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Chapter 4

LENTIC HABITATS AS STUDY MODELS FOR ASSESSING AQUATIC CONTAMINATION

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ABSTRACT

Given water scarcity and climate change caused by human activities, the degradation of water resources, such as lentic habitats, is currently a major concern worldwide because of the direct or indirect damage it can cause to the health and survival of exposed organisms. One contributing factor to water quality alteration is the discharge of effluents, mainly domestic and industrial, containing several substances potentially toxic to the aquatic environment. This review focuses on the aquatic contamination of lentic habitats, due to their importance and lack of information when compared to studies on rivers. The present paper presents information on the characterization, the legislation for the classification of these habitats in Brazil, their main contaminants and the effects on organisms, in addition to the case study of a lake in the state of São Paulo.

Keywords: Lakes, aquatic ecotoxicology, biomonitoring, genotoxicity

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1. THE CONTAMINATION OF LENTIC WATERS

Water is a priceless natural resource. Approximately 75% of the Earth's surface is covered by water, an environment with a vast number and diversity of organisms (Attemborough, 1984). Water is instrumental for the maintenance of biological and chemical cycles, and ecosystem balance (Zagatto and Bertoletti, 2006; Torres, 2008). It also plays a fundamental role in life propagation and maintenance, as the major component of organisms. More than half of plant and animal species depend on the aquatic environment to live (Ball, 1999).

Human activities have altered water quality and quantity with negative consequences to life on Earth (Smol, 2008). Because of the fast population and industrial growth in the last decades, an increase in environmental pollution has been observed. The indiscriminate use of natural resources has caused serious contamination problems in aquatic ecosystems, mainly by organic and inorganic compounds from domestic, commercial, and industrial activities (Thomé, 2007). As a result, environmental pollution and the implementation of effective measures to diagnose, monitor, and minimize it have become great concerns to the population and governmental agencies (Thomé, 2007).

Aquatic ecosystems are usually divided into two types: Marine and freshwater. The latter usually represents less than 1% of the Earth's surface and is subdivided into lotic, lentic, and swamps. Lotic habitats are characterized by water flowing quickly and unidirectionally, as in rivers, and streams. Swamps are marshes sometimes covered by water with a large diversity of plants and animals. Lentic habitats, the focus of this review, include all still water habitats, such as lakes, ponds, wells, and reservoirs (Alexander and Rodhes, 1999).

According to Abraão (2006), lentic habitats comprise rain puddles, ponds, and large lakes. These habitats have peculiar characteristics regarding the diversity of biological species. Among plants and animals that live in this habitat are microscopic algae that form the phytoplankton, higher plants normally rooted on the bottom or floating, the zooplankton comprised of protozoa, small crustaceans, and mollusks, and fish, birds, and some mammals. When they die, they accumulate on the bottom of the lake/pond and are transformed by decomposers (bacteria and fungi).

These habitats have several distinct uses, such as water supply, irrigation, aquaculture, transportation, energy production, among others. However, because of water resources mismanagement, currently deal with serious socio-environmental issues (Buss et al., 2003; Fontanetti et al., 2012).

Thousands of chemical compounds have been introduced in the environment since the early 90s, contaminating the air, soil, water, and biota. Among the most serious problems affecting the water quality of rivers and lakes are: Inadequate treatment of domestic sewage and control of industrial effluents. The latter unquestionably represents the largest portion of chemical compounds discharged in water bodies. Water quality is also affected by the loss and destruction of catchment basins, improper location of industrial plants, and deforestation (Vega et al., 1996; Moraes and Jordão, 2002; Zagatto and Bertoletti, 2006).

Vast quantities of countless xenobiotics are discharged in aquatic habitats with serious consequences to plants, animals, and even man. The physiological effects of environmental contaminants on organisms depend on their bioavailability, toxicity, concentration, duration of exposure, and species sensitivity (Fox, 2001). Toxic substances, especially pesticides and metals, constitute a threat to freshwater ecosystems (MEA, 2005). They can enter aquatic ecosystems through several pathways, such as domestic and industrial effluent discharge, crop dusting, or leaching (Niyogi et al., 2002; Sierra and Gomes, 2010), and affect aquatic communities (Liess et al., 2008). Several industries, as a result of their production process, have discharged large quantities of toxic metals in the environment, directly affecting the health of plants, animals, and humans.

Rahmani et al. (2010) reported that the use of toxic metals in the last decades resulted in an increase in metal compounds influx to aquatic ecosystems. The main concern regarding the presence of toxic metals in water bodies is their toxicity and low biodegradability. The most serious problems caused by the presence of toxic metals in aquatic habitats are brain and kidney damage, liver diseases, bone lesions (Repo et al., 2011), lung, nose and bone cancers, discomfort and weakness, headaches, dizziness, and breathing problems (Rahmani et al., 2010).

Pesticides can reach aquatic habitats through intentional application, drift, and surface drainage from treated areas (Edwards, 1973). Their excessive use in pest control represents serious consequences to ecosystems, with toxic effects on plants and animals. Several pesticides are not easily biodegradable. In addition to environmental contamination, residues can reach man through the food chain and cause health problems (Tisler et al., 2009).

Polycyclic aromatic hydrocarbons (PAH) are also found and can remain in aquatic habitats for long periods of time. This class of contaminants has a high permanence in the environment, is resistant to metabolic decomposition as they are lipophilic, and can bioaccumulate and/or biomagnify in the food chain.

Another source of contamination of aquatic habitats is PPCPs (pharmaceuticals and personal care products). These compounds have been found in high concentrations in large lakes (Ferguson et al., 2013). In addition, photodegradation and other reactions of these compounds can occur more rapidly in lentic systems, due to the extensive water surface area (Ferguson et al., 2013). Humans and other vertebrate species, by consuming fish from these habitats, can be exposed to toxic agents (Fox, 2001), as well as other compounds such as polychlorinated biphenyls (PCBs) and methylmercury (Castoldi et al., 2008). Thus, risk-benefit analysis of lake fish for human consumption is important (Turyk et al., 2012).

In Brazil, unplanned urban growth resulted in inadequate infrastructure to serve the population, especially the sewage and solid waste collection and treatment, which have been disposed into the environment (Thomé, 2007). With climate changes, longer droughts, and low rainfall levels, a gradual increase in water use is expected in the next decades (Arnell, 1999; Beniston et al., 2007; IPCC, 2007). Therefore, aquatic contamination, especially in lentic habitats, is a contemporary issue, due to human proximity and these habitats and their different uses: Fishing, water supply, domestic and industrial effluents discharge, among others. Because of still or slow moving water, lentic habitats confine pollutants, concentrating them.

This review reports on aquatic contamination, focusing on lentic habitats, due to their importance and information lack when compared to studies conducted on rivers. It presents information on the characterization and legislation for the classification of these habitats in Brazil, their main contaminants and the effects on organisms, as well as the case study of a lake in São Paulo state, Brazil.

1.1. Characterization of Lentic Habitats

1.1.1. Lentic Habitats As Study Models

According to Esteves (1998), lakes are water bodies without direct communication with the sea. Lake waters usually have low levels of dissolved ions compared to ocean waters, except for lakes in arid region or subjected to long droughts. In these conditions, the intense evaporation is not compensated by precipitation and the levels of dissolved salts can be several times higher than that of sea water.

Lakes are not permanent elements of the Earth's landscape. They are short-term phenomena in the geological scale, as they appear and disappear throughout time. Their disappearance depends on many factors. The most important one is associated with the lake's own metabolism, such as organic matter accumulation at the bottom and sediments deposition transported by affluents (Esteves, 1998). Depending on the hydraulic characteristics of the system, they can have a broad limnological instability. Their compartments are divided in: Littoral zone, limnetic or pelagic zone, profundal zone, and water-air interface. This classification is artificial, as these compartments are not isolated within the aquatic ecosystem and are in constant interaction through matter and energy exchanges (Esteves, 1998).

Although in Brazil no areas are associated with the formation of large lacustrine systems, such as those in Europe, with *lake districts*, Brazilian lakes, including several ponds, can be divided into five distinct groups:

1) *Amazonian Lakes:* With a distinction between floodplain lakes and those on dry land.
2) *Pantanal Lakes in the state of Mato-Grosso:* Very shallow freshwater lakes, regionally termed bays, that periodically (during river floods) connect with rivers and salt lakes, which are usually not reached by floods and that remain isolated, accumulating mineral salts in their waters.
3) *Coastal Lakes and Lagoons:* Extend from northeast Brazil to the Rio Grande do Sul state. They often form large ecosystems such as Patos, Mirim, and Mangueira Lagoons in Rio Grande do Sul state, and Araruama, Saquarema, and Maricá Lagoons in Rio de Janeiro state and Patos.
4) *Lakes formed along Medium and Large-sized Rivers:* Lakes formed by a natural barrier due to sediments deposition by tributaries of the larger river or through meanders erosion and sedimentation, resulting in its isolation.
5) *Artificial lakes (dams and reservoirs):* Ecosystems formed mostly by damming one or more rivers, flooding extensive areas of adjacent terrestrial ecosystems. They are found throughout Brazil and are locally known as açude, especially in Northeast, and represa, in the South and Southeast.

The formation of large urban and industrial centers, with an increasing need of water for irrigation, transportation, electricity, and leisure, makes almost all activities more dependent on water availability. Therefore, the conservation and sustainable use of aquatic ecosystems have been one of the focus of several studies on environmental monitoring, using different methodologies. The conservation of these habitats may require maintaining their natural characteristics to ensure their multiples use (Esteves, 1998; Moraes and Jordão, 2002; Christofoletti, 2008).

The ecotoxicological characteristics of lakes are difficult to be determined and interpreted. In the scientific literature, there are few studies on lakes (Nogueira et al., 1999; Grisolia and Starling, 2001; Moreira et al., 2003; Pedrozo and Rocha, 2005; Vieira et al., 2006; Moschini-Carlos et al., 2011; Medeiros et al., 2012; Pereira et al., 2012a; 2012b) when compared to those on other habitats such as rivers (Shulz and Martins-Junior, 2001; Silva et al., 2004; Matsumoto et al., 2006; Pantaleão et al., 2006; Souza and Fontanetti, 2006; Egito et al., 2007; Lemos et al., 2007; 2008; Caffetti et al., 2008; Leme and Marin-Morales, 2008; Hoshina and Marin-Morales, 2009; Adam et al., 2010; Bianchi et al., 2011, etc.).

1.2. Legislation on the Classification of Lentic Habitats

1.2.1. Physico-chemical Parameters

Water is the most important compound for the life development and maintenance. Some of its properties are peculiar when compared to those of other compounds. Its higher density in the liquid state than in solid state is crucial for terrestrial water systems. If ice did not float, lakes and rivers in cold regions would completely freeze, killing all organisms (Esteves and Santos, 2011). These and others properties are very important for the stability and circulation of tropical lakes (Tundisi and Tundisi, 2008).

Temperature is one of the main factors in the aquatic habitat, as it influences a series of physico-chemical variables. As the temperature increases, parameters such as viscosity, surface tension, compressibility, specific heat, ionization constant, and latent heat of evaporation decrease (CETESB, 2009). For aquatic life, temperature is crucial, since organisms have optimal temperatures for development, growth, and reproduction. The increase in temperature in a water body is usually caused by industrial discharge and thermal power plants (Meybeck et al., 1996). The still waters of lentic habitats make them more susceptible and sensitive to these changes. CETESB (Environmental Sanitation Technology Company) uses physical, chemical, microbiological and hydrobiological parameters to analyze water quality (Table 1). In addition to these variables, toxicological and ecotoxicological variables are also used, as well as WQI - water quality index for the protection of aquatic life and communities (Zagatto et al., 1999). This index takes into account the presence and concentration of toxicants and their effects on aquatic organisms. It also includes physico-chemical variables essential for aquatic life, pH and dissolved oxygen, which are grouped in the MVPALI - minimum variables for the preservation of aquatic life index and TSI - trophic state index.

The MVPALI is composed of two groups of variables, essential variables (pH, dissolved oxygen, and toxicity), and toxic substances (copper, zinc, lead, chromium, mercury, nickel, cadmium, surfactants, and phenols). For both groups, scores from 1 to 3 are assigned to corresponding water quality standards established by the resolution 357/2005 of the National Environmental Council (CONAMA).

The resolution 357, of March 17, 2005, presents the environmental directives for the classification of surface water bodies, and establishes the conditions and standards for effluent discharge. In chapter I, section 2, subsection IV, a lentic habitat is defined as: “Habitat with still, slow or stagnated water, such as in lakes, ponds, meanders, and tanks”. Regarding the surrounding topography, they are usually in lower areas, and thus receive organic matter and nutrients dissolved in runoff water. In these ecosystems, bacteria, algae, plants, fish, amphibians, insects, and other life forms are found. The production of organic matter in lentic bodies can be classified as oligotrophic (low production), mesotrophic (moderate production), and eutrophic (high production).

Also in this resolution, chapter II, section I, the classification of freshwater bodies is presented. According to the different classes, waters may be used for human consumption, after conventional treatment, protection of aquatic communities, primary contact recreation (swimming, water skiing, and diving), vegetables irrigation, fruit plants, and parks, gardens, sports fields, aquaculture, and fishing.

Table 1. Water quality variables of used by CETESB

Physical	Chemical		Microbiological	Hydrobiological
		Max. allowed*		
Color	Al	$0.2\ mg.L^{-1}$	Thermotolerant coliforms	Chlorophylle *a*
Series of solids	Ba	$0.7\ mg.L^{-1}$	Enterococcus	Communities
Temperature	Cd	$0.005\ mg.L^{-1}$		Phytoplankton communities
Turbidity	Pb	$0.01\ mg.L^{-1}$		Zooplankton communities
	Cu	$2\ mg.L^{-1}$		Benthic communities
	Cr	$0.05\ mg.L^{-1}$		
	Fe	$0.3\ mg.L^{-1}$		
	Hg	$0.001\ mg.L^{-1}$		
	Mn	$0.1\ mg.L^{-1}$		
	Ni	$0.07\ mg.L^{-1}$		
	Zn	$5\ mg.L^{-1}$		
	Chloride	$250\ mg.L^{-1}$		
	DDT	$2\ \mu g.L^{-1}$		
	Fluoride	$1.5\ mg.L^{-1}$		

* Maximum value allowed in drinking water (Directive 518/04 of the Ministry of Health).

1.2.2. Toxicicity, Genotoxicity and Mutagenicity

Toxicity can be defined as the ability of toxic agents to induce harmful effects on exposed organisms. Worldwide, several studies on aquatic toxicology have been conducted to evaluate the toxic effects of several environmental contaminants.

Toxicity may not simply be the result of the action of an isolated substance, but rather the interaction of various agents present in a given habitat. As a result, in 1969 the concept of Ecotoxicology was created, integrating ecological concepts. Like toxicology, aquatic ecotoxicology focuses on the processes involving the effects of contaminants on the organism and the factors influencing their fate in the environment, the identification and assessment of harmful effects, and their mechanisms of action (Zagatto, 2006).

Genotoxicity, on the other hand, focuses exclusively on the damage caused to the genetic material. Lesions induced by genotoxic agents are characterized as breaks in the genetic material, chromosome aberrations, micronucleus formation, in addition to other errors during DNA replication and gene expression (O'Brien et al., 2001). When genotoxic alterations become permanent, they may be passed on and are termed mutations (Umbuzeiro and

Roubicek, 2006). Mutations are conventionally classified as genic, when they modify the functioning of a gene, and chromosomal, when there is a reorganization of the DNA structure capable of altering the structure and/or number of the chromosomal complement (Jundi and Freitas, 2003). Thus, different contaminants can cause alterations capable of affecting not only the living biota, but also their offspring.

Genotoxicity can undoubtedly be a consequence of environmental pollution. Several tools can be used to monitor environmental pollution. However, assessing the effects of the exposure to genotoxicants on the aquatic biota is a difficult task. Potentially pollutants are frequently found as complex mixtures in the environment, which can also vary among seasons (Mitchelmore and Chipman, 1998).

1.3. Main Contaminants of Lentic Habitats and Their Effects on Organisms

There are currently various sources of contamination of aquatic habitats. The main ones are the result of human activities, such as treated or untreated sewage, residues from agricultural and cattle ranching activities, fertilizers, pesticides, industrial chemical residues, oil derived products, among others (Xagoraraki and Kuo, 2008). Rivers, lakes, and aquifers exposed to these contaminants are often near densely populated areas with a high demand for continuous supply of drinking water (Kuster et al., 2010). However, the water may be contaminated with compounds harmful to life, such as mercury, lead, cadmium, pesticides, organic toxins, and radioactive contaminants (Carr and Neary, 2008).

Metals are essential for living beings in low concentrations, as they mediate all phases of dissemination of genetic information transported in the genetic code. When present in high quantities or in incorrect places, they can induce errors in the genetic information system (Clesceri et al., 1998; Patra et al., 2004). These elements tend to be strongly associated with sediments in rivers, lakes, and reservoirs and can become available in the water due to physico-chemical factors, such as pH, reactions of oxidation-reduction and of organic matter levels, like nutrients and organic compounds (Carr and Neary, 2008).

Among metals, copper ions are known to have high affinity with the catalysis of free radicals, resulting in severe consequences during DNA repair and replication, and consequently induce genetic damage (Hartwig, 1995).

Mercury is one of the most environmentally important elements, due to its toxicity (Cai, 2003). These elements exist in the Earth's crust and reach the environment through natural or anthropogenic sources. When present in the aquatic environment, they can be converted into toxic forms, which accumulate in the food chain and become a potential hazard to the health of wildlife and humans (Hamilton, 2004; Munthe et al., 2007; Scheuhammer et al., 2007; Khokiattiwong et al., 2009).

Organic contaminants are man-made chemical substances also introduced in natural environments through the use of pesticides, industrial chemicals, and by-products of the degradation of other compounds. They are usually found in the sediment, water column, and food chain. Persistent organic pollutants (POPs) tend to remain in the environment and have wide geographic distribution. In addition, they may bioaccumulate and become hazardous to human health (Carr and Neary, 2008).

Studies using bacterial and cytogenetic bioassays revealed that human and animal feces contain mutagenic components (Stich et al., 1980; Venitt, 1982; Dion and Bruce, 1983; Venitt and Bosworth, 1983). Other studies on bacteria, however, reported that human feces can inhibit mutagenic processes, due to unsaturated fat acids (Hayatsu et al., 1981), which could mask the activity of mutagenic components in extracts of sewage effluents (Hopke et al., 1984).

1.4. Tools Available for Analyzing Aquatic Contamination

Toxicity tests with aquatic organisms are an efficient tool to evaluate possible effects of pollutants, since only the measurement of levels of chemical substances present in the aquatic environment may not be enough to assess the real consequences of contamination (Arias et al., 2007). The toxicity in the aquatic environment is evaluated through bioassays with organisms representative of the water column or of sediments (Aragão and Araújo, 2006), as bioindicators may better detect environmental imbalances (Fontanetti et al., 2012).

Among the different tools used, microscopy techniques are of special interest as they allow an in-depth perspective on morphological and physiological alterations at the tissue and cell level (Fontanetti et al., 2010). Light microscopy can be used to examine histopathological alterations caused by toxic compounds in different organs of contaminated animals (Schwaiger et al., 1997). The main technique includes hematoxylin and eosin staining for the morphological analysis, but other specific dyes can also be used to detect compounds that might aid in the investigation of physiological alterations. Electron microscopy provides a more detailed view of the cells. Scanning electron microscopy gives a tridimentional view of possible alterations in the cell surface, while transmission electron microscopy provides a bidimensional view of alterations in organelles and small cell structures (Fontanetti et al., 2010).

For the biomonitoring of environmental genotoxicity, different techniques can be used. Among them is the micronucleus test, which has been a promising methodology to monitor contamination in aquatic environments. Micronuclei are small structures of chromatin resulted from chromosome breaks or aneuploidy during cell division. They are commonly used in the detection of genetic damage caused by the exposure to mutagenic agents (Heddle et al., 1983; Grisolia and Starling, 2001). This technique can be efficiently applied to several biological systems, such as bivalves (David et al., 2008), higher plants (Yi et al., 2007; Christofoletti et al., 2013), fish (Souza and Fontanetti, 2006; Ergene et al., 2007) among others.

Like the micronucleus test, the comet assay is widely accepted by international agencies as a standardized method to evaluate DNA damage in individual cells. It has also been used in a wide variety of studies, including human and environmental biomonitoring and genotoxicity (Collins, 2004). According to Frenzilli et al. (2009), the comet assay is a tool often used in the diagnostic of genotoxicity in aquatic environments. This test is aimed at detecting DNA damage (Monteith and Vastone, 1995), single and/or double-strand breaks, as well as alkali-labile sites (Speit and Hartmanm, 1995). Although this technique can detect breaks in the genetic material of cells (Singh et al., 1988), these are considered primary lesions and possible to be repaired. Therefore they may or may not result in genetic alterations (Collins et al., 1997).

Another test used to evaluate the genotoxic potential is the Ames Test, accepted by several laboratories. The frequency of reverse mutation is measured by counting colonies that grow in the culture after exposure of cells to a potential toxicant (Umbuzeiro and Roubicek, 2006). The use of the micronucleus, comet, and Ames tests is essential for assessing genotoxicity and mutagenicity and are simple and quick techniques.

In addition, CETESB uses several tests for ecotoxicological analyses, such as the Ames test to evaluate the genotoxic potential, and bioassays with *Ceriodaphinia dubia* and *Vibrio vischeri* (Sistema Microtox®) to assess the acute and chronic toxic effects to monitor water and sediments. In the assay with *C. dubia*, the mortality of organisms is recorded for a period of 48 h (acute effect) and inhibition of reproduction, for a period of seven days (chronic effect). In assays with *V. vischeri*, bacteria are exposed to a sample during 15 minutes. In the presence of a toxicant, the luminescence of the organism decreases and the light intensity is proportional to the toxicity of the sample (CESTESB, 2009).

1.5. Biomonitoring

The use of biological parameters to evaluate water quality is based on the response of organisms to the environment they live in (Buss et al., 2003). Since rivers and lakes are subjected to several disturbances, the aquatic biota responds to these natural or anthropogenic alterations (Fontanetti et al., 2012). Biomonitoring evaluates water quality and the impact of external factors on the ecosystem, while physico-chemical analysis provides information on the actual conditions of the water body (Knie and Lopes, 2004). Therefore, biomonitoring is the systematic use of responses of organisms to evaluate changes usually caused by anthropogenic actions (Buss et al., 2003).

The use of bioindicators to monitor environmental quality as well as the health of organisms that inhabit polluted ecosystems have received much attention in recent years. Organisms of a species or group of species that reflect levels of biotic and abiotic contamination in a habitat are termed bioindicators (Fontanetti et al., 2012). These organisms present alterations that provide information on the quality of their habitat. They can accumulate compounds in concentrations higher than those considered normal or essential for their body metabolism, reducing or increasing their populations (MacFarlane et al., 2000; Hodkinson and Jackson, 2005).

Recently, bioindicators have become more commonly used to determine the abundance, bioavailability of contaminants and their harmful effects on the environment (Maia et al., 2001).

Because of still or slow moving water, the impact of anthropic activities in lentic habitats is faster and more severe. Therefore, biomonitoring of these ecosystems are extremely necessary. Several studies conducted in Latin America evaluated the lakes water quality and the impact of human actions (Figure 1).

Nogueira et al. (1999) studied the composition of planktonic algae in an artificial lake of the Chico Mendes Botanical Gardens in the state of Goiânia, Brazil. The authors focused on floristic and ecological aspects and abiotic parameters during the dry, early rainy, and rainy seasons. In the dry season, the lake exhibited meso to oligotrophic characteristics. Oligotrophic characteristics were observed early in the rainy season. Phytoplankton density was highest during the rainy season, with 18 abundant species.

Figure 1. Main studies on the water quality of lentic habitats in Latin America.

An example is the study conducted by Grisolia and Starling (2001) in Paranoá Lake, a tropical reservoir in Brasília, Brazil. The micronucleus test with fish was used to evaluate the quality of residual waters from a municipal sewage treatment plant that discharged their effluents in Paranoá Lake (Grisolia and Starling, 2001). Another study in the same lake with the MN test and the comet assay with several fish species demonstrated the importance of bioindicators to evaluate the sensitivity of aquatic organisms to different genotoxic agents that can impact lentic habitats such as lakes (Grisolia et al., 2009).

In 2003, Moreira et al. conducted several tests in the Paranoá Lake basin, among them, the granulometry of sediments of the bottom and an evaluation of the contamination level of the sediment. The authors observed that the mineralogical distribution might be associated with geology, hydrodynamic conditions, as well as anthropogenic activities. In addition, in some areas, the accumulation of sediments and enrichment of chemical elements were observed. The main sources were the discharge of effluents from a treatment plant, solid waste treatment plant, and illegal sewage dumping.

To evaluate the water quality of six lakes in Rio Grande do Sul state, Brazil, Pedrozo and Rocha (2005) used different species of zooplankton as bioindicators of aquatic pollution. The lakes analyzed were typically impacted by the discharge of organic effluents and exhibited a gradient of environmental quality, with alterations such as nutrient reduction, alkalinity, changes in BOD (biochemical oxygen demand), COD (chemical oxygen demand), and nitrogen levels, among others.

Studies by Vieira et al. (2006) reported the water quality of springs and lakes in the Dr. Fernando Costa Park (Água Branca Park) in São Paulo state, Brazil, and proposed conservation measures. After physical and chemical analysis in six different sampling sites, the authors observed that waters from springs had lower levels of dissolved oxygen and all sites evaluated had $N\text{-}NO_2$ levels above the limits (1mg/L) established by the CONAMA resolution 357/2005. However, the authors reported that the water quality of the park was adequate for ecological and landscape purposes. Unplanned urban growth without including the preservation of springs and green spaces, and the high levels of organic matter are relevant factors in the loss of water quality. Also, according to the authors, only urban planning combined with environmental education could improve the ecological and sanitary conditions of this important green space in central São Paulo City.

Barbosa et al. (2010) evaluated the genotoxic potential of the water from the Extremoz Lake, northeastern Brazil, with the chromosome aberration test and the micronucleus test using *Allium cepa*, and the micronucleus test and the comet assay with peripheral blood from tilapias (*Oreochromis ssp).* The authors also quantified the presence of heavy metals using atomic absorption spectrophotometry. The tests with *A. cepa* revealed significant alterations in the frequency of chromosomal aberrations and the mitotic index of samples exposed to the lake water, when compared to the negative control. On the other hand, only the comet assay revealed significant damage at the DNA level of *O. niloticus,* since the frequencies of micronucleated erythrocytes were not significantly different. The chemical analysis revealed the presence of metals such as Cd, Pb, Zn, Cr, Cu, Ni, and Mn. According to these authors, these results indicated a deterioration of the water quality of the Extremoz Lake, as a result of the contamination with genotoxic heavy metals. They also recommend monitoring the presence of genotoxic agents in the lake water.

Studies by Moschini-Carlos et al. (2011) evaluated the impact of mining in the water quality of three artificial lakes in the Morozini River basin (Treviso, Santa Catarina state, Brazil) formed by the flooding of mining pits after deactivation. The lakes were named 1, 2, and 3. The physico-chemical characteristics of three lakes varied, indicating that the water quality was seriously compromised. In general, the three lakes had high levels of SO_4^{-2}, Mg, Fe, Al, Ca, Ba, Si, Zn, Ni, Na, K, Sr, B, Cd, Cr, Pb, and Cu. Some variables of these lakes did not meet the criteria of the classification by the CONAMA resolution 357/2005. Based on this resolution and the values found in the surface water, lake 1 and 2 would fall into class 4, while lake 3, class 3. The authors concluded that coal mining and residue disposal used by the mining companies in the Rio Morozini Basin drastically affected the water quality of the water bodies analyzed, which became improper for human use.

Medeiros et al. (2012) evaluated communities of yeast in four lakes, Carioca Lake, Dom Helvécio Lake, Amarela Lake, and Águas Claras Lake, in the Doce River Basin, Brazil, during dry and flood periods between 2000 and 2001. The water samples were collected at the subsurface. The following physico-chemical parameters were measured: Temperature, dissolved oxygen, pH, electric conductivity, total phosphorus, orthophosphate, ammonia, nitrate, nitrite, and total nitrogen. Counts of fecal coliforms and heterotrophic bacteria were also carried out to characterize environmental quality. The highest number of species was found during the dry season of 2000 in the Águas Claras Lake. However, isolated yeast was found in all sampling dates. In the Amarela Lake, yeast samples were isolated only during the dry season of 2000. The counts of heterotrophic bacteria varied and the highest values were found during the rainy season of 2000 in all lakes. Also in this year, coliform counts were high. The lowest values for total bacteria and fecal coliforms were observed in the Carioca Lake. In the Dom Helvécio Lake, yeast was found only in the rainy season of both years examined. Counts of yeast and opportunist pathogens were positive when correlated with the parameters indicating the presence of domestic residual waters, since these unicellular species can be used as bioindicators of water quality.

Other studies conducted in Latin America used the micronucleus test in the peripheral blood of fish. One example is the study with 10 different fish species, potential bioindicators of genotoxicity in the La Alberca Lake, Michoacán, México. This lake supplies water for different areas, and is of great economic and ecological importance, where several activities are carried out, such as agriculture, cattle ranching, fishing, and recreation (Torres-Bugarín et al., 2007). Water contamination is a serious issue that affects productive activities, compromising the quantity and quality of water used for distinct uses.

Studies on wild fish from two lentic habitats have also been conducted in lowlands of Colombia (Palacio-Betancur et al., 2009). Because of the discharge of large quantities of pesticides and industrial chemical products in aquatic habitats of Colombia, these investigations can provide information on the genotoxic and ecotoxicological effects of these substances. Torres-Bugarín et al. (2007) and Palacio-Betancur et al. (2009) emphasize the importance of the use of biomarkers in sentinel species for assessing environmental contamination.

2. Azul Lake: A Case Study

Located in the city of Rio Claro, São Paulo state, Brazil, Azul Lake, (Figure 2) is an artificial lake created in 1970 by the damming of the Servidão creek. The creek was dammed to improve drainage and prevent floods in this region of the city, since water from neighboring streets above this area brought sediments and garbage, contributing for the siltation of the creek (Troppmair, 1992; Tanaka, 2001).

In the 80s, a restoration project in the lake was carried out, elevating the status of the area to Municipal Park. The project included the removal of sediments, landscaping, installation of recreational equipment, and fishing in the lake (Mauro, 1983; Pereira et al., 2012a). Currently the park encompasses 113.000 m² of total area, of which approximately 50.000 m² are covered by water and 63.000 m² by green space (Moita, 2007). The lake represents an area of leisure with sports courts, park, trails, and pedal boats. Fishing was prohibited by the Rio Claro City Hall in 2005 due to concerns with fish contamination, which could be consumed by the population. In September of 2013, Navas reported a die-off to one of the city's newspaper, with approximately 100 dead fish found floating in the lake. According to the news article, park employees said fish die-offs in Azul Lake are common, contrary to statements by city officials, describing deaths from natural causes.

Several gas stations are located around the Azul Lake. Rain water drains into the lake bringing residues from gasoline and ethanol, due to washing and possible leaks from gas stations. In addition, flooding, infiltration, erosion, and water contamination by sewage occur in several areas along the creek and also in the lake, even after channel construction (Mauro, 1983). This information supports the reported by the report-098/CEI by CETESB of August 29, 2006, which detected levels of thermotolerant bacteria 48 thousand times higher than the allowed by Brazilian laws.

Azul Lake has been the focus of several studies from different perspectives. Oliveira et al. (1996) apud Moita (2007) analyzed the perception of an open space in the urban landscape, as well its potential as a reference point in the process of cartographic representation, using the Azul Lake as study model. Also, studies conducted by Moita (2007) aimed at analyzing, describing, and interpreting the role of water bodies in the attraction or repulsion by the population in urban areas. These areas offer opportunities for outdoor activities, such as recreation, educational activities, sports, as they usually provide environmental benefits, thermal comfort, and filter air, visual, and sound pollution, among others. However, according to the author, the water of Azul Lake caused repulsion in 47.5% of individuals interviewed, due to lack of maintenance, pollution, and unpleasant odor, among other factors.

Other authors, such as Alves and Machado (1993) and Troppmair (1992), focused on the environmental quality of the city and the importance of green spaces in growing cities (Moita, 2007).

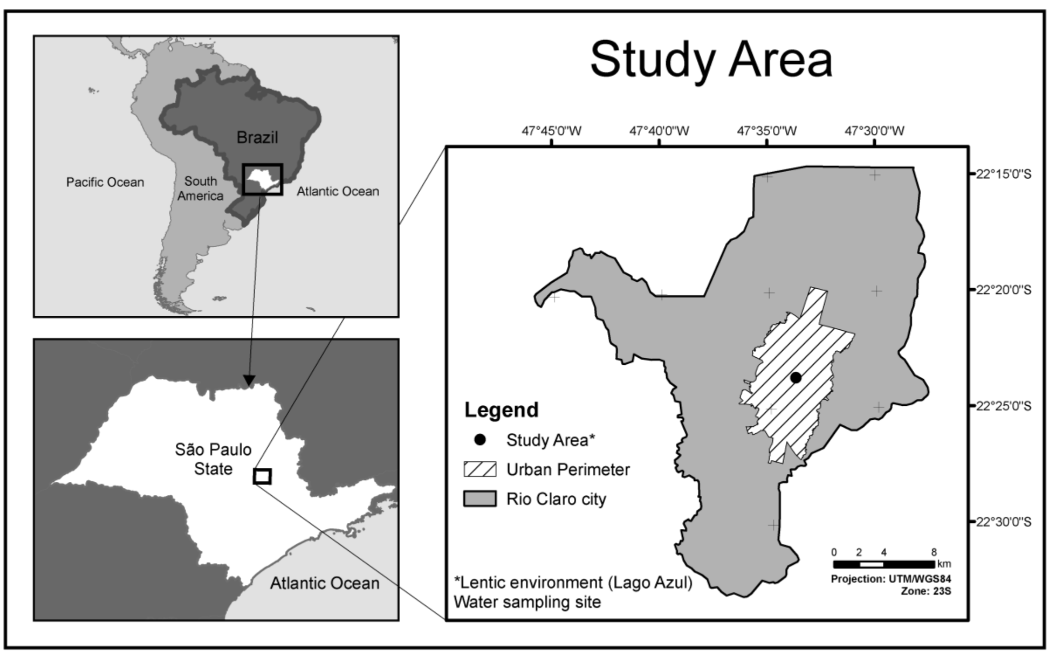

Figure 2. Location of Rio Claro, São Paulo state. *Lentic environment (Azul Lake) - water sampling site.

Regarding the environmental quality of the water and the sediment of the lake, several studies were conducted by Kataoka et al. (2003), Christofoletti (2008), Torres (2008), Brito (2009), and Pereira et al. (2012a,b).

Kataoka et al. (2003) characterized the sludge removed from Azul Lake to determine an adequate disposal of this residue. The authors conducted assays to evaluate the leaching, solubilization, and sludge biodegradation. The sludge chemical analysis revealed a high level of organic matter and 53.47 mg/dm^3 of lead (Pb), above the limit of 23 mg/Kg established by law. However, the chemical analysis of leached and solubilized extracts did not revealed parameters exceeding the limits established by law. The solubilized extract toxicity was assessed with the mortality test using *Daphnia similis*, a microcrustacean, but no toxic effects were observed.

Christofoletti (2008), Torres (2008), and Brito (2009) evaluated the lake water toxicity to the fish *O. niloticus* (tilapia). Christofoletti (2008) and Brito (2009) studied the same water samples collected between 2006 and 2008. Two collections were carried out in the dry season and two, in the rainy season. In these same periods when water samples were obtained, Torres (2008) collected tilapia from the lake.

Studies conducted by Christofoletti (2008) evaluated the cytotoxic, genotoxic, and mutagenic potentials of Azul Lake water with the chromosome aberration and micronucleus test using meristematic cells of *Allium cepa* (onion), and with the micronucleus test associated with nuclear abnormalities and the comet assay using erythrocytes of *Oreochromis niloticus*. Physico-chemical analysis was conducted for a water sample of each season. Onion seeds were directly exposed to lake water in Petri dishes and covered with filter paper. After germination, part of the roots was collected and the remainder was immersed in ultrapure water, for the recover treatment. Following the Feugen reaction, the root meristem was obtained by applying *gentle* pressure by hand on the coverslip. Alterations in the mitotic index, cells undergoing death and with chromosome aberrations, nuclear buds, and micronuclei were quantified. Water samples from all collections induced the formation of nuclear buds, polyploid cells, C-metaphases, metaphases with chromosome loss, anaphase with chromosome bridges, telophase with laggards, chromosome breaks and micronuclei.

In bioassays using fish, water samples were brought to the laboratory and oxygenated for 48h. After this period, five tilapias were placed in each tank for 96 h. After the exposure time, blood was collected with heparinized syringes by cardiac puncture and used for the micronucleus test associated with nuclear abnormalities and the comet bioassay. Brito (2009) collected the gills of these animals for later histopathological analysis. The water from Azul Lake also had cytotoxic, genotoxic, and mutagenic effects on tilapia. The author concluded that the contamination of the water of this lentic habitat is due to anthropogenic activities, as the samples from the two seasons had cytotoxic, genotoxic, and mutagenic effects on the two test organisms used. The metal analysis revealed concentrations of Ag, $Cd^{2+,}$ Cu and $Fe^{3+,}$ above the allowed by law in both seasons. Although cytotoxic, genotoxic, and mutagenic potentials were detected in both seasons, in the dry season the metal analysis showed higher concentrations of Al^{3+} and Hg. According to the author, the results may have been influenced by seasonality, since the runoff containing cytotoxic substances increased in the rainy season. In the dry season, the concentration of pollutants was higher due to the decrease in water volume, which could result in a higher interaction of elements present in the sediment with the water column.

Unlike the study by Christofoletti (2008), which evaluated the cytotoxic, genotoxic, and mutagenic potential of the lake waters using the blood of *O. niloticus*, Brito (2009) used the histopathology of gills of this fish species. After exposure, the second gill arch was extracted and fixed in different solution for morphological analysis. The histological analysis of the gills of all individuals of the group exposed to the lake water exhibited several alterations, while the histochemical analysis revealed a hyper secretion of mucus. According to the author, the alterations observed indicate a reduction in the respiratory efficiency and a defensive response. These results reflect a degradation of water quality due to metals detected by the chemical analysis reported by Christofoletti (2008).

Torres (2008) applied the same methodology used by Brito (2009) to analyze the gills of tilapias from Azul Lake during the same period of water collection conducted by Christofoletti (2008) and Brito (2009). Based on the histological analysis, several alterations were also observed, suggesting an attempt of the organisms to prevent the absorption of pollutants. Like the other authors, Torres (2008) reported the degradation of water quality, based on the induction of alterations in the gills of fish from the lake. Also, although these animals are able to survive in this environment, the function of gills is compromised.

Pereira et al. (2012a; 2012b) conducted other studies focused on the fish *Prochilodus lineatus* exposed to water from Azul Lake for 7 and 20 days. The study conducted in 2011 evaluated alterations in the pattern of white blood cells stained with Leishman's stain. The group exposed to water samples from the Azul Lake exhibited an increase in the number of leucocytes and the total number of white blood cells, suggesting that the chemical contaminants in this habitat act as antigens for this species, inducing the proliferation of defense cells. In 2012a Pereira et al. analyzed the morphology of defense cells. Like in the studies conducted by Torres (2008) and Brito (2009), Pereira et al. (2012b) observed morphological alterations, such as lamellar fusion and hyperplasy, as well as increase in collagen, variations in the nuclear volume and increase in the number of mucous and chloride cells. According to the authors, the pollutants discharged in urban rivers and lakes can induce many changes in the respiratory organ, possibly resulting in a metabolic deficit in fish.

In 2008 and 2013, small notes were published in the Rio Claro newspaper reporting the results obtained in the studies by Moita (2007) and Christofoletti (2008), warning the population about the danger of consuming fish from the lake.

3. Final Considerations

Currently, the monitoring and evaluation of environmental water quality are of extreme importance, given our dependence on this resource for the maintenance of life and the real possibility of water scarcity. Lakes are important ecosystems often used for water supply, leisure, landscaping or for effluent disposal. Studies on the biomonitoring of water from lentic habitats with sentinel organisms to detect effects of anthropogenic pollution are scarce in the scientific literature when compared that of rivers. These studies are extremely relevant, since the use of different biomarkers (genetic, morphological, etc.) can assist in the development of programs for the conservation of this ecosystem.

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