, two fish species (chub, *Leuciscus cephalus* and barb, *Capoeta capoeta)* were collected in three different sites of Karasu River. One site slightly contaminated (Dumlu), one heavily contaminated (Karasu) and one relatively not contaminated, the latter being used as control. The concentration of heavy metals (Cd, Al, As, Pb and MN) were determined in the waters collected and the micronucleus (MN) and nuclear alterations (NA) was performed in the fish with peripheral blood, gill epithelial cells and liver cells. It was observed in the fishes collected in the polluted site a significative increase in the frequencies of MN and NA when compared with the fish from the reference site. The increase in the frequencies was attributed to high levels of the metals Cd, Al, As, Pb and Mn, observed in the chemical analyses. A positive correlation was found between the NA frequencies and the heavy metals content present in the river water. The authors also state that the contaminants present in Karasu River affect not only fishes but also other aquatic organisms, which shows the need to integrate biomarkers in the monitoring of anthropogenic pollution.

Aliağa Bay is an area that receives discharges of urban sewage combined with several industries residues, including the second largest petroleum refinery of Turkey, industrial plants of paper, fertilizer, iron-steel and others. Arslan et al., [49] assessed the genotoxic effect of this contamination in fishes that inhabited this bay by analyzing cells bearing MN (MN test). Samples of peripheral blood and gills of two fish species were collected in an area considered free of pollution (clean area) and five species in the Aliağa coast (polluted area). The analysis of the MN and binucleated cells frequencies, in both cell types (peripheral blood and gills) indicated higher genotoxicity in the polluted area than in the area considered not impacted by pollutants. The authors concluded that the region studied presents contaminants with genotoxic action and, possibly, mutagenic action.

In order to evaluate the genotoxic potential of water samples collected in four different sites of the Nilufer Stream in Turkey, Summak et al., [50] determined the frequency of Micronuclei (MN) and nuclear abnormalities (NA) in blood samples of *Oreochromis niloticus* exposed in laboratorial conditions to the water samples collected. The results showed that the water samples of Nilufer Stream are contaminated with genotoxic pollutants resulted from industrial, agricultural and domestic activities of the city of Bursa.

A study conducted by Tsangaris et al., [51] in Ukrainian rivers assessed the genotoxic effects of waters of different rivers and regions using bioassays carried out with the species *Carassius gibelio.* Fishes were exposed to water samples of Dnieper River, in the region of Kiev (Dnieper-K); Dnieper River, in the region of Bortnichi (Dnieper-B); and Desna River (Desna), one of Dnieper River tributaries. Dnieper receives municipal, agro-industrial, inadequate effluents treatment discharges and contamination by radionuclides of reservoir sediments. The fishes were acclimated in laboratory conditions and the control test was performed with tap water. After exposure, blood and gill samples were collected, in which the MN test and frequency of Binucleated Cells (BC) were carried out. The researchers observed that the frequencies of MN and BC, in both tissues, were higher in the tests carried out with Dnieper-B River samples, followed by Dnieper-K River and Desna River. The authors suggest that the frequencies of MN and BC are important parameters to be used in short-term bioassays of environmental samples genotoxicity.

Danube River in Serbia receives untreated residual water of industries located along it. This impact causes negative effects on the fishes of the area. Sunjog et al., [52] detected the presence of 16 trace elements (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sr and Zn) in tissues (gills, muscle, liver and gonads) of fish collected in this river, as well as genotoxic effects in the erythrocytes of *Barbus barbus* by the comet assay. For this evaluation, fishes were collected from the Danube River and from Uvac (Special Nature Reserve), which were used as control since it receive little anthropogenic influence. After tissue analyses, higher concentrations of Sr, Mn, Fe, Ba, B, Al were observed in gills, Mo and Cu in liver and Zn in gonads. They also observed that the younger specimens presented higher concentration of Zn in the gills than older fishes. Regarding the comet assay, the three parameters analyzed (tail length, tail moment and tail intensity) presented statistically significant differences when compared to the control. The researchers point out that the metals studied do not represent the only group of contaminants in the water and that, probably, other types of contaminants contribute for the genotoxic responses observed.

Pavlica et al., [53] carried out collections of the fish species *Squalius cephalus* L in three sites of the Sava River (Croatia) in the spring and autumn of 2005 and 2006 respectively. The comet assay and MN test were performed with fish erythrocytes to evaluate the genotoxicity and mutagenicity of a location upstream the Zagreb city (site with the lowest pollution) and two other sites downstream (one moderately polluted and the other heavily polluted). Regarding the comet assay, they observed lowest genotoxic influence in the site upstream the city and highest damage in the genetic material in the two sites downstream. For the MN test, it was also observed, that the site with the lowest contamination (upstream the city) presented the lowest MN frequency. The authors consider both tests important for the assessment of contaminated sites, since the comet assay shows exposure to genotoxic contaminants and the MN test the potential mutagenic effects of the samples analyzed.

Studies conducted by Mosesso et al., [54] evaluated fishes of the species *Aphanius fasciatus* collected in the Orbetello lagoon (Tuscany, Italy), area that receives an intense pollutant load, and in the natural reserve “Saline di Tarquinia”, reference and non-polluted site. In this evaluation, the comet assay was applied on fishes collected in both sites to estimate the genotoxic potential of the Orbetello lagoon waters. A significant increase in the DNA damage levels was observed in the fishes of the lagoon, when compared with the fishes collected in the reference site, indicating the presence of pollutants with genotoxic action in the studied lagoon.

Scalon et al., [55] studied the genotoxicity of waters of Sinos River (Brazil), by the comet assay in erythrocytes of the native fish *Hyphessobrycon luetkenii*. Seasonal water samples were collected in three sites of the river and used for the genotoxicity tests with fish acquired from sites free of contamination. After exposure for a period of 48 hours, the authors observed, by the comet assay, that there was no significant difference both between the seasons and between collection sites, but the frequency of cells with DNA damage was higher in the water collected during spring in two collection sites. This suggests that in the spring, Sinos River presents a higher genotoxic potential than in the other periods. The study indicates that the river studied is contaminated with substances genotoxic for fishes.

The western region of Santa Catarina (Brazil) is impacted by residues resulted from agricultural activities, untreated domestic effluents and meat industry. Bogoni et al., [56] investigated the genotoxic effects of the waters of Engano River by the MN test in erythrocytes of *Astyanax bimaculatus*. The collections were performed in two sites along the river, in six temporal repetitions and six individuals were used per site/repetition. The negative control was carried out with fishes obtained from fish farming of recognized water quality. Only one sampling site induced genetic damages (increase in the MN frequency) in the erythrocytes of *A. bimaculatus*, when compared to the negative control. Thus, the authors inferred that the river studied could be receiving a high load of allochthonous substances.

Fuzinatto et al., [57] studied a Brazilian river (Cubatão do Sul River), which has great importance for the central region of Santa Catarina State since it is used for public supply. The authors carried out *ex situ* assays, submitting specimens of *O. niloticus,* for 24 hours, to water samples of 4 distinct sites of the river collected since the beginning of spring 2010 until winter 2011. The MN test was performed with peripheral blood samples of the fishes. Almost all samples presented significant differences in relation to the negative control, except for two samples collected in the winter. Moreover, it was possible to observe that the highest MN frequency occurred during summer for all the collection sites. This period is marked by high rainfall, which ends up increasing the leaching of chemical products used in the local agriculture. The authors concluded that the complex environmental mixtures of Cubatão do Sul River, resulted from agricultural, urban and industrial activities, confer to the water of this river a genotoxic potential, capable of inducing MN in fishes. The authors also alert to the need of evaluating rivers impacted by diversified human activities for a better understanding of the possible effects of complex mixtures on natural ecosystems, mainly aquatic.

Bühler et al., [58] conducted the MN test with peripheral blood of a Brazilian native fish (*H. luetkenii*), to evaluate the genotoxicity of two reservoirs of the Canela National Forest in southern Brazil: Reservoir 1, supplied by drainage waters from the interior of the forest and external waters from urban and industrial areas; Reservoir 2, which receives water from small uncontaminated streams (used as reference site). The samples were collected in two different seasons (winter and spring) and it was possible to observe the effect of seasonality on the MN frequencies. From the responses obtained with the samples collected in spring, in the contaminated reservoir, it could be inferred that this site is impacted with substances that present mutagenic potential, capable of altering the integrity of the genetic material of the aquatic organisms.

Researchers assessed the genotoxic potential of water samples of São Francisco River, Paraná (PR) State - Brazil, using the MN test in *A. paranae* [59]. The experiments were performed with samples collected in 3 different sites of the river, collected seasonally, during the period of 2009 to 2010: first site, located in the city of Cascavel; second, located on the border of Cascavel and Toledo; and third in the city of Toledo. All sites receive impacts from agricultural activities. Negative control was directly obtained from fish farming, recognized by the water quality. For all the sampling sites, fishes presented MN frequencies significantly higher than the negative control, with exception of site 3, during autumn. The authors infer that the waters of São Francisco River- PR, in these regions, are impacted with contaminants potentially genotoxic, derived from residues of pesticides and untreated urban effluents, besides being unprotected, due to the lack of riparian vegetation along the river. According to the authors, this impact can cause damages to the environment and to humans.

Barbosa et al., [60] assessed the genotoxic activity of the water of Extremoz Lake, Brazilian Northeast, on the site of water capitation for public supply for the city of Natal, Rio Grande do Norte State. Blood samples were collected from fishes of the species *O. niloticus*, captured in the study area, between September 2006 and July 2007. The negative control was performed with individuals maintained in aquariums with pure water. The genotoxicity was evaluated by the comet assay and MN test, which indicated significant alterations of DNA damage in the two periods studied. However, it was not observed increase in the indices of erythrocytes with micronuclei in *O. niloticus*. The authors inferred that DNA breaks, observed in the erythrocytes, were repaired and did not convert the initial damage to micronuclei.

Osório et al., [61] studied the water quality of a Brazilian river (Tubarão River, Santa Catarina State/Brazil), which receives impacts of coal mining activities; by the comet assay in *Geophagus brasiliensis*. Fishes were collected in the summer of 2009 and winter of 2010, in four locations in the river. The authors observed significant DNA damages in the fishes in all samples collected during winter, when compared with summer. In addition to the history of this river be affected by coal mining, it is also influenced by urban and rural areas, which can also induce great damage to the organisms exposed.

Omar e et al., [62] evaluated three aquatic environments in Egypt to determine the genotoxic effects of these waters in wild and captive populations of *O. niloticus* and *M.*

caphalus. The authors used fishes collected in a reference site (fish farm irrigated with Nile River water); southeast of the Qaroun Lake (collection of the wild species) and in fish farms south of Qarou Lake (that receives agricultural influence). In order to evaluate the genotoxicity the authors performed the MN test in erythrocytes; which was also used to record the frequency of Nuclear Alterations (NA), in addition, the DNA fragmentation assay in gills and liver samples were also used. Both for the MN and NA, the researchers observed a significant increase for both fish species collected southeast of the Qaroun Lake and in the fish farms in the south. Regarding the genomic DNA fragmentation, the samples of both fish species, collected in the two study sites, presented internucleosomal fragmentation (ladder pattern) mixed with smear-like pattern (these patterns are generally considered molecular markers of apoptosis and necrosis). Thus, this study shows the importance of the use of the MN test and DNA fragmentation technique to monitor impacts of pollutants with genotoxic potential to aquatic environments.

Nagpure et al., [63] evaluated the impact that tannery effluents cause in the Ganga River (India), by genotoxicity tests (comet assay and MN test) performed with the test organism *Channa punctatus*. For this assessment, the authors collected fishes in three sites: upstream the city of Kanpur (1); a site that receives tannery effluents (2); and a site 300 m downstream the effluents discharge (3). Negative control was carried out with fishes obtained from fish farm. The specimens were collected in winter 2009, spring 2010 and summer 2010. Results showed a significant increase in the MN frequency for the fishes collected in sites 2 and 3. For the comet assay, the DNA damages observed in the erythrocytes and gills were higher in site 2 than in sites 1 and 3. With these results, the authors demonstrated that the genotoxicity of the waters collected in Ganga River must be associated with impacts caused by tannery activities in the region and the comet assay and MN test are important for the monitoring of contaminated waters. The authors also point out that the results obtained can be useful for the elaboration of remediation strategies and conservation of impacted areas.

Anambra River is considered an important hydric resource of the South Central region of Nigeria. This river has been the target of several pollutant sources, in which stands out the influence of a petroleum industry. Obiakor et al., [64] investigated the genotoxicity of this river waters by the MN test in erythrocytes, gills and kidneys of fishes collected in this location. Fish collections were performed in five sites along the river, in two distinct climatic periods (dry and rainy), the species used were *Clarias Synodontis* and *Tilapia nilotica*. In this study it was observed a high frequency of MN in the fishes collected in the dry season due to an increase of the contaminants concentration present in the water. It can be concluded that climatic conditions are important factors to be considered in environmental evaluations since they can affect the water quality of rivers.

Tsangaris et al., [65] analyzed the genotoxicity of a site that receives effluents of industrial and portuary activities and a reference site, both located in the Saronikos Gulf, in Greece. The assays were performed with the bioindicator grey mullet (*M. cephalus*). Three samplings were conducted: October 2006, May 2007 and October 2007, in order to analyze the influence of seasonality on the responses of the bioindicators. In this study the authors evaluated, in peripheral blood and gills, the frequency of MN to estimate the chromosome damages and the frequency of Binucleated Cells (BC) to estimate damages in cell division. The researchers observed an induction of MN and BC in the fishes of the polluted site, when compared to the reference site, for all the sampling periods. However, the results with erythrocytes only showed statistically significant differences of MN frequency (for all the periods studied) and BC frequency in May 2007 and October 2007. Now, for the gill cells, it was observed a statistically significant difference for the BC frequency in October 2007. Thus, the authors concluded that the waters of the studied sites are impacted by genotoxic contaminants and the bioindicator used in the study was effective to assess the pollution impacts of complex contaminants in coastal environments.

Eco-Genotoxicity Using Other Organisms

Besides the test systems described above there is a series of other organisms used in the evaluation of the eco-genotoxicity of aquatic systems. In this section it will be addressed the most recent studies using some of these organisms.

Bacteria

Ames test consists in the use of *S. typhimurium* strains that are auxotrophic mutants (requires histidine to grow but are not able to synthesize this amino acid) sensitive to substances capable of inducing different types of mutation. When these strains are exposed to a mutagenic agent, they revert their auxotrophic character and start to synthesize histidine and form colonies in culture medium without this amino acid. The mutagenic action of a certain compound or sample can be estimated by the number of revertant colonies in the culture plate. Another test used to evaluate the genotoxicity of chemicals is the umuC colourimetric test, which also uses a genetically modified strain of *S. typhimurium*. In this case, when the bacterial cell is exposed to genotoxic agents, there is the induction of the activity of the umuC gene, which makes part of the SOS repair system of the prokaryote, in order to prevent DNA damage. A reporter gene (galactosidase) couples to umuC, inducing a colourimetric reaction that indicates the presence of genotoxic agents in the sample.

Hafner et al., [66] used these two test systems with the bacteria *S. typhimurium* to evaluate the toxicity and genotoxicity of German river sediments. The authors observed that the Ames test was more sensitive to assess the presence of genotoxic compounds in the samples than the umuC test. The Ames test was also used by Vincent-Hubert et al., [30] to evaluate the genotoxicity of different compartments of the Seine River (France). The authors chose this river because it is very impacted by different mutagenic compounds, previously found in the surface water and sediments of this region. Moreover, the particulate matter of the surface water is a very dynamic medium and can contribute for the contamination of the organisms. After exposure to the different samples, it was observed that from the 14 of the samples collected, 11 were genotoxic for the Ames test. From all the samples, the ones derived from effluents of chemical dye industry were the ones that induced the highest number of revertants, followed by effluents of petrochemical industry and pulp and paper mill. It was also observed genotoxic potential of the sediment of the studied site.

Siddiqui et al., [67] assessed the genotoxicity of surface and groundwater of the North of India, using three assays with mutants of *Escherichia coli* deficient in the DNA repair system (plasmid nicking assay, *E.coli* K-12 mutants survival pattern and λ prophages induction test) and observed that all the assays were able to detect the genotoxicity of the samples analyzed, showing the sensitivity of the tests. Due to this sensitivity, the authors suggest that these tests should be used as preliminary assays to evaluate the effects of substances and/or compounds.

Invertebrates

Eco-genotoxicology studies performed by Lacaze et al., [68] evaluated the efficiency of the comet assay in three cellular types of the amphipod *Gammarus fossarum* (spermatozoa, haemocytes and oocytes). In this analysis, two assays were performed, the first with *in situ* exposure in the upstream and downstream of three effluents treatment station and the second with exposure to waters collected in Riou Mort Bay (France), river impacted by polymetallic pollution derived from several industrial activities. For the first assay, it was observed high damages caused by the exposure to the downstream samples of the effluents, while the damages caused to exposure to upstream were low and the spermatozoa were the most sensitive cells for this evaluation. For the second assay, it was observed that the amphipod spermatozoa exposed to the environmental control presented low damage rates, while when exposed to the samples collected in the polluted sites, the damages were high and the values increased as the exposure period to the samples increased. The authors concluded that the comet assay with this amphipod can be used in the evaluation of the eco-genotoxicity and suggest that spermatozoa are, for this species, the most indicate cell type for this type of evaluation.

Davolos et al., [69] tested the use of the amphipod *Gammarus elvirae* as test organism in the evaluation of the genotoxicity of rivers of the Lazio region (Italy), contaminated with arsenic due to geological processes. The authors collected specimens of the amphipod in several locations of the Lazio region and observed, by the comet assay with haemocytes, that the damage in the DNA of the specimens that were living downstream the river were higher than of those living upstream, this is due to the fact that the quality of the river deteriorates along the river course, probably because there is discharge of untreated urban and agricultural effluents into this water body. The authors conclude that the comet assay with *G. elvirae* can be used to assess the genotoxicity of fresh water contaminated with arsenic.

Rocha et al., [70] also used the comet assay with haemocytes of amphipods to investigate the genotoxicity of shallow waters near the Antarctic Station “Comandante Ferraz”. The authors collected specimens of *Gondogeneia antarctica* in locations near the fuel storage tanks and sewage treatment outflow and in sites far from the “Comandante Ferraz” Station. Five different biomonitoring were performed and the authors observed that in two of them the waters collected near the station presented genotoxic effects, indicating contamination of these places and that the human presence in Antarctic ecosystems can cause environmental impacts in these ecosystems.

Aborgiba et al., [13] used the comet assay in haemocytes and coelomocytes of the oligochaete *Branchiura sowerbyi* and in the haemolymph of the mussel *Unio tumidis* to evaluate the effect of floodings in the Sava River (city of Obrenovac, Serbia) on the DNA damage frequencies. The studied area is influenced by a coal processing power plant and domestic effluents of the Obrenovac city. Assays were performed during different months of the year, comprising different rainfall periods. It was verified that during the flooding period the concentration of heavy metals was very high and concentrations of nitrate, ammonia and phosphate decreased. The comet assay results showed that floods had a significant impact in the water quality, decreasing the amount of pollutants present in urban effluents but simultaneously introducing contaminants of sites near ash disposal, which had diverse effects on the DNA damage of the organisms exposed, thus, the authors state that depending on the test organism chosen they can be exposed to different stressors due to their habits and this can influence in the responses to genotoxic agents.

Koralević et al., [71] used the comet assay in haemocytes of the mussel *Sinanodonta woodiana* to evaluate the impacts of domestic effluents in the Danube River (Serbia). For this study, the authors collected specimens of *S. woodiana* in six sites of the river and brought them to the laboratory to perform the comet assay. It was observed that the DNA damages in the organisms collected in the sampled sites were higher than the observed for the control group. From these data, the authors suggest that these damages could be mainly caused by the release of untreated domestic effluents and the presence of agricultural activities in this region can contribute to the organic pollution of this region.

Al-Shami et al., [72] evaluated the genotoxicity of river sediments of a region located in north peninsular Malaysia, using the comet assay applied in larvae of the chironomid *Chironomus kiiensis*. The authors collected sediments of the Selama River (control sediment) and of the rivers Kilang Ubi and Permatang Rawa (considered contaminated) and exposed the chironomid larvae to these samples. From the results, the authors observed that the nucleoids of the organisms exposed to the contaminated sediments presented higher DNA damage and this damage increased as the exposure period increased. The authors also observed that the possible inductors of these genotoxicity would be heavy metals (Zn, Mn and Cu) combined with the synergistic factor of other compounds present in the sediment. Moreover, the authors concluded that, for this study, sediments contaminated by industrial effluents were able to induce more damages than urban pollution.

Algae and Higher Plants

Li et al., [73] used the microalga *Euglena gracilis* to evaluate the genotoxicity of the Taihu Lake in China, which is used as drinking water source. In this study, the authors carried out assays with organic extracts obtained from water samples collected in four different seasons of the year and the microalgae were exposed to medium containing these organic extracts. The results showed a concentration-dependent response, demonstrating that the comet assay, as well as the use of the microalga can be an alternative to assess the genotoxic effects of organic compounds.

Radić et al., [74] evaluated toxic and genotoxic parameters in the aquatic plant *Lemna minor* exposed to surface waters of different sites of the Sava River (Croatia). In order to assess the genotoxic effects, the authors used the comet assay and they observed that the plant presented high sensitivity, indicating that this species can be used in the genotoxicity evaluation of pollutants present in surface waters and in effluents.

Barbosa et al., [60] used the test organism *Allium cepa* (onion) to evaluate the genotoxic potential of a lake (Extremoz Lake) located in the city of Natal, Brazil that is used as source of public water supply and recreation. The authors exposed onion bulbs to the collected waters and observed that all of them presented significant altered frequencies of chromosome aberrations, as well as the mitotic index, when compared to the negative control. However, significant frequencies of micronuclei were not observed. Furthermore, the authors detected by chemical analyses that these waters presented high levels of heavy metals (Cd, Pb, Zn, Cr, Cu, Ni and Mn) and correlated the effects found in the organism test used as probably caused by the action of these heavy metals and other contaminants present in the water of the lake.

Lacerda et al., [75] observed by physic-chemical parameters that waters samples collected in the sub-basin of High Tietê (Brazil) were not in accordance with the limits established by the legislation and the values of thermotolerant coliforms were above the limit proposed, indicating contamination by organic pollutants probably derived from domestic effluents. Thus, the authors evaluated, by assays performed with the test organism *A. cepa*, the quality of the water and sediment of small body courses of this hydrographic basin, which are used as irrigation source for leafy vegetables plantation. The tests carried out with roots of the onion bulbs exposed to the sediment samples presented high frequencies of chromosome aberrations and micronucleus. Now, the bulbs exposed to water samples presented high incidence of chromosome aberrations but not micronuclei, which, according to the authors, are responses that are due to the fact that sediments tend to accumulate pollutants. The authors conclude that the integrity of the hydric system studied is compromised due to contamination by organic pollutants.

Onion bulbs were also used by Geraskin et al., [76] to evaluate the quality of water and sediments of hydric bodies located close to mining areas of Upper Silesia (Poland). After phyisico-chemical analyses, the authors observed that all samples presented high levels of radioisotopes and several other chemical elements. For all the samples (water and sediment) there was induction of genotoxicity.

Biachi et al., [77] used seeds of *A. cepa,* and not bulbs, to evaluate the genotoxicity of Monjolinho River (São Paulo State-Brazil). The authors exposed the seeds to different river samples, collected in different seasons of the year. The samples collected in summer (season characterized by high temperatures and high rainfall index), spring and autumn induced genotoxic damages (chromosome aberrations and MN), while during winter (dry season and with low temperatures) these parameters were not statistically significant in relation to the negative control. The authors also carried out a recovery treatment (exposure of the meristems to ultra-pure water, after exposure to the water river samples) and observed that the meristems exposed to some samples collected during summer and spring were not able to recover from the genotoxic damages. The authors suggest that these observed effects could be resulted from the high concentration effect of some metals (Pb, Ni and Cu), besides other contaminants of domestic, industrial and agricultural origins, since this river receives domestic and industrial effluents and there is agricultural activity along the river course. Moreover, the authors also suggest that there is a relationship between seasonality/ temperature with the effects observed.

Kwasniewska et al., [78] compared the sensitivity of two bioassays with plants (MN test in BNL 02 and 4430 clones of *Tradescantia* and chromosome aberration test in *Crepis capillaris*) to assess two rivers of Poland (Rawa River, which suffers high impact by industries and Goczalkowiee River, which functions as drinking water reservoir). The authors observed that the waters of the two rivers induced genotoxicity in the organisms used in the bioassays. Besides these assays, it was also performed the TUNEL test (TdT-mediated dUTP nick ending labelling) in roots of *C. capillaris,* to assess the frequency of nuclei with DNA fragmentation. All water samples caused DNA fragmentation in the roots of the organism tested. The genotoxicity of the Rawa River was higher than the observed for the Goczalkowiee River. The authors justified these significative genotoxicity results of the Rawa River because this river is impacted by industries that can contain high levels of nitrites, nitrates and other nitrogen compounds and the genotoxicity of the Goczalkowice due to the high concentration of nitrites.

Yu et al., [79] studied the genotoxicity of waters of the surface microlayer and subsurface of the Guanzhou section of the Pearl River (China) by the micronucleus test with *Vicia faba.* In this study, the authors performed collections during the months of January to December 2008 and observed that all samples presented values of total nitrogen, total phosphorus and chemical oxygen demand above the allowed by the legislation, also, the authors observed that all samples induced genotoxicity and they attributed this effect to eutrophication, pollution by nitrogen and phosphorus and other organic pollutants.

Genotoxicity studies with surface waters of the Xi'an City in China using roots of *V. faba,* were conducted by Ma et al., [80]. In this study, the authors collected and analyzed water samples from three urban streams (Chanhe, Zaohe ane Weihe rivers), two urban lakes (Xingqinghu and Nanhu lakes) and effluents from wastewater treatment plants. Extracts obtained by Solid Phase Extraction (SPE extraction), in order to concentrate organic compounds, were used in the assays with *V. faba* roots. The roots were exposed to these extracts for 12 hours and later placed in milli-Q water for 24 hours for a recovery treatment. The highest genotoxicity values (number of micronuclei) were observed for the Zaohe River, followed by Canhe River, effluents, Weihe River, Zingiqinghu Lake and Nanhu Lake. The results, according to the authors, can be explained by the origin of the contaminants present in the water samples, as follows: Zaohe River receives urban runoff and untreated industrial effluents; Canhe River receives rural runoff and treated domestic effluents and the effluents can contain residual organic substances.

Amphibians

Ossana and Salivián [81] used tadpoles of *Lithobates casteianus* to evaluate the genotoxicity of surface waters of the Luján River (Argentina). The authors collected waters samples from this river, exposed the specimens to them and, later analysed the MN frequency in peripheral blood cells. It was observed, by chemical analyses, that this river is highly impacted (receives domestic, urban and agricultural effluents) and its water presented genotoxic potential since induced MN in the bioindicators used in the assays.

Final considerations

We would like to point out that in most of the studies above cited, the authors consider the endpoint micronucleus as indicative of genotoxicity. However, there is a certain controversy regarding this endpoint since many other authors consider it as indicative of mutagenicity. The term mutagenicity is used when the DNA damage cannot be repaired anymore, but as all mutagenic damage is considered genotoxic, this endpoint can be considered as genotoxic parameter, thus it can be used in eco-genotoxicity studies.

With the urban, agricultural and industrial growth, the aquatic ecosystems have suffered severe impacts by environmental pollutants. Thus, hydric resources must be constantly monitored, since many of them are used for public water supply and, according to epidemiological data, consumption of low quality water can represent a risk to the associated biota, since it can endanger the life quality and survival of these organisms. Several are the organisms and tests used to evaluate the eco-genotoxicity of waters and they are considered excellent tools for this kind of assessment, therefore, these assays should be included in the test battery used to evaluate the water quality of these ecosystems, furthermore, they can help in making decisions regarding the relevant legislation. Studies of this nature are also of extreme importance to monitor the ecosystem health and, consequently, for the well-being of all the organisms exposed to it, including man. Moreover, it is recommended the use of more than one organism in an environmental assessment, since, expanding the response for a greater diversity of organisms it is possible to better characterize better the biological responses associated to the stress caused by a xenobiont.

Due to the major environmental impacts that the anthropogenic activities have been causing into the environment and to the fact that water is a mineral resource essential to life, the assessment of the quality of hydric resources by eco-genotoxicity assays are increasingly needed. In this perspective, it should be also widely encouraged the development of new techniques and use of new organisms in order to help a better comprehension of the xenobiont effects on the biological medium so that we can estimate the possible impacts that our activities can cause on the environment.

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