Leobardo Manuel Gómez-Oliván *Editor*

Pollution of Water Bodies in Latin America

Impact of Contaminants on Species of Ecological Interest

Springer

Pollution of Water Bodies in Latin America

Leobardo Manuel Gómez-Oliván
Editor

Pollution of Water Bodies in Latin America

Impact of Contaminants on Species of Ecological Interest

Springer

Editor
Leobardo Manuel Gómez-Oliván
Laboratorio de Toxicología Ambiental, Facultad de Química
Universidad Autónoma del Estado de México
Toluca, Estado de México, Mexico

ISBN 978-3-030-27295-1 ISBN 978-3-030-27296-8 (eBook)
https://doi.org/10.1007/978-3-030-27296-8

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

The indiscriminate use of chemical substances in industrial processes and anthropogenic activities have resulted in the release of these compounds in aquatic ecosystems through municipal, hospital, and industrial discharges, causing various undesired effects on the environment and on the species of ecological interest. These compounds such as metals, pesticides, emerging pollutants, and other substances are persistent and susceptible of biotic and/or abiotic transformations, yielding metabolites that can be more toxic than the original compounds by the ecological interest species.

One of the problems of rivers, bodies of water, groundwater, and coastal areas of Latin America is the contamination of them, understanding this as the incorporation of foreign matter, microorganisms, chemicals, industrial, and other types of waste into the water or wastewater.

The issue of water pollution is a complex issue to address and of collective interest, especially when water sources, such as rivers, lakes, and aquifers, have worrisome conditions because they are overexploited, are being contaminated, and are appropriated by particular interests at a disadvantage for traditional populations settled in territories of economic interest. In addition, historically, an increase in water requirements has been identified, in quantity and quality, due to the growth of the population to carry out human activities and to provide a healthy environment for all living beings.

In the chapters included in this book are indicated data of different toxicological findings identified by the presence of contaminants such as metals, pesticides, hydrocarbons, and emerging microcontaminants, among others, in various organisms of economic and ecological interest including amphipods, amphibians, and fish in various bodies of water from countries such as Argentina, Brazil, Colombia, and Mexico.

The authors are well-known researchers from Latin America and make exhaustive reviews and show the findings identified in their investigations related to the occurrence of pollutants, the toxicity using various biomarkers, as well as some methodologies for the removal of contaminants. This compilation of research in

Latin American countries allows us to have a very specific vision of the specific water problem of this region of the world.

The authors and I hope that our book complies with the diverse and generalized expectations and needs for information about the contamination problem in Latin America.

I thank all the authors in this book for their professional expertise and thoroughness in writing up their chapters, the Faculty of Chemistry at the Universidad Autónoma del Estado de México for the unending support it has shown as my employing entity, my research group, and my family, most especially to my mother, Aida Oliván Rebollo, and friends for the enthusiasm and support they have always shown.

Toluca, Estado de México, Mexico Leobardo Manuel Gómez-Oliván

Contents

Chapter 1
Historical Findings on Presence of Pollutants in Water Bodies in Latin America and Their Ecotoxicological Impact

Alejandro Mejía García, Leobardo Manuel Gómez-Oliván, Hariz Islas-Flores, and Nely San Juan-Reyes

1.1 Introduction

In this chapter, we analyzed the historical findings related to pollution in water bodies in Latin America, focusing on the presence and effects caused to the environment, as well as on the way in which the different work groups address the problem.

We addressed the main pollutants that are present in water bodies such as heavy metals, pesticides, hydrocarbon compounds, plastics, organic compounds, and others, with emphasis on their ecotoxicological impact.

We presented a review of the works carried out in countries such as Mexico, Argentina, Brazil, Chile, and Colombia, as well as those carried out in other Latin American countries.

1.1.1 Contamination of Water Bodies in Mexico

1.1.1.1 Contamination by Heavy Metals and Its Ecotoxicological Impact

There are many studies in which the contamination produced by heavy metals in the different water bodies of the region is evaluated. These studies are focused on highlighting the presence of pollutants as well as evaluating the possible sources of contamination, and also some studies evaluate their ecotoxicological impact, because this type of pollution is one of the most frequent. Some of the studies conducted in Mexico with respect to heavy metal contamination are mentioned below (Gutierrez-Mejia et al. 2016). Among the diverse sources of aquatic contamination,

A. Mejía García (✉) · L. M. Gómez-Oliván · H. Islas-Flores · N. San Juan-Reyes
Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_1

those related to anthropogenic activities are of notable importance, especially those with social impact, for example, tourist activities. Urbanization and industrialization represent other important factors to consider. In the coastal region of the southwest in the Gulf of Mexico, industrial development, particularly the hydrocarbon extraction industry, is the key element in the contamination of water bodies (Rodríguez-Espinosa et al. 2011; Celis-Hernández et al. 2013).

In a study carried out in the Bahía de Todos los Santos (Baja California Norte), the presence of cadmium particulate and dissolved (Cdp and Cdd, respectively) and the effect it has on *Lingulodinium polyedrum*, a dominant species of proliferating dinoflagellate algae (DAB), were evaluated.

During two periods of algal blooms (2011 and 2012), *L. polyedrum* tended to concentrate near the surface; however, during the flowering period of 2011, the number of cells was twice as abundant compared to the flowering period of 2012 ($10.0 \pm 8.0 \times 10^5$ and $5.0 \pm 4.4\times 10^5$ cells/L, respectively). Cdp increased significantly in 2011 (up to 1.02 ± 0.99 nmol/kg) and correlated positively with the cellular abundance of *L. polyedrum*, suggesting that this dinoflagellate is capable of assimilating and concentrating the Cdd. Likewise, the Cdd (up to 0.71 ± 0.17 nM) increased on the days of greatest cellular abundance, which could be attributed to the uptake and subsequent regeneration of Cdd as a result of remineralization of the organic particles produced during flowering, as well as with the presence of organic ligands secreted by *L. polyedrum* that could maintain the Cdd in solution (Gutierrez-Mejia et al. 2016).

On the other hand, in 2013, Celis-Hernandez and colleagues determined the presence of heavy metals in excess in the Jamapa and Antigua Rivers, as a consequence of the discharge of sediments supplied to the rivers. The data showed the enrichment of As, Cu, Zn, Co, Cr, and V in La Antigua River and As, Cu, and Cr in Jamapa River. The concentrations of trace metals in the sediment samples scattered along the coastal area showed significant spatial variation, and higher concentration levels were observed for some metals in localized areas. The mCd data (modified pollution degree, calculated by the sum of all the metals studied) point to La Antigua as the area with the highest metal enrichment, and according to the sediment quality guidelines, Ni, As, Cu, and Cr were the metals that could cause occasional adverse effects to the aquatic organisms (Celis-Hernández et al. 2013). In 2011, the presence of various heavy metals in beach waters and sediments in Acapulco (in the Pacific coast of Mexico) was determined. As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, V, and Zn were found, and the concentration of dissolved trace metals (DTM) in beach water and acid-leachable trace metals (ALTM) in the sediments indicated that they are anthropogenic in nature due to the increase in tourist activities in overcrowded beach locations (Rodríguez-Espinosa et al. 2011).

Guerrero-Barrera et al. (2016), in regions with mining activity (Asientos, Aguascalientes), determined that, in spite of determining the presence of Cd, Pb, Cu, and Zn (105.3, 7052.8, 414.7, and 12.263.2 mg/kg, respectively), they were not leachable in water and it was considered that Cd and Pb were easily mobilized, since they were predominantly associated with interchangeable carbonate fractions, while Cu and Zn were found to be associated with Fe/Mn oxide and organic matter fractions (Guerrero Barrera et al. 2016).

There are few studies in which the intake of heavy metals is related to negative effects on wildlife, particularly fish. In a study carried out in 2016 in Chapala Lake, the concentrations of selected metals (Al, Ba, Cu, Mn, Hg, Sr, V, and Zn) in water, sediments, and fish were determined. Metal concentrations in fish ranged from 0.05 μg/g for Al and Cu to up to 64.70 μg/g for Sr. There was a positive and significant correlation between the length of the fish and the metals, particularly for Ba, Cu, Mn, and Zn; however, there were no significant correlations between the metal concentrations and the stable isotope values of nitrogen (δ15N) in the fish, indicating that there is no biomagnification through the food chain (Torres et al. 2016).

Carro et al. (2008) conducted a study in the catchment area of Pátzcuaro Lake (central Mexico), to evaluate the distributions of erosion, sediments, nutrients, and pathogens from 13 micro-basins bordering the lake. The results obtained showed a total annual contribution of 491 tons of nitrogen and 116 tons of phosphorus, mainly from micro-basins that have agricultural and livestock land uses; on the other hand, it was also determined that the cities next to the lake contribute with 10.1% of the total nitrogen load and 3.2% of phosphorus, which highlights the importance of anthropogenic activities as a source of water body contamination (Carro et al. 2008).

In the aquifers located in the Toluca Valley, Salamanca, and San Luis Potosí, elements such as fluoride, arsenic, iron, and manganese have been detected as a result of the introduction of older groundwater with longer residence times and a distinctive chemical composition. High concentrations of other elements were also observed, such as chloride, sulfate, nitrate, and vanadium, as well as pathogens, all related to anthropogenic contamination sources. Some of these elements (nitrate, fluoride, arsenic, iron, and manganese) have shown concentrations above drinking water standards in Mexico and the World Health Organization (WHO) (Esteller et al. 2012).

Among the heavy metals found most frequently in water bodies in Mexico is arsenic (As). Camacho and collaborators (2011) determined that As is not present in surface waters in the northern region of Mexico due to the affinity of the solid phases in alkaline conditions common to the arid zones but instead it accumulates in the sediments. Some methods are applied to treat contaminated water; among them, reverse osmosis, coagulation, adsorption, and electrochemical methods have been applied in the area of interest to eliminate As. Some emerging technologies have been used, especially phytoremediation; only sorghum (*Sorghum bicolor*), desert bloom (*Baccharis sarothroides* Gray), fern (*Pteris vittata*), and *Eleocharis* sp. are considered as possible hyperaccumulators for the removal of As from contaminated soil and water in the region (Camacho et al. 2011).

Mendoza-Carranza et al. evaluated the bioaccumulation of several heavy metals (Cd, Cr, Ni, Pb, V, and Zn) in different samples such as suspended load, sediments, primary producers, mollusks, crustaceans, and fish from a lagoon on the coast of Tabasco. Of the heavy metals evaluated, Zn showed the highest concentrations in suspended loads (159.58 mg/kg) and in aquatic consumers (15.43–171.71 mg/kg), in particular *Brachyura* larvae and ichthyoplankton (112.22–171.71 mg/kg), followed by *Callinectes* sp. crabs (113.81–128.07 mg/kg). These concentrations were directly related depending on the feeding path within the trophic chain. Although the other heavy metals presented minor occurrence in the food chain, high

concentrations of Ni and Cr were found in phytoplankton and sediment (37.62–119.97 mg/kg) and V in epiphytes (68.64 mg/kg), which pointed out that these concentrations exceeded the national and international limit values, as well as Cd, which entailed considerable potential risk. In the metropolitan area of Monterrey (the third largest urban area and the second largest economic city in Mexico), the content of major ions and trace elements was evaluated to investigate the main hydrochemical properties of groundwater and determine if the groundwater in the area represents a threat to population. The principal component analysis performed on groundwater data indicates the presence of Si, Co, Se, and Zn, suggesting that these are derived from rock erosion. Other trace elements such as As, Mo, Mn, U, Pb, and Cu are also present; the two last ones could be the least mobile elements in groundwater (Mendoza-Carranza et al. 2016; Mora et al. 2017).

1.1.1.2 Contamination by Pesticides and Its Ecotoxicological Impact

The state of Sinaloa, in Mexico, is an industrialized agricultural region with a documented use of pesticides (700 tons per year), of which at least 17 are classified as moderately to highly toxic. In 2017, Arellano-Aguilar and colleagues determined the presence of pesticides in river and drainage waters that flow into coastal lagoons. Several banned organochlorine pesticides such as endosulfan, hexachlorocyclohexane, and chlordane were detected in the coastal lagoons and rivers, with hexachlorocyclohexane having the highest concentrations. Organophosphorus pesticides were detected in higher concentrations than organochlorine pesticides, although a clear accumulation in the lagoons could not be identified, probably because their persistence in the environment is lower. The main organophosphorus pesticides found were diazinon (detected in all sites and in high concentrations), disulfide, methyl parathion, chlorpyrifos (the second most detected, an order of magnitude higher than diazinon), and mevinphos (Arellano-Aguilar et al. 2017).

The use of pesticides/herbicides is a common practice in Mexican agriculture. Glyphosate (GLY) is a widely used herbicide and despite being considered nontoxic, its presence in water bodies through spills, runoff, and leaching is a risk to the biota that inhabits these ecosystems. Glyphosate concentrations were determined in groundwater of the municipality of Hopelchén, in Campeche, and concentration of 1.42 μg/L was found, indicating an excessive use of glyphosate in agricultural communities (Rendón-Von Osten and Dzul-Caamal 2017).

Tremblay et al. (2017) conducted a study to establish a relationship between the presence of organochlorine pesticides and the level of several indicators of oxidative and contaminant stress of the hawksbill sea turtle (*Eretmochelys imbricata*). Endosulfans were detected in 17 samples of *E. imbricata*; compounds related to aldrin were detected in 21 samples and DDT (dichlorodiphenyldichloroethylene, a pesticide banned in Mexico, with a tendency to bioaccumulate and biomagnify) in 26. The activity of cholinesterase in washed erythrocytes and lipid peroxidation correlated positively with glutathione reductase activity, while antioxidant enzymes did not correlate with lipid damage with any organochlorine pesticides detected in the samples.

Bustillos Lagoon (Chihuahua) is recognized as one of the most important water bodies in North America and represents a valuable source of water for irrigation and livestock production. In a study carried out by Ochoa-Rivero et al. (2017), it was determined that the water in the lagoon represents a risk for the irrigation of crops and as drinking water for livestock due to the high levels of sodium adsorption ratio (SAR), nitrates, and magnesium. In addition, it was determined that concentrations of DDT were much higher than those found in other studies of Mexican water bodies (2804 ng/mL) (Tremblay et al. 2017; Ochoa-Rivero et al. 2017).

1.1.1.3 Contamination by Hydrocarbons and Its Ecotoxicological Impact

Accidents related to the oil spill in the northern region of the Gulf of Mexico have been documented. In 2010, the explosion of the Macondo caused an unprecedented oil release in the water column at a depth of approximately 1500 m. Between 2010 and 2013, samples of superficial and subsurface sediments were collected, which showed a clear pattern with total concentrations of n-alkanes, unresolved complex mixture, and petroleum biomarkers (terpanes, hopanes, steranes), indicating that they sedimented on the seabed in the subsequent months, resulting in an apparent accumulation of hydrocarbons on the seabed. In this study, the ecotoxicological impact caused by the spill was not evaluated (Babcock-Adams et al. 2017).

The presence of pollutants in water bodies has a negative effect such as a low production of aquatic specimens. Toledo-Ibarra and colleagues, who evaluated contamination in an estuary of Boca de Camichin, as well as the subsequent oxidative stress in the oysters of *Crassostrea corteziensis*, determined the presence of aromatic polycyclic hydrocarbons such as naphthalene, benzo[a]anthracene, pyrene, benzo[a]pyrene, and benzo[k]fluoranthene in water samples from oyster farms. In addition, oxidative damage, evaluated by lipoperoxidation and lipid hydroperoxide and protein oxidation, and the enzymatic activity of CAT, SOD, GPx, GST, and AChE in the gills of oysters were also evaluated. Regarding oxidative stress, the oysters of the estuary had oxidative damage to the lipids and altered antioxidant enzymatic activity, without observing any correlation between the pollutants and the evaluated oxidative stress parameters. Using the same bioindicator, Giron-Pérez et al. also evaluated the polycyclic aromatic hydrocarbon chemical contamination and oxidative stress parameters in the Camichin estuary. The results obtained showed the presence of naphthalene, pyrene, and benzo[a] pyrene in relatively higher concentrations than local and international guidelines. With respect to the oxidative stress response biomarkers (concentration of H_2O_2 and $O_2^{\bullet}$, catalase activity, lipid peroxidation, and hydroperoxide concentration), they indicate that these contaminants could be related to the oxidative stress detected in *Crassostrea corteziensis* oyster (Toledo-Ibarra et al. 2016; Girón-Pérez et al. 2013).

1.1.1.4 Contamination by Plastics and Its Ecotoxicological Impact

The ingestion of microplastics by fish could be an emerging environmental crisis due to the proliferation of plastic pollution in aquatic environments. Phillips and Bonner (2015) found that of 535 fish examined 8% and 10% of the fish (freshwater and marine, respectively) had microplastics in their intestinal tract, while the percentage of occurrence of microplastics ingested by fish in undeveloped streams was lower than that of one of the urbanized streams (5% and 29%, respectively) (Phillips and Bonner 2015).

The accumulation of marine debris is a global problem that affects the oceans in multiple scales. The majority of floating marine debris is made up of microplastics, plastic particles up to 5 mm in diameter, which is a serious problem since they have sizes and appearances similar to natural foods; these small fragments present potential risks for many marine organisms, including zooplankton and phytoplankton. In the study carried out by Fossi et al. (2017), in skin biopsy samples of whale shark (*Rhincodon typus*), the levels of polybrominated diphenyl ether (PBDE) plastic additives were evaluated. The average abundance pattern for the target pollutants was PCBs > DDTs > PBDEs > HCB, with concentration values of 8.42, 1.31, 0.29, and 0.19 ng/g, respectively. Di Mauro et al. characterized the microplastics present in the waters off the coast of Louisiana (northern Gulf of Mexico); the estimated concentrations of microplastics collected are among the highest reported worldwide, ranging from 4.8 to 18.4 particles/m3. The sizes of microplastics and zooplankton overlap partially or completely, suggesting the possibility of confusion with natural prey (Fossi et al. 2017; Di Mauro et al. 2017).

1.1.1.5 Other Sources of Contamination and Their Ecotoxicological Impact

The contamination of water bodies not only implies an impact to the aquatic organisms but also the consequences at other levels. This is the case of antimony (Sb) present in PET bottles, which can be leached to the medium it contains. The group of Ruiz-Ruiz et al. (2016) determined the concentration of Sb in 12 samples of bottled water in Mexico, finding concentrations ranging from 1.1 to 18.5 μg/L, suggesting that the release of PET material brings toxic effects on the aquatic environment.

On the other hand, mitochondrial activity has become a key tool in the evaluation of the ecosystem health, in particular, water quality. Rodriguez-Romero et al. (2017) used this biomarker in spores of the native fern *Cyathea costaricensis* to evaluate water quality at 11 sites along the Bobos River, Veracruz.

The results showed that mitochondrial activity (measured through the reduction of 2,3,5-triphenyl tetrazolium chloride to the formation of formazan salts) is a good point of reference for the evaluation of water quality, reflecting the effects of its physicochemical characteristics; finding the lowest mitochondrial activity

(63.8777.47%) related to the geological nature of the basin and high levels of hardness. Mitochondrial activity peaked in September (98.32% ± 9.01), probably as a result of nutrient enrichment in the rainy season (Rodríguez-Romero et al. 2017; Ruiz-Ruiz et al. 2016).

1.1.2 Contamination of Water Bodies in Argentina

1.1.2.1 Contamination by Heavy Metals and Its Ecotoxicological Impact

One of the most complete studies for the determination of heavy metals in water bodies was carried out by Avigliano and colleagues (2015), who evaluated the presence of As, Ag, B, Ba, Bi, Ca, Cd, Co, Cr, Fe, Ga, Hg, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Se, Sn Sr, Te, Ti, U, V, and Zn in samples of *Odontesthes bonariensis* from four important fishing commercial sites: the Plata River, the Barranga and Adela Lagoons, and the Chasicó Lake. Obtained data indicated that the concentrations of trace elements in the water were above the recommended maximum levels established for the protection of aquatic biota, for example, for As 28.4 to 367 μg/L, for Cd 0.17 to 1.05 μg/L, for Hg 0.07 to 0.63 μg/L, and for Zn 71.3 to −90.0 μg/L, while the highest concentrations of heavy metals found in *Odontesthes bonariensis* muscles were for As, Hg, and Pb (0.03–0.76, 0.03–0.42, and 0.04–0.19 mg/kg of wet weight, respectively) (Avigliano et al. 2015).

The behavior of heavy metals, evaluated by Jakomin et al. (2015) in samples obtained from the Pampeano and Puelche aquifers, showed that the Pampeano aquifer presented values of Kd higher than the Puelche aquifer (Pb > Zn > Cd and Pb > Cd > Zn, respectively). From these data it was possible to predict the behavior of heavy metals in the two aquifer systems; for example, if the reactive transport of these metals is considered, Pb (having a higher Kd value) would have a higher delay factor, followed by Cd and Zn; this means that Pb would migrate within a smaller area, while Cd and, to a large extent, Zn, would migrate within a larger area, considering similar flow times (Jakomin et al. 2015).

Many heavy metals and compounds present as contaminants of water bodies have the tendency to be bioaccumulated through the food chain, directly affecting aquatic life. Griboff et al. (2018) evaluated in water, sediment, plankton, shrimp (*Palaemonetes argentinus*), and fish muscle (silverside, *Odontesthes bonariensis*) the bioaccumulation and the transfer of these inorganic elements through the food chain, in the lake Los Molinos. According to the results, it was determined that Al, Cr, Mn, Fe, Ni, Cu, Zn, Ag, Cd, Hg, Pb, As, and Se are subjected to bioaccumulation, especially in organisms such as plankton, while the fish muscle was characterized by the highest bioaccumulation factor for Se and the invertebrates were characterized by the highest bioaccumulation factor for Cu, Zn, As, Cd, and Hg. On the other hand, a significant decrease was observed in the concentrations of Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Cd, and U through the trophic network analyzed.

Despite being located far from point sources of Hg contamination, Arcagni et al. (2017) recorded high concentrations of Hg in the plankton of the deep oligotrophic lake Nahuel Huapi (North Patagonia), in which two species of predatory fish (native and introduced) with different eating habits co-inhabit and, therefore, present different trophic chains. As expected, high values of total Hg were obtained in the plankton; however, mercury was not biomagnified in the food chain because most of it was found in inorganic form. It was also observed that, when evaluating the molar relationship between Hg and Se, all ratios were greater than 1, which indicates that it could be offering a natural protection against the toxicity of Hg (Griboff et al. 2018; Arcagni et al. 2017).

1.1.2.2 Contamination by Pesticides and Its Ecotoxicological Impact

The indiscriminate use of chemical products in agricultural activity and livestock waste have been identified as point sources of contamination of water bodies. It is estimated that the use of pesticides in the La Plata basin (region with agricultural activities) has increased to 900% in the last two decades, which represents a serious environmental problem in the area. Etchegoyen and colleagues determined the presence of endosulfan, cypermethrin, and chlorpyrifos in superficial waters and deep sediments of major affluents of the Paraguay-Paraná River, finding a generalized but variable distribution in concentrations ranging from 0.004 to 6.62 μg/L and 0.16 to 221.3 μg/kg, in water and sediments, respectively. All the concentrations detected in the water exceeded the recommended guidelines for the protection of aquatic biota, and agricultural activity was identified as the main source of pollution loads by pesticides. The water of the agro-ecosystems has an adverse effect on diverse biological parameters (mortality, growth, development, morphological anomalies, behavior, and parameters of the blood cells) of the amphibians. As reported by Babini et al. (2016), adverse effects were found on *Rhinella arenarum* tadpoles (early stage of development), and there was higher prevalence of morphological anomalies, as well as presence of micronuclei and alterations in swimming, indicating that poor water quality of the evaluated ponds has an impact on the health of the tadpoles, and this could affect the persistence of the populations (Etchegoyen et al. 2017; Babini et al. 2016).

There is little information about contamination by pesticides in surface waters in Argentina. Of the few studies conducted in surface waters of four agricultural subbasins of San Vicente, Azul (southeast of Buenos Aires and Mista), it was found that the sub-basin of southeast Buenos Aires was the site with the highest frequency of pesticide detection followed by Azul and San Vicente micro-basins. The most detected pesticides were atrazine, tebuconazole, and diethyltoluamide with levels of maximum concentration of 1.4, 0.035, and 0.701 μg/L, respectively (De Gerónimo et al. 2014).

1.1.2.3 Contamination by Plastics and Its Ecotoxicological Impact

Plastic industries, industrial effluents, wastewater outlets, and domestic wastewater are considered point sources of contamination of water bodies. In fact, a study carried out in 2017 by Quintas and colleagues showed that the sources mentioned above contribute to the contamination of the Bahía Blanca estuary (Argentina), placing the species that live there at environmental risk. In native mussels (*Brachidontes rodriguezii*) total values of butyltin (TBts = TBT + DBT + MBT) that oscillated between 19.64 and 180.57 ng for each gram of Sn were found, indicating that 73.9% of the mussels could be under the effects of the biological hazards associated with TBT contamination and that approximately 56% of the samples appeared to have accumulated TBT through the sediments (Quintas et al. 2017).

1.1.2.4 Other Sources of Contamination and Their Ecotoxicological Impact

There are water bodies that are used for both agricultural/livestock and recreational activities. This is the case of the Dulce River (Santiago del Estero), whose water is used for drinking, as well as recreational use and irrigation through a vast network of channels and ditches. By analyzing samples from the river, the presence of phenolic compounds (5 mg/L), which exceeded established limits for recreational purposes, was determined, and depending on the sampling epoch and due to activities of anthropogenic origin, the phenolic compounds contribute to the contamination of the channel mainly in the months of April and September (400 ± 110 mg/L and 240 ± 20 mg/L, respectively). A high concentration of sulfate was also found that was higher than the limit allowed by the legislation (Acosta et al. 2018).

The Luján River basin (northwest of Buenos Aires) is characterized by receiving different anthropogenic inputs before reaching the estuary of the Río de la Plata. To evaluate the adverse impact on the aquatic life of the river, the responses of the hepatic biomarkers in standardized (*Cyprinus carpio*) and native (*Pimelodella laticeps*) species were evaluated. The results indicate that the biomarkers liver somatic index and GST increased and the levels of thiobarbituric acid-reactive substances (TBARS) decreased in both biomarkers. An increase in SOD was observed in the nurseries of *Cyprinus carpio* (14 days) and CAT in those of *Pimelodella laticeps* (7 days), demonstrating that a period of 14 days of exposure could lead to antioxidant and biotransformation processes in *C. carpio* and to phase II biotransformation responses in *P. laticeps.* Using the *P. lineatus* fish as biomarker, Cazenave et al. (2009) evaluated the water quality of the Salado River basin through multiple biomarkers including morphological indexes (condition factor, somatic liver index), hematological parameters (red and white blood cells), biochemical parameters (glucose, total protein, and cholinesterase activity), and markers of detoxification and oxidative stress (antioxidant enzymes and lipid peroxidation) in various organs such as liver, gills, and kidneys. Although the water quality assessment showed no marked differences between the sampling sites, the results of the analysis of glucose

levels, the activity of glutathione S-transferase, the levels of lipid peroxidation, and the counting of blood cells indicate that fish lives in stressful environmental conditions, suggesting that the use of a set of specific biomarkers in *P. lineatus* represents a sensitive and effective tool to reflect adverse environmental conditions for fish health (Scarcia et al. 2014; Cazenave et al. 2009).

Fernandez et al. (2007) carried out an evaluation of the physicochemical composition and presence of heterotrophic aerobic microorganisms of the Sauce Grande Lagoon. Ninety-six samples were analyzed and results showed that the presence of microorganisms was influenced by high temperatures (in the warmer months) and more intense recreational use. Of the microorganisms analyzed, the bacteria count indicated that fecal contamination was statistically low; however, *P. aeruginosa*, an opportunistic pathogen, was present in densities higher than those allowed in all determinations (Fernández et al. 2007).

Santucci et al. (2018) report that industrial waste from a sulfuric acid industry is dispersed in a local area of the coast of the Río de la Plata. Through the collection of samples of surface and groundwater, both in unconfined and semi-confined aquifers, parameters such as electrical conductivity, pH, and the main elements present in the samples were determined, in which minerals composed of associated sulfur are present with jarosite and iron oxides in surface sediments; in addition, the high concentration of SO_4^{-2} recorded in the semi-confined aquifer due to its infiltration from the unconfined aquifer showed that industrial pollution has a significant impact at the local level (Santucci et al. 2018).

1.1.3 Contamination of Water Bodies in Brazil

1.1.3.1 Contamination by Heavy Metals and Its Ecotoxicological Impact

One way to evaluate the water quality of the different water bodies is to evaluate the composition of their sediments, which are formed by the deposition of organic and inorganic particles in the depth of the water, playing an important role in the health of aquatic ecosystems. An analysis carried out in Lake Guaíba (metropolitan region of Porto Alegre, Rio Grande do Sul State, Brazil) provided data indicating that heavy metals such as Zn, Pb, Cu, Cr, Ni, Cd, and Co were present in the sediments obtained from 12 sampling sites along the river, while Pb, Cu, Cr, Ni, total organic carbon, and P were mainly present in sediments (de Andrade et al. 2018).

1.1.3.2 Contamination by Organic Compounds and Its Ecotoxicological Impact

Furtado and Von-Müller (2015) performed the analysis of samples of residential water filters collected in the basin region of the Dos Sinos River to determine the presence of compounds with endocrine-disrupting activity, which have the

particularity of generating dysfunctions or changes harmful to human and animal health by similarly acting hormones produced endogenously. Among the endocrine-disrupting compounds found in the analyzed samples, estrone and 17-α-ethinyl estradiol (0.68–17.79 and 0.63–16.86 μg/L, respectively) stand out. The presence of phenolic compounds such as 2,4-dichlorophenol, 2,5-dichlorophenol, and 2,4,6-trichlorophenol, in concentrations ranging from 1.60 to 70.37 μg/L, was also determined in the samples (Furtado and von Mühlen 2015).

1.1.3.3 Other Sources of Contamination and Their Ecotoxicological Impact

The São Francisco River is the largest in Brazil and problems of water quality are a concern and have worsened with recent urbanization and industrialization. Da Costa and collaborators reported in 2017 that several parameters evaluated in the river, during a monitoring period of 14 years, were found above the water quality standards established by local legislation. Some of these parameters could be identified as a cause of concern, such as the fecal coliform indicator and phosphorus, which are related to domestic and effluent removal without treatment or insufficient treatment, as well as manganese and total suspended solids, which can be affected by erosive processes of natural and anthropogenic causes. Some metallic parameters such as iron and arsenic may be related to the mining activities typical of the area (da Costa et al. 2017).

A large amount of organic pollutants that damage the ecosystem are transported to wastewater treatment plants (WWTPs). Silva et al. (2014) evaluated samples of wastewater from a treatment plant located in Sao Carlos and found that several compounds are present in the samples with a concentration that varies considerably with treatment and seasonality. Factors such as abnormal discharge, the influence of rainwater on the composition of wastewater, and the presence of recalcitrant compounds (alkyl benzene sulfonate surfactant homologues) were identified as sources of variation of found compounds in the samples; other compounds such as amino acids (serine, leucine, and alanine), organic acids (lactic, glutamic, phenylacetic, propionic, and formic), sucrose, glycine, and taurine were also found in the samples (Silva et al. 2014).

The anthropogenic influence in the Murucupi River (Barcarena, state of Pará) makes the quality of the water to be considered bad to good, since, according to the results obtained by Medeiros and colleagues in 2017, various values of physicochemical and biological parameters, including pH, total nitrogen and phosphorus, dissolved oxygen, and thermostable coliforms, were found elevated compared to controls; however, in order to have a more complete evaluation panorama, the authors suggest that other important variables be evaluated, such as sulfate, benzene, toluene, ethylbenzene, xylenes, aluminum, manganese, iron, lead, cadmium, mercury, and other toxic elements, which are associated with the elimination of domestic and industrial effluents to the river basins. Rigotto et al. (2015) reported the presence of different adenovirus species including human adenovirus (HAdV),

bovine adenovirus (BAdV), canine adenovirus (CAV1-2), porcine adenovirus (PAdV), and aviary adenovirus (AvAdV), in the upper, middle, and lower sections of the Sinos River basin, during the 24-month period, which indicates fecal contamination from different sources and demonstrates the inefficiency of wastewater treatment in river waters and the intensification of the strong influence of human activities that may contribute to the presence of inhibitory substances such as organic acids on the surface of these waters (Medeiros et al. 2017; Rigotto et al. 2015).

1.1.4 Contamination of Water Bodies in Chile

1.1.4.1 Contamination by Heavy Metals and Its Ecotoxicological Impact

The rivers of central-northern Chile are characterized by being exposed to various sources of pollution, mainly those related to mining, volcanic, and geological activities. A study conducted by Pizarro and collaborators (2010) showed the highest historical average concentrations of As, Cu, and Pb found in the Elqui River, the highest concentrations of Hg and Cr in the El Aconcagua River, and high concentrations of Cu and Mo in the Rapel River. It was also determined that the concentration of sulfates exceeded 100 mg/L in nine rivers, and in seven of them it had positive annual slopes, suggesting that mining pollution is the main process contributing to this growing annual trend in As, Cu, and SO_4^{-2} (Pizarro et al. 2010).

Parra and colleagues evaluated the content of heavy metal on sediments of Quintero Bay (2015). In the 14 sampling tested sites, both major and minor metals were found in different concentrations; among the determined metals are Al (65,522 ± 3300 mg/kg), Zn (360 ± 26 mg/kg), Cu (307 ± 10 mg/kg), Se (0.90 ± 0.33 mg/kg), As (11.1 ± 2.4 mg/kg), Pb (179.3 ± 4 mg/kg), and Cr (88.4 ± 3.2 mg/kg), among others. The concentrations of metals found suggest an anthropogenic origin related to Cu, Se, Mo, As, Sb, and Pb, which are probably associated with copper smelting. Rivera et al. collected and analyzed 384 samples of coastal waters from the San Jorge Bay (Antofagasta, northern Chile). Although the distribution of Cu, Cr, Ni, Zn, Cd, and Pb (1.25, 1.29, 1.90, 2.60, 0.03, and 0.03 μg/L, respectively) along the coast of the bay provides evidence of the effects of industrial activity, the results suggest that the coastal waters of San Jorge Bay are of very good quality and suitable for recreational activities that involve contact with the human body (Parra et al. 2015; Rivera et al. 2015).

The industrial activity is one of the main sources of point pollution of water bodies as evidenced by Mansilla et al. (2013), who demonstrated the presence of elevated levels of mercury in sediments of the Lenga estuary. Total mercury concentrations (Hg-total) ranged from 0.5 to 129 mg/kg and organic mercury (Hg-org) from 11 to 53 μg/kg; these results show that the proportion of Hg-org/Hg-total in the sediment varies in more than two orders of magnitude (0.02–5.7%) according to the concentration of Hg-total. Other chemical elements have been

presented in different water bodies, for example, lithium, which has been reported in trace amounts in the groundwater with few important exceptions. Figueroa and collaborators (2012) report that, in the northern region of Chile, drinking water and many foods have high levels of lithium, with values between 100 and 10,000 times higher than most rivers in North America (Mansilla et al. 2013; Figueroa et al. 2012).

More specific studies have been reported related not only to the uptake of heavy metals by aquatic plants but also to the speciation of captured metals. Barbero and colleagues in 2012 reported the speciation of arsenic by two species of green algae (*Cladophora* sp. and *Chara* sp.) and six aquatic plants (*Azolla* sp., *Myriophyllum aquaticum*, *Phylloscirpus* cf. desserticola, *Potamogeton pectinatus*, *Ruppia filifolia*, and *Zannichellia palustris*) located in the basin of the Loa River. The results showed that the inorganic arsenic compounds were the main arsenic species measured in all the samples (arsenite and arsenate); however, algae species accumulated more arsenic than aquatic plants. The total content of arsenic varied from 182 to 11,100 and from 20 to 248 mg As/kg in algae and freshwater plants, respectively, and *Cladophora* sp. was the only algae that showed hyperaccumulation behavior (>0.1%). Finally, in the study carried out by Queirolo et al. (2000), it was determined that the concentrations for the soluble elements were for Cd <0.1, for Pb <0.5, and for Zn and Cu between 1 and 10 ng/ml; and with respect to the particulate material present in the samples analyzed, the concentrations were for Cd <0.1 ng/ml, for Pb <0.3 ng/ml, and for Zn and Cu <1 ng/ml. The total content of these elements is well below international recommendations (WHO) and national standards; however, in several rivers high concentrations of arsenic (up to 3000 ng/ml) were found, which exceed the national standard by more than 50 times (Barbero et al. 2012; Queirolo et al. 2000).

1.1.4.2 Contamination by Pesticides and Its Ecotoxicological Impact

The concern about the contamination of rivers is a phenomenon that has increased in recent years. An evaluation of five pesticides was carried out (2001–2003) in the waters of the Traigue River basin. According to the results, the compounds found were the herbicides simazine, hexazinone, 2,4-D, and picloram and the fungicide carbendazim; the concentrations varied depending on the sampling period; for example, during the first sampling (2001), the highest concentrations of pesticides were 3.0 μg/L for simazine and hexazinone and 1.8 μg/L for carbendazim, while, in 2003, the highest concentrations of pesticides were 4.5 μg/L for carbendazim and 2.9 μg/L for 2,4-D. A study carried out by Gajardo and colleagues, in 17 fluvial stations located throughout the Chillán River basin and in which *Daphnia* spp. were used as a bioindicator, showed that most of the observed toxic effects were directly related to the discharge of water urban residuals, while toxicity in rural areas was detected mainly during the winter period, when rainfall and river flow are high, suggesting that the agricultural and forestry activities in the basin, characterized by an intensive use of pesticides, play an important role in the generation of non-point pollution. Parameters such as mortality and alterations in the reproductive success of *Daphnia* spp. were evaluated and not directly related to the chemical contamination

detected; and only with the exception of the pesticide atrazine, the concentrations of pesticides detected were below the known levels of toxicity, but the additive and synergistic effects of the presence of a pesticide mixture were not excluded as one of the possible causes of observed toxicity (Palma et al. 2004; Gajardo et al. 2005).

1.1.4.3 Contamination by Hydrocarbons and Its Ecotoxicological Impact

In the Lenga estuary (San Vicente Bay), the surface sediments of nine sites were analyzed by Pozo et al. (2011) with the purpose of determining the components that formed them. The results obtained from the sediment analysis showed that the total concentrations of polycyclic aromatic hydrocarbons ranged between 290 and 6118 (2025 ± 1975) ng/g, and the percentages of organic carbon varied from 1% to 7%. A comparison of the results obtained and international guidelines established that sediments from the Lenga estuary showed no ecotoxicological risk to benthic organisms in which high levels of polycyclic aromatic hydrocarbons were detected (Pozo et al. 2011).

1.1.4.4 Other Sources of Contamination and Their Ecotoxicological Impact

Valenzuela et al. (2006), through a study carried out in *Oncorhynchus mykiss*, showed the toxic effects caused by industrial discharges, from a cellulose and paper plant in the Biobio River, by the analysis of three zones of discharge: pre-impact, impact, and post-impact (less influenced). In the study, no significant changes were observed in the physiological and somatic indexes of the liver during different sampling times (11, 21, and 30 days of exposure); however, the activities of ethoxyresorfurin-O-deethylase were significantly higher in the trout in the impact and postimpact areas (2–4 times) compared to the pre-impacted area (considered as reference), and a strong inhibition of acetylcholinesterase activity was observed, which reached 50%. Likewise, the trout from the impact and postimpact areas showed significant increases in the gonadal somatic index and plasma vitellogenin levels combined with an induction of gonadal maturation (Valenzuela et al. 2006).

1.1.5 Contamination of Water Bodies in Colombia

1.1.5.1 Contamination by Heavy Metals and Its Ecotoxicological Impact

Valdelamar-Villegas and Olivero-Verbel (2018) evaluated the content of heavy metals in beaches of the Caribbean coast of Colombia (Riohacha, Berrugas, and Cartagena), using as a bioindicator the mollusk *Donax denticulatus*, which is a key organism for the ecology of sandy beaches, acting as controller of organic matter

and microorganisms. The metal analysis revealed that the tissue concentrations of heavy metals varied according to the location (Hg = 0.018 ± 0.004 μg/g, in Riohacha Beach; Pb = 0.110 ± 0.060 μg/g, in Berrugas Beach; and Cd = 0.040 ± 0.010 μg/g, in Cartagena Beach). On the other hand, in a study conducted by Marrugo-Negrete et al. (2008), the concentrations of Hg-total in samples of water, sediment, seston, phytoplankton, and zooplankton from a polluted swamp located in southern Colombia (municipality of Montecristo) were determined; the concentrations obtained were 0.33 ± 0.03, 0.71 ± 0.03, 1.20 ± 0.06, 0.52 ± 0.03, and 0.94 ± 0.05 μg/L, respectively. It was also determined that the concentrations of Hg-total varied significantly, in fish, according to consumption within the trophic chain; for example, the highest average values of total mercury were found in carnivorous species such as *Caquetaia kraussii* (1.09 ± 0.17 μg/g), *Hoplias malabaricus* (0.58 ± 0.05 μg/g), and *Plagioscion surinamensis* (0.53 ± 0.07 μg/g), while the lowest were detected in non-carnivorous species such as *Prochilodus magdalenae* (0.157 ± 0.01 μg/g), indicating that not only mercury contamination directly affects the species that inhabit the bodies of water where they are present but also that it represents a serious concern for human health; besides, Alvarez and colleagues found that the highest average level of mercury in muscle tissue was found in *Pimelodus blochii* (non-carnivorous fish). However, the group of carnivorous fish had significantly higher levels of mercury in their muscle tissue, compared to non-carnivores. No differences were observed in the total mercury concentration according to species or genus (Valdelamar-Villegas and Olivero-Verbel 2018; Marrugo-Negrete et al. 2008; Alvarez et al. 2012).

One of the most important rivers in Colombia is the Magdalena River (it supplies more than 70% of the fish and drinking water), and with the purpose of evaluating its toxicity profile, Tejeda-Benitez et al. (2016) exposed wild-type strains of *Caenorhabditis elegans* to aqueous sediment extracts to evaluate end points such as survival, locomotion, and growth. Metals such as Cd, Cu, and Ni were present in the sediments (values above the permitted limits) (Tejeda-Benitez et al. 2016).

The concentrations of polycyclic aromatic hydrocarbons and heavy metals were evaluated by Marrugo-Negrete et al. (2017) in different samples from Cispata Bay: sediments, water, and fish. The concentrations of heavy metals in the sediment were in the following order: Cu > Pb > Hg > Cd; the highest concentration of mercury in fish was 0.67 μg/g, while the total concentration of polycyclic aromatic hydrocarbons ranged between 7.0 and 41 ng/g in sediments and between 0.03 and 0.34 ng/mL in water samples; in fish concentration of 53.24 ng/g was reported, and finally, the data presented by Alonso and colleagues (2014) reveal that As is present in matrices such as soil, sediments, and water and in the food chain; some of the As concentrations exceed the limits specified by national and international regulations. The highest concentrations of arsenic are associated with mining regions (e.g., soils up to 148 mg/kg and sediments up to 1400 mg/kg); this highlights the importance of focusing research on the understanding of the occurrence, origin, and distribution of As to better understand its environmental and public health impact (Marrugo-Negrete et al. 2017; Alonso et al. 2014).

1.1.5.2 Contamination by Pesticides and Its Ecotoxicological Impact

Jaramillo-Colorado et al. (2015) were the first to relate the presence of organochlorine pesticides with the development of alterations in fish from the Cartagena Bay and the presence of nematodes in the samples analyzed. The organochlorine compounds β-HCH, γ-HCH, heptachlor, aldrin, endosulfan, 4,4′-DDE, and dieldrin, among others, were detected in the liver and spleen of *Mugil incilis* fish. They produced histological changes (characterized by the presence of melano-macrophages and granulomas) that are associated with contamination by organochlorine pesticides and their possible influence with the presence of larvae of *Anisakis* spp. (1.6%), *Pseudoterranova* spp. (25.3%), and *Contracaecum* spp. (57.8%); however, the parasite found in the fish studied is not statistically associated with the concentration of chlorinated pesticides. The presence of fecal and total coliforms in Cartagena Bay could indicate poor quality of water due to fecal pollution, which could encourage parasite infections in the fish (Jaramillo-Colorado et al. 2015).

1.1.5.3 Contamination by Hydrocarbons and Its Ecotoxicological Impact

In particular, the contamination of rivers with polycyclic aromatic hydrocarbons is a subject that has received little attention in the country; however, Sarria-Villa and collaborators, in 2016, carried out an analysis in the water and sediments of the Cauca River to determine the presence of aromatic polycyclic hydrocarbon compounds. The results showed total concentrations of polycyclic aromatic hydrocarbons of 4476.5 ng/L and 1582.7 ng/g in water and sediments, respectively, with benzo[b]fluoranthene, benzo[k]fluoranthene, and pyrene in sediments and fluorene, acenaphthylene, and anthracene in water being the most detected compounds (Sarria-Villa et al. 2016).

Ahrens et al. (2017) conducted a study using the mangrove oyster *Crassostrea rhizophorae* as a sentinel species, evaluating the biological effects at different levels of complexity (stress response in stress, reproduction, condition index, biomarkers at tissue level, and histopathology). Different mixtures of persistent pollutants (As, Cd, and polycyclic aromatic hydrocarbons) and emerging chemical contaminants (musk fragrances), in combination with different levels of organic and particulate matter resulting from seasonal oceanographic variability and wastewater discharges, and environmental factors (salinity, temperature) caused a different degree of alteration in the health condition of the ecosystem, as reflected in *C. rhizophorae* sentinel (Ahrens et al. 2017).

1.1.5.4 Other Sources of Contamination and Their Ecotoxicological Impact

Endocrine disruptors have been studied for their high incidence in different environments, including aquatic ones; however, their occurrence, magnitude, and potential threat have been little studied, mainly in developing countries. In a study carried out

by Bedoya-Ríos and Lara-Borrero (2018), it is clear that the compounds with the greatest presence are plasticizers such as phthalates and bisphenol A, while among the drugs, carbamazepine had the highest concentrations (0.68–31.45 μg/L); the analysis of the hazard coefficient showed the importance of bis(2-ethylhexyl) phthalate (BEHP) and estrone (E1) that can reach the surface waters of domestic and industrial discharges (Bedoya-Ríos and Lara-Borrero 2018).

There are compounds that are known to be generalized contaminants of toxicological importance, such as perfluorinated compounds, which have been detected in water lees. Olivero-Verbel et al. (2006) determined the presence of perfluorinated compounds in the bile of fish *Mugil incilis* and in tissues of pelicans (*Pelecanus occidentalis*) of Cartagena Bay. The results showed that the compounds detected were perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonate (PFHxS), and perfluorooctanesulfonamide (PFOSA), indicating that in fish, the average concentrations obtained for PFOS, PFOA, and PFHxS were 3,673, 370, and 489 ng/mL, respectively, while the concentrations of PFOS in the pelican organs decreased in the order of the spleen > liver > lung > kidney > brain > heart > muscle (Olivero-Verbel et al. 2006).

1.1.6 Contamination of Water Bodies in Other Countries in Latin America

1.1.6.1 Contamination of Water Bodies in Peru

A clear example that anthropogenic activities contribute to the contamination of the various water bodies in Latin America is reported by Yusta-Garcia et al. (2017). Through a meta-analysis of 2951 samples of water collected in four basins of Amazonian rivers (Marañón, Tigre, Corrientes, and Pastaza) and 652 chemical analyses of water produced from government institutions and reports of oil companies located in the region, it was determined that discharges of produced water had much higher concentrations of chloride, barium, cadmium, and lead than those typically found in freshwater, resulting in widespread contamination of natural watercourses. A significant number of samples showed levels of cadmium, barium, hexavalent chromium, and lead that did not comply with Peruvian and international water standards, which represents a serious risk for the indigenous population and the wildlife of the region, the same risk that has been present for several decades (Yusta-García et al. 2017).

1.1.6.2 Contamination of Water Bodies in Ecuador

Voloshenko-Rossin et al. (2015) evaluated the water quality of the San Pedro-Guayllabamba-Esmeraldas River. The river has an annual flow of approximately 22,000 mm^3 per year and collects wastewater from Quito in the Andes and supplies drinking water to the city of Esmeraldas, near the Pacific Ocean. The presence of

persistent emerging pollutants such as carbamazepine and acesulfame was determined, which were stable along the river flow. Conversely, other contaminants such as caffeine, sulfamethoxazole, venlafaxine, O-desmethylvenlafaxine, and steroid estrogens were found to be greatly degraded over the 300-km flow. These last pollutants showed to be persistent even after a filtration treatment in the potable water system of the city of Esmeraldas, being below 20 ng/L, a lower level than those found in Europe and North America. This study also determined the presence of benzoylecgonine, a cocaine metabolite, presumably due to the fact that coca plantations and wild coca trees are located in the region, which shows that anthropogenic activities contribute significantly to pollution of bodies of water (Voloshenko-Rossin et al. 2015).

1.2 Conclusions

In this chapter we made a review of the studies conducted in Latin America through which the presence and toxic effects of the contaminants present in the different bodies of water are determined. In general, the main pollutants that were subject of the studies reviewed in this chapter were heavy metals, pesticides, hydrocarbon compounds, plastics, organic compounds, and others, and anthropogenic activities were identified as the main sources of contamination, in particular those related to various industries, as well as tourism and recreation activities, and drug abuse, among others.

Heavy metals were a constant in all the studies reviewed; metals such as As, Ag, B, Ba, Bi, Ca, Cd, Co, Cr, Fe, Ga, Hg, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Se, Sn, Sr, Te, Ti, U, V, and Zn were, in most cases, found in values that exceeded local and international regulations, cataloging heavy metals as one of the main sources of water body contaminants in Latin America, representing a serious problem at the local level with direct effects on organisms that inhabit the ecosystems and, probably, becoming a human health problem.

Another recurrent source of contamination of water bodies reported in countries of Latin America is the indiscriminate use of pesticides. As reported in several works, these compounds are used mainly in agricultural activities, and although most of them are banned or have not been reported to have toxic effects, we find that their use is frequent nowadays; the pesticides that were reported in different sources evaluated include simazine, hexazinone, 2,4-D, picloram, endosulfan, cypermethrin, and chlorpyrifos, among others, which were found in surface waters and waters with irrigation use of crops and livestock.

Hydrocarbons such as benzo[b]fluoranthene, benzo[k]fluoranthene, and pyrene and other polycyclic aromatic hydrocarbons were reported by various working groups in water bodies in Latin America; the main sources of contamination of these compounds were spills in the extraction of hydrocarbons, but other sources from the industry are not ruled out.

Other sources of contamination reported in water bodies were contamination by plastics and their derivatives. Compounds such as butyltin (TBts = TBT + DBT + MBT) and micro- and macroplastics were present and were identified as an important source of contamination that, like the aforementioned, can have toxic effects on the biota of water bodies. Contaminants of biological origin such as adenovirus (HAdV, BAdV, CAV1-2, PAdV, and AvAdV) and endocrine-disrupting compounds (estrone and 17-α-ethinyl estradiol) were also present in the studies reviewed in this chapter.

It is important to note that in the reviewed studies not only physicochemical parameters were evaluated to determine the quality of the water, but also bioindicators were used to evaluate the toxic effects caused by the contaminants (heavy metals, pesticides, hydrocarbon compounds, plastics, organic compounds, and others). Among the most used bioindicators are *Lingulodinium polyedrum*, *Baccharis sarothroides* Gray, *E. imbricata*, *Crassostrea corteziensis*, *Cyathea costaricensis*, *Rhinella arenarum*, *Cyprinus carpio*, *Pimelodella laticeps*, and *Daphnia* sp., among others.

Civil society, government, and science and technology need to work together to reestablish the state of health of water bodies in Latin America, creating effective programs to prevent and/or redeem the toxic effects produced by the various pollutants. It is important to continue with studies on the ecotoxicological impact and, in selected cases, to continue existing studies, to generate a complete picture about the problem that, although this chapter is focused on Latin America, is a global problem.

References

Acosta CA, Pasquali CEL, Paniagua G, Garcinuño RM, Hernando PF (2018) Evaluation of total phenol pollution in water of San Martin Canal from Santiago del Estero, Argentina. Environ Pollut 236:265–272

Ahrens MJ et al (2017) Assessment of ecosystem health disturbance in mangrove-lined Caribbean coastal systems using the oyster Crassostrea rhizophorae as sentinel species. Sci Total Environ 618:718–735

Alonso DL, Latorre S, Castillo E, Brandão PFB (2014) Environmental occurrence of arsenic in Colombia: a review. *Environ Pollut* 186:272–281

Alvarez S, Kolok AS, Jimenez LF, Granados C, Palacio JA (2012) Mercury concentrations in muscle and liver tissue of fish from marshes along the Magdalena river, Colombia. Bull Environ Contam Toxicol 89(4):836–840

Arcagni M et al (2017) Mercury and selenium in the food web of Lake Nahuel Huapi, Patagonia, Argentina. Chemosphere 166:163–173

Arellano-Aguilar O, Betancourt-Lozano M, Aguilar-Zárate G, Ponce de Leon-Hill C (2017) Agrochemical loading in drains and rivers and its connection with pollution in coastal lagoons of the Mexican Pacific. Environ Monit Assess 189(6):270

Avigliano E, Schenone NF, Volpedo AV, Goessler W, Fernández Cirelli A (2015) Heavy metals and trace elements in muscle of silverside (Odontesthes bonariensis) and water from different environments (Argentina): aquatic pollution and consumption effect approach. Sci Total Environ 506–507:102–108

Babcock-Adams L, Chanton JP, Joye SB, Medeiros PM (2017) Hydrocarbon composition and concentrations in the Gulf of Mexico sediments in the 3 years following the Macondo well blowout. Environ Pollut 229:329–338

Babini MS, C de Bionda L, Salas NE, Martino AL (2016) Adverse effect of agroecosystem pond water on biological endpoints of common toad (Rhinella arenarum) tadpoles. *Environ Monit Assess* 188(8):459

Barbero M et al (2012) Occurrence of arsenic species in algae and freshwater plants of an extreme arid region in northern Chile, the Loa River Basin. Chemosphere 90(2):556–564

Bedoya-Ríos DF, Lara-Borrero JA (2018) Occurrence of endocrine disruptor chemicals in the urban water cycle of Colombia. Endocr Disruptors 1:1–15

Camacho LM, Gutiérrez M, Alarcón-Herrera MT, M de Villalba L, Deng S (2011) Occurrence and treatment of arsenic in groundwater and soil in northern Mexico and southwestern USA. Chemosphere 83(3):211–225

Carro MM et al (2008) Importance of diffuse pollution control in the Patzcuaro Lake Basin in Mexico. Water Sci Technol 58(11):2179–2186

Cazenave J, Bacchetta C, Parma MJ, Scarabotti PA, Wunderlin DA (2009) Multiple biomarkers responses in Prochilodus lineatus allowed assessing changes in the water quality of Salado River basin (Santa Fe, Argentina). Environ Pollut 157(11):3025–3033

Celis-Hernández O, Rosales-Hoz L, Carranza-Edwards A (2013) Heavy metal enrichment in surface sediments from the SW Gulf of Mexico. Environ Monit Assess 185(11):8891–8907

da Costa EP, Pinto CC, Soares ALC, Melo LDV, Oliveira SMAC (2017) Evaluation of violations in water quality standards in the monitoring network of São Francisco River basin, the third largest in Brazil. *Environ Monit Assess* 189(11):590

de Andrade LC, Tiecher T, de Oliveira JS, Andreazza R, Inda AV, de Oliveira Camargo FA (2018) Sediment pollution in margins of the Lake Guaíba, Southern Brazil. *Environ Monit Assess* 190(1):3

De Gerónimo E, Aparicio VC, Bárbaro S, Portocarrero R, Jaime S, Costa JL (2014) Presence of pesticides in surface water from four sub-basins in Argentina. Chemosphere 107:423–431

Di Mauro R, Kupchik MJ, Benfield MC (2017) Abundant plankton-sized microplastic particles in shelf waters of the northern Gulf of Mexico. Environ Pollut 230:798–809

Esteller MV, Rodríguez R, Cardona A, Padilla-Sánchez L (2012) Evaluation of hydrochemical changes due to intensive aquifer exploitation: case studies from Mexico. Environ Monit Assess 184(9):5725–5741

Etchegoyen M, Ronco A, Almada P, Abelando M, Marino D (2017) Occurrence and fate of pesticides in the Argentine stretch of the Paraguay-Paraná basin. *Environ Monit Assess* 189(2):63

Fernández C, Salerno CM, Paoloni JD, Laurent GC (2007) Water quality in a lagoon in the southeast pampa region of Argentina. Rev Argent Microbiol 39(1):51–56

Figueroa L et al (2012) Environmental Lithium exposure in the North of Chile-I. Natural water sources. Biol Trace Elem Res 149:280

Fossi MC et al (2017) Are whale sharks exposed to persistent organic pollutants and plastic pollution in the Gulf of California (Mexico)? First ecotoxicological investigation using skin biopsies. Comp Biochem Physiol C Toxicol Pharmacol 199:48–58

Furtado C, von Mühlen C (2015) Endocrine disruptors in water filters used in the Rio dos Sinos Basin region, Southern Brazil. Braz J Biol 75(2 suppl):85–90

Gajardo M, Urrutia R, Barra R, Debels P, Cooman K (2005) Use of Daphnia spp. for the ecotoxicological assessment of water quality in an agricultural watershed in South-Central Chile. Arch Environ Contam Toxicol 48(2):191–200

Girón-Pérez MI et al (2013) Evaluation of pollution in Camichin estuary (Mexico): pro-oxidant and antioxidant response in oyster (Crassostrea corteziensis). Comp Biochem Physiol A Mol Integr Physiol 165(4):476–482

Griboff J, Horacek M, Wunderlin DA, Monferran MV (2018) Bioaccumulation and trophic transfer of metals, As and Se through a freshwater food web affected by antrophic pollution in Córdoba, Argentina. *Ecotoxicol Environ Saf* 148:275–284

Guerrero Barrera AL, Yamamoto Flores L, Ramos Gómez MS, Flores de la Torre JA, Avelar González FJ, Mitchell KN (2016) Evaluation of environmental risk of metal contaminated soils and sediments near mining sites in Aguascalientes, Mexico. Bull Environ Contam Toxicol 97(2):216–224

Gutierrez-Mejia E, Lares ML, Huerta-Diaz MA, Delgadillo-Hinojosa F (2016) Cadmium and phosphate variability during algal blooms of the dinoflagellate Lingulodinium polyedrum in Todos Santos Bay, Baja California, Mexico. Sci Total Environ 541:865–876

Jakomin LM, Marbán L, Grondona S, Glok Galli M, Martínez DE (2015) Mobility of heavy metals (Pb, Cd, Zn) in the Pampeano and Puelche aquifers, Argentina: partition and retardation coefficients. Bull Environ Contam Toxicol 95(3):325–331

Jaramillo-Colorado BE, Arroyo-Salgado B, Ruiz-Garcés LC (2015) Organochlorine pesticides and parasites in Mugil incilis collected in Cartagena Bay, Colombia. Environ Sci Pollut Res 22(22):17475–17485

Mansilla HD, Miranda C, Flegal AR, Yáñez J, Guajardo M, Soto C (2013) New assessment of organic mercury formation in highly polluted sediments in the Lenga estuary, Chile. Mar Pollut Bull 73(1):16–23

Marrugo-Negrete J, Benitez LN, Olivero-Verbel J (2008) Distribution of mercury in several environmental compartments in an aquatic ecosystem impacted by gold mining in northern Colombia. Arch Environ Contam Toxicol 55(2):305–316

Marrugo-Negrete J, Urango-Cárdenas I, Navarro-Frómeta A, Burgos-Núñez S, Enamorado-Montes G (2017) Polycyclic aromatic hydrocarbons and heavy metals in the Cispata Bay, Colombia: a marine tropical ecosystem. Mar Pollut Bull 120(1–2):379–386

Medeiros AC et al (2017) Quality index of the surface water of Amazonian rivers in industrial areas in Pará Brazil. Mar Pollut Bull 123(1–2):156–164

Mendoza-Carranza M, Sepúlveda-Lozada A, Dias-Ferreira C, Geissen V (2016) Distribution and bioconcentration of heavy metals in a tropical aquatic food web: a case study of a tropical estuarine lagoon in SE Mexico. Environ Pollut 210:155–165

Mora A, Mahlknecht J, Rosales-Lagarde L, Hernández-Antonio A (2017) Assessment of major ions and trace elements in groundwater supplied to the Monterrey metropolitan area, Nuevo León, Mexico. Environ Monit Assess 189(8):1–15

Ochoa-Rivero JM et al (2017) Levels and distribution of pollutants in thewaters of an aquatic ecosystem in Northern Mexico. Int J Environ Res Public Health 14(5). https://doi.org/10.3390/ijerph14050456

Olivero-Verbel J et al (2006) Perfluorooctanesulfonate and related fluorochemicals in biological samples from the north coast of Colombia. Environ Pollut 142(2):367–372

Palma G, Śnchez A, Olave Y, Encina F, Palma R, Barra R (2004) Pesticide levels in surface waters in an agricultural-forestry basin in Southern Chile. Chemosphere 57(8):763–770

Parra S, Bravo MA, Quiroz W, Querol X, Paipa C (2015) Distribution and pollution assessment of trace elements in marine sediments in the Quintero Bay (Chile). Mar Pollut Bull 99(1–2):256–263

Phillips MB, Bonner TH (2015) Occurrence and amount of microplastic ingested by fishes in watersheds of the Gulf of Mexico. Mar Pollut Bull 100(1):264–269

Pizarro J, Vergara PM, Rodríguez JA, Valenzuela AM (2010) Heavy metals in northern Chilean rivers: spatial variation and temporal trends. J Hazard Mater 181(1–3):747–754

Pozo K et al (2011) Levels and spatial distribution of polycyclic aromatic hydrocarbons (PAHs) in sediments from Lenga Estuary, Central Chile. Mar Pollut Bull 62(7):1572–1576

Queirolo F et al (2000) Total arsenic, lead, cadmium, copper, and zinc in some salt rivers in the northern Andes of Antofagasta, Chile. Sci Total Environ 255:85

Quintas PY et al (2017) Organotin compounds in Brachidontes rodriguezii mussels from the Bahía Blanca Estuary, Argentina. *Ecotoxicol Environ Saf* 145:518–527

Rendón-Von Osten J, Dzul-Caamal R (2017) Glyphosate residues in groundwater, drinking water and urine of subsistence farmers from intensive agriculture localities: a survey in Hopelchén, Campeche, Mexico. Int J Environ Res Public Health 14(6). https://doi.org/10.3390/ijerph14060595

Rigotto C et al (2015) Surface water quality in the Sinos River basin, in Southern Brazil: tracking microbiological contamination and correlation with physicochemical parameters. Environ Sci Pollut Res 22(13):9899–9911

Rivera L et al (2015) Trace metal variability in coastal waters of San Jorge Bay, Antofagasta, Chile: an environmental evaluation and statistical approach to propose local background levels. Mar Pollut Bull 100(1):544–554

Rodríguez-Espinosa PF et al (2011) Metal concentrations in water and sediments from tourist beaches of Acapulco, Mexico. Mar Pollut Bull 62(4):845–850

Rodríguez-Romero AJ, Rico-Sánchez AE, Catalá M, Sedeño-Díaz JE, López-López E (2017) Mitochondrial activity in fern spores of Cyathea costaricensis as an indicator of the impact of land use and water quality in rivers running through cloud forests. Chemosphere 189:435–444

Ruiz-Ruiz E, Hernández-Ramírez A, Maya-Treviño L, Chapa-Martínez CA, Guzmán-Mar JL, Hinojosa-Reyes L (2016) An evaluation of the migration of antimony from polyethylene terephthalate (PET) plastic used for bottled drinking water. Sci Total Environ 565:511–518

Santucci L, Carol E, Tanjal C (2018) Industrial waste as a source of surface and groundwater pollution for more than half a century in a sector of the Río de la Plata coastal plain (Argentina). Chemosphere 206:727–735

Sarria-Villa R, Ocampo-Duque W, Páez M, Schuhmacher M (2016) Presence of PAHs in water and sediments of the Colombian Cauca River during heavy rain episodes, and implications for risk assessment. Sci Total Environ 540:455–465

Scarcia P, Calamante G, De la Torre F (2014) Responses of biomarkers of a standardized (Cyprinus carpio) and a native (Pimelodella laticeps) fish species after in situ exposure in a periurban zone of Luján river (Argentina). Environ Toxicol 29(5):545–557

Silva LMA et al (2014) Non-targeted analyses of organic compounds in urban wastewater. Magn Reson Chem 53(9):704–710

Tejeda-Benitez L, Flegal R, Odigie K, Olivero-Verbel J (2016) Pollution by metals and toxicity assessment using Caenorhabditis elegans in sediments from the Magdalena River, Colombia. Environ Pollut 212:238–250

Toledo-Ibarra GA et al (2016) Assessment of pollution of the Boca de Camichin Estuary in Nayarit (Mexico) and its influence on oxidative stress in Crassostrea corteziensis oysters. Comp Biochem Physiol A Mol Integr Physiol 200:47–55

Torres Z, Mora MA, Taylor RJ, Alvarez-Bernal D (2016) Tracking metal pollution in Lake Chapala: concentrations in water, sediments, and fish. Bull Environ Contam Toxicol 97(3):418–424

Tremblay N, Ortíz Arana A, González Jáuregui M, Rendón-von Osten J (2017) Relationship between organochlorine pesticides and stress indicators in hawksbill sea turtle (Eretmochelys imbricata) nesting at Punta Xen (Campeche), Southern Gulf of Mexico. Ecotoxicology 26(2):173–183

Valdelamar-Villegas J, Olivero-Verbel J (2018) Bioecological aspects and heavy metal contamination of the mollusk Donax denticulatus in the Colombian Caribbean coastline. Bull Environ Contam Toxicol 100(2):234–239

Valenzuela A et al (2006) Effects of pulp and paper mill discharges on caged rainbow trout (Oncorhynchus mykiss): biomarker responses along a pollution gradient in the Biobio River, Chile. *Environ Toxicol Chem* 25(9):2280

Voloshenko-Rossin A et al (2015) Emerging pollutants in the Esmeraldas watershed in Ecuador: discharge and attenuation of emerging organic pollutants along the San Pedro-Guayllabamba-Esmeraldas rivers. Environ Sci Process Impacts 17(1):41–53

Yusta-García R, Orta-Martínez M, Mayor P, González-Crespo C, Rosell-Melé A (2017) Water contamination from oil extraction activities in Northern Peruvian Amazonian rivers. *Environ Pollut* 225(November):370–380

Chapter 2
Toxicity Produced by an Industrial Effluent from Mexico on the Common Carp (*Cyprinus carpio*)

Nely San Juan-Reyes, Leobardo Manuel Gómez-Oliván, Hariz Islas-Flores, Marcela Galar-Martínez, Sandra García-Medina, and Ricardo Pérez-Pastén Borja

2.1 Introduction

More than 4000 pharmaceuticals are commercially produced each year up to hundreds of tons for human and animal care; these products enter the environment through various sources, among which are wastewater treatment plants (WWTPs), hospitals, landfills, and pharmaceutical production plants (Lillenberg et al. 2010; Rehman et al. 2015). Pharmaceutical industry wastewaters proceed for the most part from production process and the cleaning of machinery and may contain organic solvents, catalysts, additants, reactants, intermediates, raw materials, and active pharmaceutical ingredients (APIs) at different concentrations (Sreekanth et al. 2009). In the environment, APIs and their metabolites are normally found in the range from ng L^{-1} to μg L^{-1}; these compounds resist their complete removal in WWTPs, due to their lipophilic and non-biodegradability nature, as well as their biological activities (Velagaleti and Burns 2006).

Among the main pharmacotherapeutic groups detected in water systems in Mexico are beta-blockers (0.01–3.10 μg L^{-1}) (Siemens et al. 2008; Pérez-Alvarez et al. 2018; Rivera-Jaimes et al. 2018; Luja-Mondragón et al. 2019), lipid-lowering agents (0.001–

N. San Juan-Reyes (✉)
Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

Laboratorio de Toxicología Acuática, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, Mexico City, Mexico

L. M. Gómez-Oliván · H. Islas-Flores
Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

M. Galar-Martínez · S. García-Medina · R. P.-P. Borja
Laboratorio de Toxicología Acuática, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, Mexico City, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_2

3.445 μg L^{-1}) (Siemens et al. 2008; Rivera-Jaimes et al. 2018; Gibson et al. 2010; Félix-Cañedo et al. 2013), anti-infective agents (0.01–4.01 μg L^{-1}) (Siemens et al. 2008; Pérez-Alvarez et al. 2018; Rivera-Jaimes et al. 2018; Luja-Mondragón et al. 2019), hormones (0.018–10.38 μg L^{-1}) (Pérez-Alvarez et al. 2018; Luja-Mondragón et al. 2019; Díaz-Torres et al. 2013), antiepileptics and psychoanaleptics (0.052–0.476 μg L^{-1}) (Rivera-Jaimes et al. 2018; Gibson et al. 2010), antidiabetic medications (1.31–2.03 μg L^{-1}) (Pérez-Alvarez et al. 2018; Luja-Mondragón et al. 2019), and anti-inflammatory agents (0.0004–14.9 μg L^{-1}) (Siemens et al. 2008; Pérez-Alvarez et al. 2018; Rivera-Jaimes et al. 2018; Luja-Mondragón et al. 2019; Gibson et al. 2010; Félix-Cañedo et al. 2013; González-González et al. 2014; Neri-Cruz et al. 2014). The latter group includes nonsteroidal anti-inflammatory drugs (NSAIDs). In Mexico, these drugs are marketed in various pharmaceutical forms and are irrationally used since they are sold without prescription (SanJuan-Reyes et al. 2013). NSAIDs are commonly used for the relief of fever, inflammation, and reduction of pain, since they act as selective inhibitors of the enzyme cyclooxygenase (COX); this enzyme is responsible for production of prostaglandins, prostacyclins, and thromboxanes (Gonzalez-Rey and Bebianno 2011). During the biotransformation of NSAIDs, reactive oxygen species (ROS) are produced and can elicit and/or contribute to oxidative stress generation and, consequently, DNA damage, as well as embryotoxicity and teratogenicity if oxidative stress occurs during embryonic development (Dennery 2007).

The occurrence of NSAIDs in the environment is of great concern due to their potential ecotoxicological effect on the aquatic organisms at different trophic levels (Cycoń et al. 2016). Several studies in Mexico have shown the damage generated to lipids, proteins, and DNA by NSAIDs (diclofenac (DCF), paracetamol (PCT), ibuprofen (IBP), naproxen (NPX), acetylsalicylic acid and ketorolac) in different aquatic species like *Daphnia magna*, *Hyalella azteca*, and *Cyprinus carpio* (Oviedo-Gómez et al. 2010; Gómez-Oliván et al. 2012, 2014a, b, c; Islas-Flores et al. 2013, 2014; Nava-Álvarez et al. 2014; Saucedo-Vence et al. 2015; Galar-Martínez et al. 2016). Bioindicators are an effective tool for the monitoring of environmental contamination. Fish interact extensively with water and its sediments; for this reason they may reflect the effects of pollution (SanJuan-Reyes et al. 2017). The common carp (*C. carpio*) is a freshwater fish and is found in a wide geographic distribution. This organism has high economic importance for the global aquaculture industry due to fish protein is one of the most important protein sources for human consumption (Xu et al. 2014).

The present study aimed to evaluate the damage induced by an industrial effluent on common carp (*Cyprinus carpio*).

2.2 Methods

2.2.1 Test Substances

Reagents in this section were obtained from Sigma-Aldrich (St. Louis MO).

2.2.2 *Sampling of Industrial Effluent and Physicochemical Characterization*

Effluent from an NSAID-manufacturing plant in Toluca (State of Mexico) was sampled using the procedure stipulated in the official Mexican norm for wastewater sampling (NMX-AA-003-1980 1980). It is worth noting that industrial waste receives no treatment and goes directly to the municipal effluent from the city of Toluca. After sampling, the physicochemical characterization of effluents was realized following the official Mexican norms that set the maximum permissible levels of contaminants in wastewater discharges arising in the pharmaceutical and pharmacochemical industries (NOM-073-ECOL-1994 1994) and entering, respectively, domestic waters and resources and receiving water bodies (NOM-001-SEMARNAT-1996 1996). Also, APHA, AWWA, and WPCF (1995) set that the standard methods were used for the examination of water and wastewater.

2.2.3 *Quantification of NSAIDs by Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS)*

The high-performance liquid chromatography (HPLC)–MS/MS system used was an Agilent 1290 Infinity HPLC unit (Santa Clara, CA). The RRHD Eclipse Plus C18 chromatography column (2.1 × 50 mm, 1.8 μm) was maintained at 40 °C. Standards of 10 μg mL^{-1} of DCF, IBP, NPX, and PCT were prepared in a 60:40 mixture of acetonitrile and ammonium formate at pH 6 and stored in the dark at −8 °C in amber glass bottles. The mobile phase was a 60:40 v/v mixture of acetonitrile and 10 mM ammonium formate. Flow rate was 0.3 mL min^{-1}, run time 1.8 min, and injection volume 2 μL. DCF, IBP, NPX, and PCT were quantified on an Agilent 6430 Triple Quadrupole MS equipped with electrospray ionization (ESI). The ESI-positive mode was used throughout. Electrospray voltage operated at 4000 V as the MS collected data in the negative ion mode. MS optimization was performed by direct infusion of a 10 μg mL^{-1} standard solution of DCF, IBP, NPX, and PCT; thereafter, the ionization mode and precursor ion mode were selected.

2.2.3.1 Water

Water samples (5 mL) were collected from the different test systems in glass vials and refrigerated at 4 °C. Samples were vacuum-filtered through 1–0 μm GF/C glass microfiber filters, followed by 0.45 μm nylon membrane filters. A liquid–liquid extraction with 5 mL (1:1, v/v) hexane/ethyl acetate was performed to extract DCF, IBP, NPX, and PCT from 1 mL water samples. These samples were centrifuged at 1800× *g* for 10 min, and the upper organic layer was extracted again. This extraction was repeated, and organic layers were combined and evaporated to dryness. Results were expressed as time-weighted average concentrations of DCF, IBP, NPX, and PCT.

2.2.4 Fish Procurement

Carps were obtained from the aquaculture facility in Tiacaque (State of Mexico), with the following characteristics: 18.39 ± 0.31 cm in length and weight 50.71 ± 7.8 g. Prior to the toxicity evaluation, organisms were maintained for 40 days in water complemented with salts: $NaHCO_3$ (174 mg L^{-1}), $MgSO_4$ (120 mg L^{-1}), KCl (8 mg L^{-1}), and $CaSO_4 \cdot 2H_2O$ (120 mg L^{-1}), at 20 ± 2 °C and exposed to natural light/dark photoperiods.

2.2.5 Determination of Median Lethal Concentration

The median lethal concentration (LC50) of the industrial effluent was determined. The systems containing different proportions of industrial effluent in water (previously reconstituted with salts; see Sect. 2.4) and an effluent-free control system were set up, and ten carps randomly selected from the stock were placed in each system. Static systems were used with natural light/dark photoperiod, and no food was provided to specimens during the exposure period. Duration of the exposure period was 96 h, at the end of which the number of dead specimens in each system was counted. The assay was performed in quintuplicate. The 96 h LC50 of industrial effluent and its 95% confidence limits ($P < 0.05$) were estimated by Probit analysis (EPA, v1.5).

2.2.6 Experimental Design

2.2.6.1 Oxidative Stress, Genotoxicity, and Cytotoxicity

Test systems consisting of 120 × 80 × 40 cm glass tanks filled with water were maintained at room temperature with constant aeration and a natural light/dark photoperiod. Static systems were used, and no food was provided to specimens during the exposure period. Industrial effluents were added to each system at a concentration equal to the lowest level of adverse effects observed, that is, 0.1173%, to five test systems with six tents each. The kinetics were performed for the following exposure periods: 12, 24, 48, 72, and 96 h. An effluent-free industrial control system was established with six tents for each exposure period, and sublethal tests were carried out in triplicate. A total of 180 fish were used in these trials. At the end of the period, the fish were removed from the systems and placed in a tank containing a xylocaine solution (0.02 mg mL^{-1}, AstraZeneca, State of Mexico, Mexico). The anesthetized samples were placed in lateral position. Blood was removed with a heparinized 1 mL hypodermic syringe by puncture of the caudal vessel performed laterally near the base of the caudal peduncle, at mid-height of the anal fin and ventral to the lateral line. After puncture, specimens were placed in an ice bath and

sacrificed. The gill, brain, and liver were removed, placed in phosphate buffer solution pH 7.4, and homogenized. The supernatant was centrifuged at 12500× *g* and −4 °C for 15 min. The following biomarkers were then evaluated: hydroperoxide content (HPC) (Jiang et al. 1992), lipid peroxidation (LPX) (Büege and Aust 1978), protein carbonyl content (PCC) (Levine et al. 1994; Parvez and Raisuddin 2005; Burcham 2007), the activity of the antioxidant enzymes superoxide dismutase (SOD) (Misra and Fridovich 1972), catalase (CAT) (Radi et al. 1991) and glutathione peroxidase (GPX) (Gunzler and Flohe-Clairborne 1985; Stephensen et al. 2000), genotoxicity (DNA damage was evaluated by comet assay (Tice et al. 2000), micronucleus test (Çavaş and Ergene-Gözükara 2005; Kim and Hyun 2006; Bolognesi et al. 2006)), and cytotoxicity (caspase-3 activity using colorimetric assay CaspACE™, Promega kit, and TUNEL assay with the ApopTag Fluorescein S7110 kit).

The importance of the organs studied is the following: the *gills* are the main organs in contact with the water and consequently with the xenobiotics; additionally, gills are known to be a site with a high oxidative metabolism; the *brain* and the nervous systems are inadequately equipped with antioxidant defense systems to prevent oxidative damage and therefore are prone to oxidative stress; the *liver* is the main organ of biotransformation of the xenobiotics; the *blood* transports proteins and other biomolecules to all tissues of the body, in addition to xenobiotics.

2.2.7 Statistical Analysis

In the acute toxicity assay (96 h LC50 of industrial effluent), Probit analysis was performed and significantly assessed by the degree of 95% LC50 overlap (EPA Analysis Program v3.3; US-EPA 2013). Results of oxidative stress biomarkers were statistically evaluated by one-way analysis of variance (ANOVA), followed by Tukey–Kramer multiple comparisons test, with P set at <0.05. For geno- and cytotoxicity, data normality and homoscedasticity were verified using the Shapiro–Wilk and Levene's tests, respectively. A Bonferroni post hoc test was applied in order to evaluate significant differences, with P set at <0.05. Statistical determinations were made with SPSS v10 software (SPSS, Chicago IL, USA).

2.3 Results

2.3.1 Physicochemical Characteristics of the Industrial Effluent

Table 2.1 shows the physicochemical characteristics of the industrial effluent, which do not exceed the limits established in the official Mexican norms NOM-001-SEMARNAT-1996 (1996) and NOM-073- ECOL-1994 (1994). Nevertheless, there

Table 2.1 Physicochemical characteristics of the industrial effluent analyzed

Physicochemical characteristics	Industrial effluent
Temperature (°C)	15.6
Dissolved oxygen (mg L^{-1})	12.2
Conductivity (μS cm^{-1})	143.2
pH	6.3
Chlorides (mg L^{-1})	101
Fluorides (mg L^{-1})	3.8
Hardness (mg L^{-1})	245.7
Ammonia (mg L^{-1})	0.73
Total suspended solids (mg L^{-1})	36
Total P (mg L^{-1})	7.3
Total N (mg L^{-1})	18
Biochemical oxygen demand (mg L^{-1})	33
NaClO (mg L^{-1})	1.0

are four parameters that are not considered in either of these norms: dissolved oxygen was 12.2 mg L^{-1}; conductivity, 143.2 μS cm^{-1}; ammonia, 0.73 mg L^{-1}; and NaClO, 1.0 mg L^{-1}.

2.3.2 Quantification of NSAIDs

The NSAIDs detected in the effluent are shown in Fig. 2.1. DCF, IBP, NPX, and PCT were detected at concentrations of 27.27–3034.41 μg L^{-1}. A decrease in the NSAIDs content in the water is observed as the exposure time increases.

2.3.3 Determination of LC50

The 96 h LC50 of the industrial effluent was 1.173%, with a 95% confidence interval of (1.059–1.283). The $\chi 2$ linear adjustment test was not significant at $P < 0.05$.

2.3.4 Evaluation of Oxidative Damage and Antioxidant Enzymes Activity

HPC, LPX, and PCC results and activity of SOD, CAT, and GPX enzymes are shown in Table 2.2. Significant increases with respect to the control group ($P < 0.05$) were observed in: *HPC* in gill and blood from 24 to 96 h, brain at 96 h, and liver at 24 and 48 h; *LPX* in gill from 12 to 48 h, liver at 48 h, and blood at 24 h; *PCC* in gill at all exposure times, brain and liver at 48 h, and blood at 12 h; *SOD* in

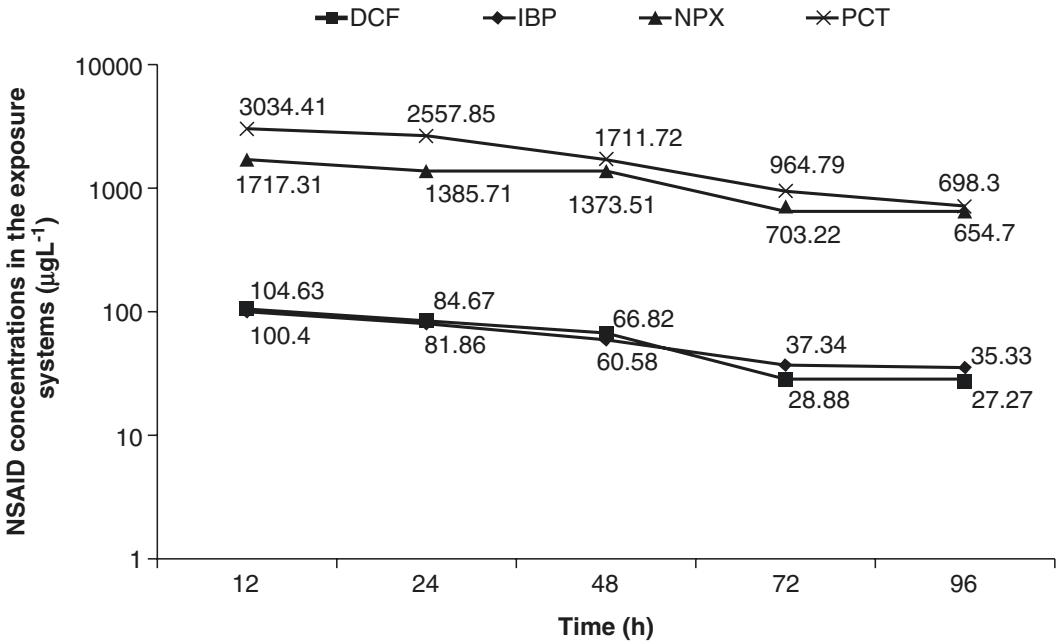

Fig. 2.1 NSAID concentrations in the exposure systems. Values are the mean of five replicates

Table 2.2 Values and (percentages) of increases and decreases of oxidative damage and antioxidant activity against the control group. Values are the mean

Oxidative damage				Antioxidant activity			
Biomarkers	**Organs**	**Time (h)**	**Industrial effluent**	**Biomarkers**	**Organs**	**Time (h)**	**Industrial effluent**
HPC (nM CHP/mg protein)	Gill	12	0.145 (↑3)	*SOD* (IU/mg protein)	Gill	12	8.318 (↑11)
		24	0.155 (↑28*)			24	8.978 (↑18)
		48	0.199 (↑45*)			48	10.182 (↑40*)
		72	0.220 (↑51*)			72	10.541 (↑43*)
		96	0.486 (↑238*)			96	13.137 (↑59*)
	Brain	12	0.054 (↑4)		Brain	12	4.508 (↓19)
		24	0.059 (↓1)			24	5.229 (↓3)
		48	0.076 (↑43)			48	5.965 (↑9)
		72	0.090 (↑49)			72	4.439 (↓19)
		96	0.128 (↑112*)			96	4.010 (↓30)
	Liver	12	0.009 (↑37)		Liver	12	2.405 (↑35)
		24	0.017 (↑206*)			24	3.099 (↑80)
		48	0.017 (↑180*)			48	5.286 (↑203*)
		72	0.009 (↑51)			72	2.143 (↑53)
		96	0.006 (↑20)			96	2.168 (↑17)
	Blood	12	0.249 (↑9)		Blood	12	5.773 (↑72*)
		24	0.290 (↑23*)			24	7.909 (↑112*)
		48	0.333 (↑32*)			48	4.707 (↑36)
		72	0.370 (↑53*)			72	3.643 (↑11)
		96	0.684 (↑224*)			96	3.702 (↑5)

(continued)

Table 2.2 (continued)

Oxidative damage				Antioxidant activity			
Biomarkers	**Organs**	**Time (h)**	**Industrial effluent**	**Biomarkers**	**Organs**	**Time (h)**	**Industrial effluent**
LPX (mM MDA/ mg protein)	Gill	12	33.426 (↑126*)	*CAT* (μM H_2O_2/ mg protein)	Gill	12	3744.199 (↑2)
		24	27.660 (↑67*)			24	5970.476 (↑93*)
		48	22.321 (↑44*)			48	6830.450 (↑105*)
		72	19.083 (↑22)			72	7260.104 (↑95*)
		96	19.196 (↑19)			96	10073.130 (↑190*)
	Brain	12	7.470 (↑11)		Brain	12	2638.755 (↑8)
		24	7.724 (↑18)			24	2670.879 (↑12)
		48	7.497 (↑11)			48	3205.007 (↑34)
		72	7.267 (↑17)			72	3671.960 (↑78*)
		96	7.430 (↑1)			96	3857.562 (↑39)
	Liver	12	4.135 (↑23)		Liver	12	1294.923 (↑6)
		24	4.255 (↑13)			24	1454.212 (↑8)
		48	8.838 (↑151*)			48	1456.637 (↑8)
		72	3.457 (↑12)			72	1516.269 (↑11)
		96	3.469 (↓1)			96	1571.431 (↑12)
	Blood	12	6.331 (↑60)		Blood	12	544.385 (↑215)
		24	10.312 (↑156*)			24	761.439 (↑419)
		48	6.494 (↑50)			48	777.948 (↑433)
		72	5.700 (↑50)			72	803.899 (↑334)
		96	5.636 (↑37)			96	1966.799 (↑1041*)

(continued)

Table 2.2 (continued)

Oxidative damage				Antioxidant activity			
Biomarkers	**Organs**	**Time (h)**	**Industrial effluent**	**Biomarkers**	**Organs**	**Time (h)**	**Industrial effluent**
PCC (μM reactive carbonyls/mg protein)	Gill	12	3.838×10^{-9} (↑172*)	*GPX* (mM NADPH/mg protein)	Gill	12	0.022 (↑145*)
		24	8.046×10^{-9} (↑417*)			24	0.017 (↑72*)
		48	6.468×10^{-9} (↑457*)			48	0.009 (↑4)
		72	4.115×10^{-9} (↑162*)			72	0.010 (↑26)
		96	4.148×10^{-9} (↑103*)			96	0.010 (↑12)
	Brain	12	8.369×10^{-10} (↑7)		Brain	12	0.002 (↑9)
		24	8.905×10^{-10} (↑18)			24	0.002 (↑23)
		48	1.269×10^{-9} (↑60*)			48	0.002 (↑14)
		72	1.208×10^{-9} (↑56)			72	0.003 (↑15)
		96	1.114×10^{-9} (↑64)			96	0.003 (↑31)
	Liver	12	1.099×10^{-9} (↑18)		Liver	12	0.001 (↑24)
		24	1.213×10^{-9} (↑67)			24	0.002 (↑11)
		48	1.788×10^{-9} (↑125*)			48	0.002 (↑103*)
		72	1.299×10^{-9} (↑62)			72	0.001 (↑4)
		96	1.300×10^{-9} (↑104)			96	0.001 (↑12)
	Blood	12	1.610×10^{-9} (↑196*)		Blood	12	0.001 (↓30)
		24	8.722×10^{-10} (↑105)			24	0.003 (↑91*)
		48	7.827×10^{-10} (↑34)			48	0.002 (↑52)
		72	6.027×10^{-10} (↑13)			72	0.002 (↑6)
		96	4.800×10^{-10} (↑10)			96	0.001 (↓2)

The up arrow and the down arrow indicate an increase or decrease in percentage with respect to the control group.

HPC hydroperoxide content, *CHP* cumene hydroperoxide, *LPX* lipid peroxidation, *MDA* malondialdehyde, *PCC* protein carbonyl content, *SOD* superoxide dismutase activity, *IU* international units, *CAT* catalase activity, *GPX* glutathione peroxidase activity

*Significantly different from control group. ANOVA and Tukey–Kramer ($P < 0.05$).

Table 2.3 Values and (percentages) of increases in geno- and cytotoxicity biomarkers in blood of *C. carpio*. Values are the mean

Biomarkers	Time (h)	Industrial effluent
Comet assay (damage index)	12	1.059 (↑2)
	24	1.300 (↑25*)
	48	1.401 (↑28*)
	72	1.173 (↑13*)
	96	1.163 (↑19*)
Micronucleus test (Micronuclei/1000 cells)	12	6.333 (↑19)
	24	19.333 (↑480*)
	48	25.667 (↑208*)
	72	128.667 (↑1508*)
	96	62.333 (↑1338*)
Caspase-3 activity ((nM free pNA h^{-1}) μg $protein^{-1}$)	12	110.427 (↑1)
	24	306.549 (↑198*)
	48	423.931 (↑316*)
	72	157.672 (↑49)
	96	283.404 (↑138*)
TUNEL assay (%TUNEL-positive cells)	12	13 (↑8)
	24	38 (↑159*)
	48	56.333 (↑333*)
	72	68.333 (↑356*)
	96	64.333 (↑286*)

Up arrow indicates an increase (percentage) with respect to control group
*Significantly different from control group. Bonferroni post hoc ($P < 0.05$)

gill from 48 to 96 h, liver at 48 h, and blood at 12 and 24 h; *CAT* in gill from 24 to 96 h, brain at 72 h, and blood at 96 h; and *GPX* in gill at 12 and 24 h, liver at 48 h, and blood at 24 h.

2.3.5 Evaluation of Geno- and Cytotoxicity

The results of comet assay, micronucleus test, caspase-3 activity, and TUNEL assay are shown in Table 2.3. Significant increases with respect to the control group ($P < 0.05$) were observed in *comet assay, micronucleus test*, and *TUNEL assay* from 24 to 96 h and in *caspase-3 activity* at 24, 48, and 96 h.

2.4 Discussion

The pharmaceutical industry is dedicated to the development, manufacture, and marketing of pharmaceuticals for human and animal health. In recent years, attention has been raised in the direction of presence, sources, discharge, and potentially

harmful pharmaceuticals on the environment (Sharma and Kaushik 2017). The pharmaceutical plant emanating the effluent analyzed in our study is used exclusively for NSAIDs manufacture, so that the manufacturing of this group of pharmaceuticals is continuous. The physicochemical characteristics of the industrial effluent analyzed in this study do not exceed the limits established in the official Mexican norms NOM-001-SEMARNAT-1996 (1996) and NOM-073-ECOL-1994 (1994). Nevertheless, toxic effects were observed in this study due to the presence of NSAIDs (DCF, IBP, NPX, and PCT) and NaClO. The decreasing content of the NSAIDs in the water (Fig. 2.1) may be explained because of the carp may be able to obtain and metabolize these pharmaceuticals in a similar manner to mammals. Besides, NSAIDs undergo both abiotic transformations by photodegradation and biotic transformations by cytochrome P450-mediated biotransformation and by bacterial activity probably due to diversification of the metabolic activity of microorganisms (Ternes et al. 2004; Jiang et al. 2017). Also, the activity of microorganisms that can biodegrade NSAIDs plays the most important role in the fate of NSAIDs in wastewater than their inherent physicochemical properties and environmental factors (Caracciolo et al. 2015). The LC50 value obtained in the present study is due to the mechanism of action of NSAIDs, which consists in blocking the action of the enzyme COX and results in inhibition of prostaglandin synthesis, the latter compounds being involved in pain management, blood flow regulation, neurotransmission, vascular permeability, renal function, and ion transport (Sali 2005), and by the presence of NaClO, this compound is a highly toxic chemical widely used because of its disinfectant properties. NaClO can form highly toxic products such as haloalkanes, haloacetic acids, haloacetonitriles, haloketones, and haloaldehydes (WHO 1996). The cytochromes P450 constitute the major enzyme family capable of catalyzing the oxidative biotransformation of most drugs and other lipophilic xenobiotics (Nelson 2004; Guengerich 2007; Zanger et al. 2008). Different P450 gene families have been characterized in fish, such as CYP1, CYP2, CYP3, CYP4, CYP11, CYP17, and CYP19 (Stegeman and Livingstone 1998). NSAIDs are metabolized mainly by subfamily CYP2C9 (Zanger et al. 2008; Blanco et al. 2005), and during this process ROS are generated as hydroxyl radicals (OH•) and oxygenated intermediates like the oxy-cytochrome P450 complex [P450 (Fe^{3+})] as a result of release of the superoxide anion by reaction decoupling. The latter radical reacts rapidly with the nitric oxide (NO) derived from arginine metabolism, forming peroxynitrite (ONOO−) (Halliwell 1997; Doi et al. 2002; Jifa et al. 2006) which has a high binding affinity for proteins and lipids. In addition, during the photodegradation and biotransformation of NSAIDs, benzoquinones are formed; these molecules are highly electrophilic with a high affinity for the binding of lipids, proteins, and DNA and altering the function of these macromolecules (Baillie 2006; Wilhelm et al. 2009). These facts may explain the damage to lipids and proteins that was observed in this study (Table 2.2). A time-dependent increase in HPC was observed in the gill, brain, liver, and blood, the latter organ displaying the highest value for this biomarker, while the increase in LPX was evident in all organs and is highest in the gill. During LPX, polyunsaturated fatty acids with double bonds react with ROS, particularly with the OH• and ONOO−, through a chain reaction mechanism. This permits the formation of hydroperoxides which are degraded to low molecular

weight products, including malondialdehyde (MDA) (Wilhelm Filho et al. 2005). Further, PCC increased in all organs evaluated. ROS can cause oxidation in both amino acid side chains and protein backbones, resulting in protein fragmentation or protein–protein cross-linkages (Zhang et al. 2013). Results obtained in the present study are consistent with those reported in previous studies by our research team. Studies conducted by (Oviedo-Gómez et al. 2010; Gómez-Oliván et al. 2012) found increases in LPX and PCC in *H. azteca* exposed to DCF and PCT, respectively; (Islas-Flores et al. 2013; Islas-Flores et al. 2017) report increased LPX in *C. carpio* with exposure to DCF and mixture of DCF and IBP, while (Nava-Álvarez et al. 2014) found increases in LPX in the brain and gill of *C. carpio* exposed to a mixture of DCF and PCT.

Oxidative stress is a disturbance in the balance between the production of ROS and antioxidant defenses. Antioxidant enzymes are an important protective mechanism against ROS; SOD catalyzes the conversion of superoxide anion (O_2•) to hydrogen peroxide (H_2O_2) which is reduced to O_2 and water by CAT and GPx (Van der Oost et al. 2003). In this study (Table 2.2), an increase in SOD activity was observed in the liver, blood, and gill; the increase may be explained by the fact that during the biotransformation of the NSAIDs the O_2• is generated. In contrast, SOD activity decreased in brain with respect to the control group. This may be due to the fact that when high concentrations of reactive species are present, antioxidant enzymes are inactivated, inducing major damage on cell components (Azzi et al. 2004) (Islas-Flores et al. 2013). report inhibition of SOD in brain of *C. carpio* following exposure to DCF. On the other hand, CAT activity increases in the gill, brain, and blood, and also increased GPx activity occurred in the gill, liver, and blood. These increases can be attributed to the antioxidant capacity of organisms to offset H_2O_2-induced oxidative damage. The present results are consistent with those obtained by (Oviedo-Gómez et al. 2010), who report increases in the activity of CAT and GPx in *H. azteca* exposed to an NSAID.

The oxidatively induced DNA damage associated with ROS typically are apurinic/apyrimidinic (abasic) DNA sites, oxidized purines and pyrimidines, and single-strand and double-strand DNA breaks (Kryston et al. 2011). This DNA damage is shown in Table 2.3. Comet assay or single cell gel electrophoresis assay detects single- or double-strand breaks measured at the individual cell level (Beedanagari et al. 2014). In this study DNA damage increased significantly compared to the control group from 24 h on, reaching peak values at 48 h. This may be explained by the fact that OH• is highly reactive and has the capacity to attract hydrogen atoms from the 2-deoxyribose leading to the formation of carbon-based radicals which under the presence of oxygen can be converted to peroxyl radicals (ROO•). The peroxyl radicals through different reactions can also abstract hydrogen atoms from sugar moieties, thus leading to DNA strand breaks (Kryston et al. 2011; Medeiros 2008; Valko et al. 2004). $ONOO^-$ is another highly reactive molecule capable of oxidizing DNA, and it may be contributing to the damage observed in our study. ROS and RNS induce diverse types of DNA damage, including damage to bases and sugars, protein–DNA and DNA–DNA crosslinks, single and double-strand breaks, and abasic site formation (Medeiros 2008; Bolognesi and

Cirillo 2014). Besides, compounds formed during chlorination by the addition of NaClO induce DNA damage and mutagenic effects in fish (Gustavino et al. 2001; Minissi et al. 1996). On the other hand, NSAID metabolites – particularly NSAID acyl glucuronides – may be involved in DNA adduct formation (Gómez-Oliván et al. 2014a).

Micronuclei (MN) are a common outcome of many cell division defects, including mitotic errors that missegregate intact chromosomes and errors in DNA replication or repair that generate acentric chromosome fragments (Zhang et al. 2015). A significant increase with respect to the control group was found from 24 h on, with maximum values recorded at 72 h. These results may be due to breakage of DNA and/or chromosome missegregation. A MN may be formed following direct DNA damage (clastogenic mechanism) or following secondary interaction with DNA replication apparatus (indirect aneugenic mechanism); these effects are elicited by ROS and some xenobiotics, including NSAIDs (Beedanagari et al. 2014; Canistro et al. 2012). However, at 96 h a significant reduction was observed with respect to 72 h, which may be explained by the fact that the antioxidant system inactivates oxy radicals before their interaction with DNA and can reduce micronuclei formation (Çavaş and Ergene-Gözükara 2005; de Lemos et al. 2007).

Our genotoxicity results are consistent with those of others, such as (Parolini et al. 2009; Parolini et al. 2010) in which DCF (60, 156, and 250 μg L^{-1}), PCT (0.154, 0.75, 1.51, 30, 150, and 450 μg L^{-1}), and IBP (45, 450, and 909 μg L^{-1}) were found to induce genotoxicity in *D. polymorpha* (Gómez-Oliván et al. 2014a, b). suggest that ROS may be associated with increased genotoxicity of DCF, IBP, NPX, and acetylsalicylic acid in *D. magna*. In addition, (Sapone et al. 2007) showed that exposure to NaClO induces DNA damage in *C. carpio*.

The term cytotoxicity is more accurately applied to the pre-lethal changes and events that occur in cells before necrosis is histologically evident (Vasquez 2012). Lesions to DNA and inefficient repair mechanisms are crucial in the unleashing of apoptosis (Roos and Kaina 2013). Apoptosis is important for normal development and tissue homeostasis. The family of cysteine proteases known as caspases is critical mediators of programmed cell death (Nakagawa et al. 2000). Caspase-3 is an effector caspase in which both apoptosis pathways (intrinsic and extrinsic) converge. Our results of cytotoxicity are shown in Table 2.3. A significant increase in the activity of caspase-3 was observed at 24, 48, and 96 h, with peak activity occurring at 48 h; this result can be explained because any stressful stimulus, such as exposure to ROS, DNA damage, or increased extracellular calcium induced by prostaglandin inhibition, can initiate the intrinsic pathway of apoptosis (Islas-Flores et al. 2017). As COX inhibition is the mechanism of action of NSAIDs, it has been suggested that decreased cellular levels of prostaglandin E2 (PGE2) and increased levels of arachidonic acid may be involved in inhibition of cell proliferation and induction of apoptosis (Chan et al. 1998). Also, an increase in the cellular concentration of arachidonic acid can alter mitochondrial membrane permeability and elicit cytochrome c release, leading to apoptosis (Cao et al. 2000; Scorrano et al. 2001). It has been shown that arachidonic acid also increases the production of ceramide, a potent apoptosis inducer (Hannun 1996). However, a decrease in the

activity of caspase-3 was observed in our study at 72 and 96 h with respect to 48 h; this result is explained because in the apoptosis pathway, there are inhibitors of apoptosis (IAP), heat shock (HSP), and anti-apoptotic (bcl-2) proteins that can block the apoptosis cascade at various places (Bandala et al. 2001; Danial and Korsmeyer 2004; Hengartner 2000; Reed et al. 2004).

Apoptosis is associated with a distinct set of biochemical and physical changes involving the cytoplasm, nucleus, and plasma membrane; in this process the nucleus becomes convoluted and buds off into several fragments, which are encapsulated within the forming apoptotic bodies (Lawen 2003). TUNEL assay is a method used for in situ detection of DNA damage and is able to detect cells in the initial stages of apoptosis and those in which morphologic changes, including apoptotic bodies, have already occurred (Gavrieli et al. 1992; Kylarová et al. 2002). A significant increase in apoptotic or TUNEL-positive cells occurred beginning at 24 h, with maximum values being attained at 72 h. This increase is related to the increase in caspase-3 activity due to contaminants in the effluent which induced DNA damage. Nevertheless, the results of caspase-3 activity at 72 h and the significant increase in TUNEL-positive cells at the same exposure time suggest that a potential inhibition of apoptosis was not carried out completely. When damage is such that the cell is unable to repair itself, mechanisms directing the cell to die are activated. In the present study, a reduction was found in the percentage of apoptotic cells at 96 h with respect to 72 h. This may be explained by the fact that apoptotic bodies, immediately after their formation, are phagocytosed by macrophages without eliciting an inflammatory response, preventing that molecules in the cytoplasm, organelles, and genetic material remain free between the cells (Kurokawa and Kornbluth 2009).

The results of cytotoxicity are consistent with those of others such as (Caminada et al. 2006), who showed that genotoxicity was induced by a mixture of 34 pharmaceuticals in aquatic systems, including the NSAIDs (IBP, DCF, PCT, and NPX) on *Poeciliopsis lucida* and *Oncorhynchus mykiss*, while (Parolini et al. 2009, 2010) state that PCT (0.154, 0.75, 1.51, 30, 150, and 450 μg L^{-1}) and IBP (45, 450, and 909 μg L^{-1}) induced cytotoxicity in *D. polymorpha*. On the other hand, in a study by (Matozzo et al. 2012), exposure to IBP (100, 500, and 1000 μg L^{-1}) did not induce cytotoxicity on hemocytes of *R. philippinarum*.

2.5 Conclusions

Industrial effluent containing NSAIDs and NaClO induces oxidative stress and geno- and cytotoxicity in *C. carpio*. Bioassays combined with physicochemical analyses commonly performed for water quality assessment may be helpful in evaluating the ecotoxicological effects of this type of effluents.

References

APHA, AWWA, WPCF (1995) Standard methods for the examination of water and wastewater, 19th edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC

Azzi A, Davies KJ, Kelly F (2004) Free radical biology–terminology and critical thinking. FEBS Lett 558:3–6

Baillie TA (2006) Future of toxicology metabolic activation and drug design: challenges and opportunities in chemical toxicology. Chem Res Toxicol 19:889–893

Bandala E, Espinosa M, Maldonado V, Meléndez-Zajgla J (2001) Inhibitor of apoptosis-1 (IAP-1) expression and apoptosis in non-small-cell lung cancer cells exposed to gemcitabine. Biochem Pharmacol 62:13–19

Beedanagari S, Vulimiri SV, Bhatia S, Mahadevan B (2014) Genotoxicity biomarkers: molecular basis of genetic variability and susceptibility. In: Gupta RC (ed) Biomarkers in toxicology, pp 729–742. Academic Press

Blanco G, Martínez C, García-Martín E, Agúndez JA (2005) Cytochrome P450 gene polymorphisms and variability in response to NSAIDs. Clin Res Regul Aff 22:57–81

Bolognesi C, Cirillo S (2014) Genotoxicity biomarkers in aquatic bioindicators. Zoology 60:273–284

Bolognesi C, Perrone E, Roggieri P, Pampanin DM, Sciutto A (2006) Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. Aquat Toxicol 78:S93–S98

Büege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310

Burcham PC (2007) Modified protein carbonyl assay detects oxidised membrane proteins: a new tool for assessing drug- and chemically-induced oxidative cell injury. J Pharmacol Toxicol Methods 56:18–22

Caminada D, Escher C, Fent K (2006) Cytotoxicity of pharmaceuticals found in aquatic systems: comparison of PLHC-1 and RTG-2 fish cell lines. Aquat Toxicol 79:114–123

Canistro D, Melega S, Ranieri D, Sapone A, Gustavino B, Monfrinotti M, Rizzoni M, Paolini M (2012) Modulation of cytochrome P450 and induction of DNA damage in *Cyprinus carpio* exposed in situ to surface water treated with chlorine or alternative disinfectants in different seasons. Mutat Res Fundam Mol Mech Mutagen 729:81–89

Cao Y, Pearman AT, Zimmerman GA, McIntyre TM, Prescott SM (2000) Intracellular unesterified arachidonic acid signals apoptosis. Proc Natl Acad Sci 97:11280–11285

Caracciolo AB, Topp E, Grenni P (2015) Pharmaceuticals in the environment: biodegradation and effects on natural microbial communities. A review. *J Pharm Biomed Anal* 106:25–36

Çavaş T, Ergene-Gözükara S (2005) Induction of micronuclei and nuclear abnormalities in *Oreochromis niloticus* following exposure to petroleum refinery and chromium processing plant effluents. Aquat Toxicol 74:264–271

Chan TA, Morin PJ, Vogelstein B, Kinzler KW (1998) Mechanisms underlying nonsteroidal anti-inflammatory drug-mediated apoptosis. Proc Natl Acad Sci 95:681–686

Cycoń M, Borymski S, Żołnierczyk B, Piotrowska-Seget Z (2016) Variable effects of non-steroidal anti-inflammatory drugs (NSAIDs) on selected biochemical processes mediated by soil microorganisms. Front Microbiol 7:1969

Danial NN, Korsmeyer SJ (2004) Cell death: critical control points. Cell 116:205–219

de Lemos CT, Rödel PM, Terra NR, de Oliveira NCDA, Erdtmann B (2007) River water genotoxicity evaluation using micronucleus assay in fish erythrocytes. Ecotoxicol Environ Saf 66:391–401

Dennery PA (2007) Effects of oxidative stress on embryonic development. Birth Defects Res C Embryo Today 81:155–162

Díaz-Torres E, Gibson R, González-Farías F, Zarco-Arista AE, Mazari-Hiriart M (2013) Endocrine disruptors in the Xochimilco wetland, Mexico City. *Water Air Soil Pollut* 224:1586

Doi H, Iwasaki H, Masubuchi Y, Nishigaki R, Horie T (2002) Chemiluminescence associated with the oxidative metabolism of salicylic acid in rat liver microsomes. *Chem Biol Interact* 140:109–119

Félix-Cañedo TE, Durán-Álvarez JC, Jiménez-Cisneros B (2013) The occurrence and distribution of a group of organic micropollutants in Mexico City's water sources. Sci Total Environ 454:109–118

Galar-Martínez M, García-Medina S, Gómez-Olivan LM, Pérez-Coyotl I, Mendoza-Monroy DJ, Arrazola-Morgain RE (2016) Oxidative stress and genotoxicity induced by ketorolac on the common carp *Cyprinus carpio*. Environ Toxicol 31:1035–1043

Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119:493–501

Gibson R, Durán-Álvarez JC, Estrada KL, Chávez A, Cisneros BJ (2010) Accumulation and leaching potential of some pharmaceuticals and potential endocrine disruptors in soils irrigated with wastewater in the Tula Valley, Mexico. *Chemosphere* 81:1437–1445

Gómez-Oliván LM, Neri-Cruz N, Galar-Martínez M, Vieyra-Reyes P, García-Medina S, Razo-Estrada C, Dublán-García O, Corral-Avitia AY (2012) Assessing the oxidative stress induced by paracetamol spiked in artificial sediment on *Hyalella azteca*. Water Air Soil Pollut 223:5097–5104

Gómez-Oliván LM, Galar-Martinez M, García-Medina S, Valdés-Alanís A, Islas-Flores H, Neri-Cruz N (2014a) Genotoxic response and oxidative stress induced by diclofenac, ibuprofen and naproxen in *Daphnia magna*. Drug Chem Toxicol 37:391–399

Gómez-Oliván LM, Galar-Martínez M, Islas-Flores H, García-Medina S, SanJuan-Reyes N (2014b) DNA damage and oxidative stress induced by acetylsalicylic acid in *Daphnia magna*. Comp Biochem Physiol C Toxicol Pharmacol 164:21–26

Gómez-Oliván LM, Neri-Cruz N, Galar-Martínez M, Islas-Flores H, García-Medina S (2014c) Binary mixtures of diclofenac with paracetamol, ibuprofen, naproxen, and acetylsalicylic acid and these pharmaceuticals in isolated form induce oxidative stress on *Hyalella azteca*. Environ Monit Assess 186:7259–7271

González-González ED, Gómez-Oliván LM, Galar-Martínez M, Vieyra-Reyes P, Islas-Flores H, García-Medina S, Jiménez-Vargas JM, Razo-Estrada C, Pérez-Pastén R (2014) Metals and nonsteroidal anti-inflammatory pharmaceuticals drugs present in water from Madín Reservoir (Mexico) induce oxidative stress in gill, blood, and muscle of common carp (*Cyprinus carpio*). Arch Environ Contam Toxicol 67:281–295

Gonzalez-Rey M, Bebianno MJ (2011) Non-steroidal anti-inflammatory drug (NSAID) ibuprofen distresses antioxidant defense system in mussel *Mytilus galloprovincialis* gills. Aquat Toxicol 105:264–269

Guengerich FP (2007) Cytochrome p450 and chemical toxicology. Chem Res Toxicol 21:70–83

Gunzler W, Flohe-Clairborne A (1985) Glutathione peroxidase. In: Green-Wald RA (ed) Handbook of methods for oxygen radical research. CRC Press, Boca Raton, pp 285–290

Gustavino B, Scornajenghi KA, Minissi S, Ciccotti E (2001) Micronuclei induced in erythrocytes of *Cyprinus carpio* (teleostei, pisces) by X-rays and colchicine. Mutat Res Genet Toxicol Environ Mutagen 494:151–159

Halliwell B (1997) Antioxidants and human disease: a general introduction. Nutr Rev 55:S44–S49

Hannun YA (1996) Functions of ceramide in coordinating cellular responses to stress. Science 274:1855–1859

Hengartner MO (2000) The biochemistry of apoptosis. Nature 407:770

Islas-Flores H, Gómez-Oliván LM, Galar-Martínez M, Colín-Cruz A, Neri-Cruz N, García-Medina S (2013) Diclofenac-induced oxidative stress in brain, liver, gill and blood of common carp (*Cyprinus carpio*). Ecotoxicol Environ Saf 92:32–38

Islas-Flores H, Gómez-Oliván LM, Galar-Martínez M, García-Medina S, Neri-Cruz N, Dublán-García O (2014) Effect of ibuprofen exposure on blood, gill, liver, and brain on common carp (*Cyprinus carpio*) using oxidative stress biomarkers. Environ Sci Pollut Res 21:5157–5166

Islas-Flores H, Gómez-Oliván LM, Galar-Martínez M, Sánchez-Ocampo EM, SanJuan-Reyes N, Ortíz-Reynoso M, Dublán-García O (2017) Cyto-genotoxicity and oxidative stress in common carp (*Cyprinus carpio*) exposed to a mixture of ibuprofen and diclofenac. Environ Toxicol 32:1637–1650

Jiang ZY, Hunt JV, Wolff SP (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 202:384–389

Jiang C, Geng J, Hu H, Ma H, Gao X, Ren H (2017) Impact of selected non-steroidal anti-inflammatory pharmaceuticals on microbial community assembly and activity in sequencing batch reactors. PLoS One 12:e0179236

Jifa W, Yu Z, Xiuxian S, You W (2006) Response of integrated biomarkers of fish (*Lateolabrax japonicus*) exposed to benzo [a] pyrene and sodium dodecylbenzene sulfonate. Ecotoxicol Environ Saf 65:230–236

Kim IY, Hyun CK (2006) Comparative evaluation of the alkaline comet assay with the micronucleus test for genotoxicity monitoring using aquatic organisms. Ecotoxicol Environ Saf 64:288–297

Kryston TB, Georgiev AB, Pissis P, Georgakilas AG (2011) Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res Fundam Mol Mech Mutagen 711:193–201

Kurokawa M, Kornbluth S (2009) Caspases and kinases in a death grip. Cell 138:838–854

Kylarová D, Procházková J, Mad'arová J, Bartoš J, Lichnovský V (2002) Comparison of the TUNEL, lamin B and annexin V methods for the detection of apoptosis by flow cytometry. Acta Histochem 104:367–370

Lawen A (2003) Apoptosis—an introduction. BioEssays 25:888–896

Levine RL, Williams JA, Stadtman ER, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–357

Lillenberg M, Yurchenko S, Kipper K, Herodes K, Pihl V, Lõhmus R, Ivask M, Kuu A, Kutti S, Litvin SV, Nei L (2010) Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting. Int J Environ Sci Technol 7:307–312

Luja-Mondragón M, Gómez-Oliván LM, SanJuan-Reyes N, Islas-Flores H, Orozco-Hernández JM, Heredia-García G, Galar-Martínez M, Dublán-García O (2019) Alterations to embryonic development and teratogenic effects induced by a hospital effluent on *Cyprinus carpio* oocytes. Sci Total Environ 660:751–764

Matozzo V, Rova S, Marin MG (2012) The nonsteroidal anti-inflammatory drug, ibuprofen, affects the immune parameters in the clam Ruditapes philippinarum. Mar Environ Res 79:116–121

Medeiros MHG (2008) DNA damage. In: Konigsberg-Fainstein M (ed) Free radicals and oxidative stress, pp 122–124. Medical Applications. Manual Moderno

Minissi S, Ciccotti E, Rizzoni M (1996) Micronucleus test in erythrocytes of *Barbus plebejus* (Teleostei, Pisces) from two natural environments: a bioassay for the in situ detection of mutagens in freshwater. Mutat Res Genet Toxicol 367:245–251

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J (2000) Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-β. Nature 403:98

Nava-Álvarez R, Razo-Estrada AC, García-Medina S, Gómez-Olivan LM, Galar-Martinez M (2014) Oxidative stress induced by mixture of diclofenac and acetaminophen on common carp (*Cyprinus carpio*). *Water Air Soil Pollut* 225:1873

Nelson DR (2004) Cytochrome P450 nomenclature, 2004. In: Cytochrome P450 protocols. Humana Press, Totowa, pp 1–10

Neri-Cruz N, Gómez-Oliván LM, Galar-Martínez M, Romero-Figueroa MS, Islas-Flores H, García-Medina S, Jiménez-Vargas JM, SanJuan-Reyes N (2014) Oxidative stress in *Cyprinus carpio* induced by hospital wastewater in Mexico. Ecotoxicology 24:181–193

NMX-AA-003-1980 (1980) Wastewater, sampling. Procuraduría Federal de Protección al Ambiente. Diario Oficial de la Federación (25 Mar)

NOM-001-SEMARNAT-1996 (1996) Official Mexican norm setting the maximum permissible limits of contaminants in wastewaster discharges entering national waters and resources. Procuraduría Federal de Protección al Ambiente. Diario Oficial de la Federación (30 Oct)

NOM-073-ECOL-1994 (1994) Official Mexican norm setting the maximum permissible limits of contaminants in wastewater discharges arising in the pharmacochemical and pharmaceutical industries and entering receiving water bodies. Secretaría de Desarrollo Social. Diario Oficial de la Federación (10 June)

Oviedo-Gómez DGC, Galar-Martínez M, García-Medina S, Razo-Estrada C, Gómez-Oliván LM (2010) Diclofenac-enriched artificial sediment induces oxidative stress in *Hyalella azteca*. Environ Toxicol Pharmacol 29:39–43

Parolini M, Binelli A, Cogni D, Riva C, Provini A (2009) An *in vitro* biomarker approach for the evaluation of the ecotoxicity of non-steroidal anti-inflammatory drugs (NSAIDs). Toxicol In Vitro 23:935–942

Parolini M, Binelli A, Cogni D, Provini A (2010) Multi-biomarker approach for the evaluation of the cyto-genotoxicity of paracetamol on the zebra mussel (*Dreissena polymorpha*). Chemosphere 79:489–498

Parvez S, Raisuddin S (2005) Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish *Channa punctata* (Bloch). Environ Toxicol Pharmacol 20:112–117

Pérez-Alvarez I, Islas-Flores H, Gómez-Oliván LM, Barceló D, López De Alda M, Pérez Solsona S, Sánchez-Aceves L, SanJuan-Reyes N, Galar-Martínez M (2018) Determination of metals and pharmaceutical compounds released in hospital wastewater from Toluca, Mexico, and evaluation of their toxic impact. Environ Pollut 240:330–341

Radi R, Turrens JF, Chang LY, Bush KM, Crapo JD, Freeman BA (1991) Detection of catalase in rat heart mitochondria. J Biol Chem 266:22028–22034

Reed JC, Doctor KS, Godzik A (2004) The domains of apoptosis: a genomics perspective. *Sci Signal* 2004:re9

Rehman MSU, Rashid N, Ashfaq M, Saif A, Ahmad N, Han JI (2015) Global risk of pharmaceutical contamination from highly populated developing countries. Chemosphere 138:1045–1055

Rivera-Jaimes JA, Postigo C, Melgoza-Alemán RM, Aceña J, Barceló D, López De Alda M (2018) Study of pharmaceuticals in surface and wastewater from Cuernavaca, Morelos, Mexico: occurrence and environmental risk assessment. *Sci Total Environ* 613:1263–1274

Roos WP, Kaina B (2013) DNA damage-induced cell death: from specific DNA lesions to the DNA damage response and apoptosis. Cancer Lett 332:237–248

Sali T (2005) Prostaglandins. In: Vohr HW (ed) Encyclopedic reference of immunotoxicology. Springer, Heidelberg, pp 537–540

SanJuan-Reyes N, Gómez-Oliván LM, Galar-Martínez M, Vieyra-Reyes P, García-Medina S, Islas-Flores H, Neri-Cruz N (2013) Effluent from an NSAID-manufacturing plant in Mexico induces oxidative stress on *Cyprinus carpio*. *Water Air Soil Pollut* 224:1689

SanJuan-Reyes N, Gómez-Oliván LM, Islas-Flores H, Castro-Pastrana LI (2017) Control of environmental pollution caused by pharmaceuticals. In: Ecopharmacovigilance. Springer, Cham, pp 255–264

Sapone A, Gustavino B, Monfrinotti M, Canistro D, Broccoli M, Pozzetti L, Affatato A, Valgimigli L, Forti GC, Pedulli GF, Biagi GL, Abdel-Rahman SZ, Paolini M (2007) Perturbation of cytochrome P450, generation of oxidative stress and induction of DNA damage in *Cyprinus carpio* exposed in situ to potable surface water. *Mutat Res Genet Toxicol Environ Mutagen* 626:143–154

Saucedo-Vence K, Dublán-García O, López-Martínez LX, Morachis-Valdes G, Galar-Martínez M, Islas-Flores H, Gómez-Oliván LM (2015) Short and long-term exposure to diclofenac alter oxidative stress status in common carp *Cyprinus carpio*. Ecotoxicology 24:527–539

Scorrano L, Penzo D, Petronilli V, Pagano F, Bernardi P (2001) Arachidonic acid causes cell death through the mitochondrial permeability transition implications for tumor necrosis factor-α apoptotic signaling. J Biol Chem 276:12035–12040

Sharma K, Kaushik G (2017) NSAIDS in the environment: from emerging problem to green solution. Ann Pharmacol Pharm 2:1077

Siemens J, Huschek G, Siebe C, Kaupenjohann M (2008) Concentrations and mobility of human pharmaceuticals in the world's largest wastewater irrigation system, Mexico City–Mezquital Valley. Water Res 42:2124–2134

Sreekanth D, Sivaramakrishna D, Himabindu V, Anjaneyulu Y (2009) Thermophilic treatment of bulk drug pharmaceutical industrial wastewaters by using hybrid up flow anaerobic sludge blanket reactor. Bioresour Technol 100:2534–2539

Stegeman JJ, Livingstone DR (1998) Forms and functions of cytochrome P450. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 121:1–3

Stephensen E, Svavarsson J, Sturve J, Ericson G, Adolfsson-Erici M, Förlin L (2000) Biochemical indicators of pollution exposure in shorthorn sculpin (*Myoxocephalus scorpius*), caught in four harbours on the southwest coast of Iceland. Aquat Toxicol 48:431–442

Ternes TA, Joss A, Siegrist H (2004) Peer reviewed: scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environ Sci Technol 38:392A–399A

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35:206–221

US-EPA (2013) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. U.S. Environmental Protection Agency; Washington, DC, USA

Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004) Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 266:37–56

Van der Oost R, Beyer J, Vermeulen NP (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149

Vasquez MZ (2012) Recommendations for safety testing with the in vivo comet assay. Mutat Res Genet Toxicol Environ Mutagen 747:142–156

Velagaleti R, Burns PK (2006) The industrial ecology of pharmaceutical raw materials and finished products with emphasis on supply chain management activities. [cited 25 Jan 2019]. Available from http://www.epa.gov/esd/chemistry/ppcp/images/ecology.pdf

WHO (1996) Revision of the WHO guidelines for drinking water quality. World Health Organization, Geneva

Wilhelm Filho D, Torres MA, Zaniboni-Filho E, Pedrosa RC (2005) Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant status in piapara, *Leporinus elongatus* (Valenciennes, 1847). Aquaculture 244:349–357

Wilhelm EA, Jesse CR, Leite MR, Nogueira CW (2009) Studies on preventive effects of diphenyl diselenide on acetaminophen-induced hepatotoxicity in rats. Pathophysiology 16:31–37

Xu J, Zhao Z, Zhang X, Zheng X, Li J, Jiang Y, Kuang Y, Zhang Y, Feng J, Li C, Yu J, Li Q, Zhu Y, Liu Y, Xu P, Sun X (2014) Development and evaluation of the first high-throughput SNP array for common carp (*Cyprinus carpio*). BMC Genomics 15:307

Zanger UM, Turpeinen M, Klein K, Schwab M (2008) Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. Anal Bioanal Chem 392:1093–1108

Zhang W, Xiao S, Ahn DU (2013) Protein oxidation: basic principles and implications for meat quality. Crit Rev Food Sci Nutr 53:1191–1201

Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, Meyerson M, Pellman D (2015) Chromothripsis from DNA damage in micronuclei. Nature 522:179

Chapter 3
Pesticide Contamination in Southern Brazil

Barbara Clasen, Camila Murussi, and Tamiris Storck

3.1 Introduction

The increasing global demand for food in recent years has put pressure on the food production system, which needs to pursue greater production efficiency in the same crop area. However, agriculture has resources to respond to this expectation, such as new crop varieties and increasingly efficient mechanisms adopted for purposes such as pest control and the correction of areas eroded by intense cultivation systems.

Brazil is globally acknowledged for its agricultural production and food diversity. According to the US Department of Agriculture – USDA (2018), Brazil is the world leader in orange juice and coffee production, the second largest soybean producer – based on estimates for the 2018/2019 crop, the country will become the largest soybean producer in the world (CONAB 2018) – and the third largest bean and maize producer (CONAB 2018). Besides, the country stands out among the leading beef, chicken and cotton producers in the world (USDA 2018). However, different climatic regions lead to production inequalities in the country: 78.6% of leguminous and oilseed cereals are produced in the Central-Western and Southern regions, 75% of the rice and 95% of the wheat grown in the country are produced in the Southern region (CONSEA 2014; IBGE 2014). The agricultural model adopted

B. Clasen (✉)
Laboratory of Environmental Toxicology, Department of Environmental Sciences, State University of Rio Grande do Sul, Três Passos, RS, Brazil

Department of Sanitary and Environmental Engineering, Technology Center, Federal University of Santa Maria, Santa Maria, RS, Brazil

C. Murussi
Department of Biochemistry and Molecular Biology, Center for Natural and Exact Sciences, Federal University of Santa Maria, Santa Maria, RS, Brazil

T. Storck
Department of Sanitary and Environmental Engineering, Technology Center, Federal University of Santa Maria, Santa Maria, RS, Brazil

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_3

in Brazil since the 1950s is called “Green Revolution”, which is defined by the use of genetically modified plants and inputs such as agrochemicals and fertilizers. The Brazilian Southern region comprises Paraná, Santa Catarina and Rio Grande do Sul States. It was initially colonized by European immigrants, who developed small estates to cultivate their lands for their own subsistence; nowadays, these properties form the family farming system. According to the 2006 Census of Agriculture (IBGE 2006), this system accounts for 80% of the total production properties in this region. The diversity of crops grown in these properties puts in evidence the variety of agrochemicals used in them. Soybean, wheat, tobacco, rice and corn crops, as well as beef (cattle, pork and chicken) breeding and fruit growing, are worth mentioning among the most important activities performed in these properties.

The intense cultivation in these producing areas also demands constant pest control and continuous soil correction through fertilizers. Thus, fertilizer residues can be found in a variety of sources such as water, food, areas adjacent to applications, soil and sediment, as well as in non-target animals such as fish, birds, earthworms, among others, and in the air. Contamination takes place by what is known as "technical drift". Drift is the term used to describe pesticide dispersion by the environment (wind or water). Technical drift is a pesticide dispersion means that contaminates the environment around the application site, regardless of all precaution procedures adopted during application. This drift can range from 30% to 70% (Chaim et al. 2004, Londres 2011). However, different crops are planted in Summer and Winter due to crop rotation and climatic variations in the Brazilian Southern region. These factors can explain the use of certain pesticides only in a specific season, as well as the great variety of adopted pesticides; among them, one finds glyphosate, carbofuran, atrazine, clomazone, tebuconazole, diphenoconazole, tetraconazole, propoxur, 2,4-D, propanil, mancozeb, penoxsulam, imazethapyr, imazapique, mancozeb, fipronil and quinclorac.

The increased agricultural productivity was followed by the increased use of agrochemicals. Brazil is the global leader in the use of agricultural pesticides; the country has even surpassed the United States since 2008 (EMBRAPA 2014). According to Aenda (2011), who listed the main companies selling these products, 4 multinational companies account for approximately 50% of fertilizer sales: Syngenta accounts for 20.5% of the sales (glyphosate is its main product applied to genetically modified soybeans), and it is followed by Bayer (16.2%), BASF (12.4%) and FMC (6.9%).

Worse than the excessive use of agrochemicals in the most diverse crops, the use of these substances in Rio Grande do Sul State is almost twice (8.3 L per inhabitant) the national mean (4.5 L per inhabitant) (Cassal et al. 2014). Among the Brazilian Southern states, Paraná ranks third in fertilizer consumption (14.3%) and it is followed by Rio Grande do Sul (10.8%) (IBGE 2006; SINDAG 2011); soybean crops prevail in both states. Santa Catarina State does not make significant use of agrochemicals in comparison to the other states, since its agricultural activity is based on pork and chicken livestock. High volumes of pesticides are used in agriculture because pest became resistant to these products over time; thus, farmers need to use larger fertilizer doses, or new products recently launched by the companies, and it constitutes a vicious cycle for them.

Intoxication is another alarming fact regarding the use of agrochemicals in Southern Brazil. From 2007 to 2014, the region recorded the largest number of intoxication cases due to agricultural use of pesticides (Bombardi 2017). According to SINITOX (National System of Toxic and Pharmacological Information), the Brazilian Southern region accounted for approximately 5% of total poisoning cases in the region due to agricultural and domestic use of pesticides in 2012 (total $n = 20{,}237$). Human poisoning caused by pesticides used in agriculture is two times higher in men than in women. However,, it is known that many poisoning cases are not notified; this, the number of intoxications can be much larger than that reported by SINITOX.

Data reported by SINITOX refer to management procedures adopted by producers at the time to pack and use these pesticides, whether in product transportation, storage and application or in package disposal. Studies conducted with farmers in Northern Rio Grande do Sul State have shown that they use some protective equipment such as hat, gloves, boots and glasses; however few of them use the complete Personal Protective Equipment (PPE) as recommended (Murussi et al. 2014). Therefore, it is possible seeing to what extent it is necessary improving the awareness and knowledge that must be transmitted to farmers about the handling of agrochemicals in their properties, since they are the ones who have direct contact with these products, which can lead to health damages in the long term.

3.2 Environmental Impacts of Agrochemicals

The application of agrochemicals presents positive aspects such as increased crop yield and, consequently, increased food production and significant decrease in the number of vector-borne diseases. However, the indiscriminate and uncontrolled use of pesticides has raised serious concerns with possible environmental damages, mainly to the quality of the water and to human and animal health. Although the application of some of the most environmentally persistent and less biodegradable agrochemicals (such as organochlorines) was forbidden in many countries, their use is always on the rise. Agrochemicals pose serious risks to the health of living systems due to the presence of fast-solubility fats and bioaccumulation in nontarget organisms (Agrawal et al. 2010).

Agrochemicals can cause several adverse effects, even when they are used at low concentrations; these effects can be monitored through biochemical, molecular, and/or behavioral assessments. Factors such as drainage, rainfall, microbial activity, soil temperature, surface treatment, and fertilizer application rate, as well as pesticide solubility, mobility, and half-life, affect water contamination with pesticides, their residues, and secondary metabolites (Agrawal et al. 2010; Clasen et al. 2014). Thus, one of the major impacts of agriculture on the quality of water resources (groundwater and surface water) lies on the contamination of the water with agrochemical residues. Unfortunately, there has been increasing evidence on the incidence of pesticide residues in groundwater and surface water samples collected in agricultural areas, or even in areas of water abstraction for human consumption purposes (Scorza Júnior et al. 2010).

The widespread use of agrochemicals is a serious public health issue in many developing countries, mainly in those whose economy is based on agriculture, such as Brazil (Melo et al. 2010). According to the US Environmental Protection Agency (USEPA), there are more than 18,000 licensed products, and approximately 1 billion liters of pesticides are applied to agricultural production, homes, schools, parks, and forests every year.

As Brazil has been the largest pesticide consumer in the world since 2008, monitoring and evaluating the impacts caused by the use of these substances is fundamental to assure the sustainability of agricultural production systems that use such inputs (Embrapa 2014).

According to the National Agency of Sanitary Surveillance (ANVISA), the intense use of pesticides has led to the long-term degradation of natural resources, soil, water, flora, and fauna; in some cases, this degradation is irreversible and leads to biological and ecological imbalances. The main disadvantage in the incorrect use of agrochemicals lies on the environmental imbalance caused in the application region and in its ecosystem. Agrochemicals can affect living organisms that are not harmful to crops, as well as extinguish certain species that are fundamental to the environmental balance in the application region.

The first report referring to the standard adopted by conventional agriculture was presented by marine biologist Rachel Carson in her book entitled *Silent Spring*, published in 1962, and it had significant repercussion worldwide. The book questioned the secondary impacts of toxic substances on the environment. Carson was able to sensitize American and world public opinion about the side effects of pesticides on the environment, fact that significantly marked the environmental movement. In 1964, Robert Rudd published the book entitled *Pesticides and the Living Landscape*, in which he corroborated the findings by Carson. In his book, the author proved that biological systems, unlike physical systems, tend to concentrate persistent toxic products found in the environment. Thus, hardly degradable products such as dichlorodiphenyltrichloroethane (DDT) penetrate food chains, as well as accumulate and concentrate at every trophic level. In addition, they can reach fatal levels, mainly in predatory vertebrates, including humans. Thus, fish, amphibians, reptiles, birds, and mammals, which are final consumers in the food chain, can present pesticide concentrations up to millions of times larger than those found in the water they live in or have access to (Paschoal 1979; Vail 2015).

The impact of pesticides on the environment depends, to a large extent, on the amount of active ingredients applied to the crop; on the place of application; on pesticide partition coefficient and concentration in the air, soil, surface water, and groundwater; on pesticide degradation rate in each environmental compartment; and on its toxicity to the species living in compartments of the soil-water-plant-atmosphere system (Primel et al. 2005).

Most issues associated with the use of agrochemicals due to noncompliance with safety regulations focused on product handling, application, and recommended doses. Consequently, food and the environment may present residual levels above the ones recommended in the legislation, which turns the intake of certain products into a significant risk to human health (ANVISA).

The indiscriminate use of agrochemicals can generate pest resistance to these products, besides playing a determining role in the emergence of new pests and in the imbalance in the prey-predator chain. Biological imbalance, in its turn, takes place because pesticides are much more harmful to pests' natural enemies than to the pests themselves. Paschoal (1979) has mentioned three reasons that lead pests to present advantages over predators. According to the first reason, natural enemy populations are often smaller than the pest populations they feed on, since natural enemies occupy higher trophic levels in the food chain. For example, nonselective insecticides applied to fight pests kill more predators and parasites than the target pests themselves; it happens because they exist in smaller numbers. The second reason is associated with reduced populations of predatory and parasitic species, who present lower genetic variability than large pest populations. Thus, chemical resistance genes are more easily transmitted to new generations in pest populations and less so to natural enemies, because the likelihood of survival of individuals carrying resistance genes is higher in pest populations. Finally, the last reason is linked to the incidence of pests over millennia of natural selection and evolution, which enables them to develop a certain resistance to pesticides. Because predators and parasites do not face this type of selective pressure, resistance is not required to be developed as pre-adaptive mechanism, and, consequently, they are more sensitive to insecticides.

Therefore, pesticides and pests eliminate useful organisms, animals, and plants; consequently, they reduce biodiversity and generate significant instability in different ecosystems. Resulting changes in these ecosystems make farmers use increasing amounts of pesticides, which leads to pest resistance to these inputs (Borges Filho 2004).

3.3 Water Contamination with Pesticides

According to IBGE (2011), agrochemical residues are the second main source of contamination in Brazilian water sources, second only to sanitary sewage. However, the use of these substances has increased, fact that requires improving the knowledge about the chemical risks, mainly for water sources. Agrochemicals comprise substances acknowledged for their toxicity to biological organisms. Regular re-evaluations are the only way to achieve acceptable safety levels for the incidence of such substances in water (EMBRAPA 2014). Approximately one-third of all produced organic compounds are discharged in the environment, mainly in water. Approximately 700 chemical compounds, including more than 600 organic compounds, many of which are biologically active, have been identified in water samples (Primel et al. 2005).

The main crops in Southern Brazil comprise soybeans, rice, potatoes, tobacco, corn, wheat, and grapes. The first four crops stand out in the use of pesticides; however, there are few studies available for all of them. Grützmacher et al. (2008) monitored pesticides in the water of São Gonçalo and Piratini river channels in Southern Rio Grande do Sul State and found carbofuran, quinclorac, clomazone, and fipronil residues in it.

Rice crops prevail in this region, where the drainage of the irrigated area after sowing in pre-germinated crop systems (widely used in Rio Grande do Sul State) can trigger serious environmental issues. At the same time, it can cause losses of nutrients and/or herbicides suspended in the irrigation water to be released.

Mattos et al. (2002) have conducted a study in Rio Grande do Sul State and found incidence of glyphosate in rice fields irrigated with water from Mirim Lake, at concentrations higher than 7 μg L^{-1} – the maximum concentration allowed by the American Environmental Protection Agency (USEPA), carried out studies about the monitoring of pesticides in irrigated rice crop areas in the coastal plain and in the western border of Rio Grande do Sul State, in the 2007/2008 crop. They found residues of 3 hydroxy-carbofuran, clomazone, 2,4-D, azoxystrobin, bentazone, difenoconazole, edifenphos, ethoxysulfuron, fipronil, glyphosate, imazethapyr, mancozeb, oxadiazone, oxyfluorfen, penoxsulam, propanil, tebuconazole, tetraconazole, thiabendazole, and thiobencarb (11 herbicides, 2 insecticides, and 7 fungicides). Glyphosate and 2,4-D were found at concentrations below the limits established by CONAMA. According to the aforementioned authors, 2,4-D herbicide residues were also found in a rice crop area located in the western border of Rio Grande do Sul State in the 2006/2007 crop, at the concentration of 0.001 mg.kg $^{-1}$ of soil (Mattos et al. 2007, EMBRAPA 2014).

Silva et al. (2009) found residues of several agrochemicals after the drainage of crops, which indicated that this technique must be improved in order to avoid the contamination of water bodies receiving the water from the rice crop. The aforementioned study also observed that crops in Santa Catarina State presented the same contamination pattern found in Rio Grande do Sul State. Studies available in the literature have shown that pesticide residues found in the water drained from rice fields were also found in all producing regions of Rio Grande do Sul State, including the central depression (Marchesan et al. 2010) and the southern region of the state (Grützmacher et al. 2008). Irrigated rice crops pose a potential risk of spring contamination because they flood the crop areas. Therefore, studies about the adequacy of these production systems must be prioritized (EMBRAPA 2014).

Organophosphates are mainly used in tobacco plantations grown in small family-owned properties in Rondinha County, Rio Grande do Sul State. Griza et al. (2008) conducted a study in this county, where they found contamination in superficial waters and in a source of mineral water used for human consumption purposes. Bortoluzzi et al. (2006) also observed impaired quality of the surface water in watercourses located in a micro-basin near a tobacco crop grown in Agudo County, Rio Grande do Sul State, due to the incidence of active principles of pesticides. Furthermore, Sequinatto et al. (2006) reported that the surface water used for human consumption in a small rural river basin where tobacco was grown was contaminated with agrochemical residues. Of the seven active principles analyzed in their study, three (imidacloprid, atrazine, and clomazone) were found in streams and in water sources used for human consumption.

Data about water contamination with pesticides deriving from soybean and corn crops grown in the states of the southern region remain scarce and require further studies focused on quantitatively analyzing such contamination. It is known that the

contamination happens because many streams are silted by sediments transported from these plantations.

Paraná and Rio Grande do Sul states are the third and fourth largest potato producers in Brazil; the first two states are Minas Gerais and São Paulo (EMBRAPA 2005). Potato crops often require the exaggerated use of agrochemicals. Consumers often select potatoes based on visual features such as the shape and color of tubers; they prefer the smooth and shiny ones (EMBRAPA 2005). Thus, potato farms often make abusive use of agrochemicals in order to grow products suitable to the market, mainly the ones located in the Brazilian southern region, where up to 30 sprays per production cycle are applied (Lopes e Buso 1999).

3.4 Fish Contamination with Pesticides

Aquatic ecosystems are increasingly vulnerable to pesticide contamination. Overall, the surroundings of river basins in Rio Grande do Sul State are occupied by small farmers, who practice intensive agriculture in areas presenting slope terrain and erosions. These conditions are favorable for soil and water contamination. Thus, the aquatic biota is constantly exposed to agricultural runoff processes; consequently, agrochemicals and fertilizers eventually reach the water of rivers and streams and contaminate water bodies.

The toxicity of agrochemicals is overall expressed as the effective concentration, or dose, capable of producing a specific effect on 50% of a test species population (EC 50 or ED 50). Terms such as LC50 and LD50 are used when the recorded effect is the death of individuals. The non-observed-effect concentration (NOEC or NOEL) is the dose immediately below the lowest dose causing some kind of toxicological response. Agrochemicals can cause severe damage to living organisms found in aquatic ecosystems, with emphasis on fish mortality (Sissino and Oliveira-Filho 2013).

When pesticides reach water bodies, they contaminate these sites and cause serious damage to nontarget organisms such as fish, fact that can lead to significant changes in certain physiological and biochemical processes taking place in these animals (Matthiessen 1995; Shweta et al. 2007). It happens because fish are particularly sensitive to the influence of pesticides, since they are able to absorb and retain these xenobiotics dissolved in water via active or passive transport. Physiological changes observed in fish are not only a response to low pesticide concentrations in the environment, but they also help better understanding these pollutants in biological terms and enable the development of a toxicity model for vertebrates, including humans (Sancho et al. 2010).

Besides natural water courses, fish farming systems are also vulnerable to contamination – this practice is often adopted in Southern Brazil. Most breeding sites are near, or within, agricultural plantation areas, fact that enables the direct contact between animal agrochemicals used in crops. Thus, the continuous incidence of toxic components in the water can cause several changes in fish, including changes

in their reproductive behavior; these components can even kill these individuals. The extinction of species that are more susceptible to this type of environmental change can be a long-term effect of the abusive use of agrochemicals (Soso et al. 2007).

Fish can make contact with pesticides through the intake of contaminated food and water, as well as through breathing and skin contact. These chemical substances overcome several barriers of the body and reach tissues where they can be metabolized or deposited. Fish can accumulate pesticides at concentrations much higher than the ones found in the water they live in; this process is known as biomagnification. Several tests are used to evaluate different pesticide concentrations and the time of exposure to these pesticides in test organisms such as fish as well as to measure their response to particular substance. Among these tests, one finds the ones in which fish die due to the toxic effect of the tested agrochemical, as well as sublethal tests applied to analyze biochemical, physiological, histological, and behavioral changes in animals exposed to pesticides. Biochemical responses can be determined through the assessment of oxidative stress and of the antioxidant defense system of these organisms, based on enzymatic and non-enzymatic tests. The toxic effect of pesticides on fish depends on their susceptibility level, on the time of exposure to the chemical features and concentrations of the toxicant, and on environmental factors.

Fish are extremely important components of freshwater ecosystems, because they are at the top of the aquatic food chain, besides being consumed by humans. Thus, fish communities have been widely used to evaluate and monitor the possible contamination of these environments. Many national and international surveys have reported the effects of several pesticides on different fish species. These studies include field surveys and research conducted under controlled laboratory conditions.

Spacie and Hamelink (1985) reported the bioaccumulation and biomagnification of insecticides such as DDT (dichlorodiphenyltrichloroethane), DDD (dichlorodiphenyldichloroethane), and methylmercury both in fish and in wild animals. Bueno-Krawczyk et al. (2015) used Astyanax bifasciatus as bioindicators to evaluate the ecological integrity of Iguaçu Mid River basin in Paraná State, since it receives agricultural effluents and is used for public water supply. Although the results of the physical and chemical analyses were within the limits recommended by the Brazilian legislation on water bodies, changes in genetic and biochemical biomarkers were observed, fact that indicated changes in fish health status. This outcome is concerning for aquatic and human populations, since this water is used for fishing and drinking water supply purposes.

Different studies have been conducted in irrigated rice farming systems based on rizipisciculture models. Clasen et al. (2012, 2014) used insecticides such as fipronil and carbofuran; Toni et al. (2011, 2013) used the fungicide tebuconazole and the herbicide quinclorac, respectively; and Cattaneo et al. (2011a, b) used herbicides penoxsulam and clomazone, respectively. All these studies found changes in enzymatic biochemical parameters and oxidative stress in carp belonging to species *Cyprinus carpio*.

Changes in the antioxidant system and oxidative stress have also been reported in fish (*Cyprinus carpio* and *Rhamdia quelen*) exposed to different pesticide formu-

lations in studies conducted under laboratory conditions (Moraes et al. 2009; Menezes et al. 2011; Cattaneo et al. 2011a, b; Murussi et al. 2013; Antes et al. 2013; Clasen et al. 2014, 2018).

Based on these and on other studies carried out to evaluate the toxicological potential of different pesticides, it was possible seeing that biochemical tests are important to help assessing the quality of aquatic ecosystems, since, in many cases, even when the physical and chemical parameters and the concentration of agrochemicals are within the values recommended by the Brazilian legislation, damaging effects to organisms living in these water bodies may happen, thus threatening and even endangering some species.

3.5 Conclusions

The indiscriminate use of pesticides in the Brazilian countryside, mainly in Southern Brazil, is concerning. Thus, urgent measures need to be taken in order to avoid irreversible damages in the near future.

In light of the aforementioned, the agricultural activity has been seen as one of the main activities causing environmental contamination; the indiscriminate use of agrochemicals is the determining factor for such contamination, mainly when in water resources. The identification of pesticide residues in surface and groundwater in Southern Brazil has been reported by several researchers, fact that reinforces the need of monitoring these water bodies, since many of them are used as source of water supplied to the population. It is worth emphasizing that the determination of biomarkers in organisms acting as bioindicators of these environments is essential to enable the evaluation of the real conditions of these ecosystems, as well as to avoid false-negative results.

However, besides encouraging research, it is essential raising population awareness, mainly farmers' awareness, about the rational use of pesticides in order to reverse this situation. They need to be informed about the acute and chronic health effects of the exposure to these agrochemicals, as well as about environmental damages caused by the abusive use of these products, since they contaminate the water and accumulate in certain species, such as fish, which can be consumed by humans. This situation also happens in countless other contaminated food such as fruits and vegetables, which arrive at our tables with high residual pesticide levels.

Moreover, the international market already demands, and will increasingly demand, food with reduced agrochemical residue levels, as well as production systems that have reduced environmental impacts. Given this scenario, Brazil needs to adapt to these conditions in order to keep exporting its products.

According to Gomes and Barizon (2014), Brazil needs to improve the control of agrochemicals used in agriculture. It is essential to implement new regulatory measures focused on the use of agrochemicals and encourage further studies in order to generate complementary information about this issue. Nowadays, it may be the most important procedure to enable sustainability in rural areas.

References

Aenda – Associação Brasileira dos Defensivos Genéricos (2011) Disponível em: http://www.aenda.org.br/. Acesso em 08 set 2015

Agrawal A, Pandey RS, Sharma B (2010) Water pollution with special reference to pesticide contamination in India. J Water Res Protection 2:432–448

Antes FG, Becker AG, Parodi TV, Clasen B, Lópes T, Loro VL, Baldisserotto B, Flores EMM, Dressler VL (2013) Toxicity of triphenyltin hydroxide to fish. Arch Environ Contam Toxicol 65:733–741

ANVISA – Agência Nacional de Vigilância Sanitária. Disponível em: http://portal.anvisa.gov.br/wps/content/Anvisa+Portal/Anvisa/Inicio/AgrotoxicoseToxicologia accessed in: 09 apr 2017

Barbara Clasen, Vania L. Loro, Camila R. Murussi, Tadeu Luis Tiecher, Bibiana Moraes, Renato Zanella, (2018) Bioaccumulation and oxidative stress caused by pesticides in Cyprinus carpio reared in a rice-fish system. Science of The Total Environment 626:737–743

Bombardi LM (2017) Geografia do uso de agrotóxicos no Brasil e conexões com a união europeia. São Paulo, FFLCH – USP, 296p

Borges Filho EL (2004) Impactos ambientais ocasionados pelo uso de defensivos agrícolas: a escassez de pesquisas no programa nacional de pesquisa agropecuária. In: Congresso Brasileiro de Economia e Sociologia Rural, 2004. XLII Congresso da sociedade brasileira de economia e sociologia rural, Cuiabá

Bortoluzzi EC, Rheinheimer DS, Gonçalves CS, Pellegrin JBR, Zanella R, Copetti ACC (2006) Contaminação das águas superficiais por agrotóxicos em função do uso do solo numa microbacia de Agudo, RS. Rev bras eng agríc ambient 10:881–886

Bueno-Krawczyk ACD, Guiloski IC, Piancini LDS, Azevedo JC, Ramsdorf WA, Ide AH, Guimarães ATB, Cestari MM, Silva de Assis HC (2015) Multibiomarker in fish to evaluate a river used to water public supply. Chemosphere 135:257–264

Cassal VB, Azevedo LF, Ferreira RP, Silva DG, Simão RS (2014) Agrotóxicos uma revisão de suas consequências para a saúde pública. Revista Eletrônica em Gestão Educação e Tecnologia Digital 18:437–445

Cattaneo R, Clasen B, Loro VL, de Menezes CC, Moraes B, Santi A, Toni C, de Avila LA, Zanella R (2011a) Toxicological responses of Cyprinus carpio exposed to the herbicide penoxsulam in rice field conditions. J Appl Toxicol 31:626–632

Cattaneo R, Moraes BS, Loro VL, Pretto A, Menezes C, Sartori GMS, Clasen B, Avila LA, Marchesan E, Zanella R (2011b) Tissue biochemical alterations of Cyprinus carpio exposed to commercial herbicide containing clomazone under rice-field conditions. Arch Environ Contam Toxicol 61:97–106

Chaim A (2004) Tecnologia de Aplicação de Agrotóxicos: Fatores que afetam a eficiência e o impacto ambiental. Disponível em: http://www.agencia.cnptia.embrapa.br/Repositorio/Chaim_AgrotoxicoAmbiente_000fgp2794702wyiv8020uvkp2st4aal.pdf

Clasen B, Loro VL, Cattaneo R, Moraes B, Lópes T, de Avila LA, Zanella R, Reimche GB, Baldisserotto B (2012) Effects of the commercial formulation containing fipronil on the non-target organism Cyprinus carpio: implications for rice fish cultivation. Ecotoxicol Environ Saf 77:45–51

Clasen B, Leitemperger J, Murussi C, Pretto A, Menezes C, Dalabona F, Marchesan E, Adaime MB, Zanella R, Loro VL (2014) Carbofuran promotes biochemical changes in carp exposed to rice field and laboratory conditions. Ecotoxicol Environ Saf 101:77–82

CONAB – Companhia Nacional de Abastecimento (2018) Perspectivas para a agropecuária. Brasília 6:1–112

CONAMA – Conselho Nacional de Meio Ambiente. Resolução 357 de 17 de março de 2005. Disponível em: http://www.mma.gov.br/port/conama/res/res05/res35705.pdf. Acesso em: 25 set 2015

CONSEA – Análise dos indicadores de Segurança Alimentar e Nutricional. 4ª Conferência Nacional de Segurança Alimentar e Nutricional. Brasília, 2014

EMBRAPA – Empresa Brasileira de Pesquisa Agropecuária. Circular Técnica 48: Produção de Batata no Rio Grande do Sul. Ministério da Agricultura, Pecuária e Abastecimento. 2005. ISSN 1516-8832

EMBRAPA – Empresa Brasileira de Pesquisas Agropecuárias. Documento 98. Panorama da Contaminação Ambiental por Agrotóxicos e Nitrato de Origem Agrícola no Brasil: cenário 1992/2011. 2014

Gomes MAF, Barizon RRM (2014) Panorama da contaminação ambiental por agrotóxicos e nitrato de origem agrícola no Brasil: cenário 1992/2011. Embrapa Meio Ambiente, Jaguariúna, p 35p

Griza FT, Ortiz KS, Geremias D, Thiesen FV (2008) Avaliação da contaminação por organofosforados em águas superficiais no munícipio de Rondinha – Rio Grande do Sul. Quím Nova 31:1631–1635

Grützmacher DD, Grützmacher AD, Agostinetto D, Loeck AE, Roman R, Peixoto SC, Zanella R (2008) Monitoramento de agrotóxicos em dois mananaciais hídricos no sul do Brasil. Rev bras eng agríc ambient 12(6):632–637

IBGE – INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. Censo Agropecuário (2006) Available in: http://www.ibge.gov.br. accessed in: 22 aug 2018

IBGE – INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. Atlas de Saneamento (2011) Available in: http://www.ibge.gov.br/home/estatistica/populacao/atlas_saneamento/default_zip.shtm. Accessed em 02 jan. 2018

IBGE – Indicadores IBGE. Estatística da produção agrícola (2014)Available in: http://www.ibge.gov.br. Accessed in: 23 out 2017

Londres F Agrotóxicos no Brasil – Um guia para ação em defesa da vida. Rio de Janeiro (2011) Available in: http://www4.planalto.gov.br/consea/biblioteca/documentos/agrotoxicos-no-brasil.-um-guia-para-acao-em-defesa-da-vida. Accessed in: 14 sep 2017

Lopes CA, Buso JA (1999) A Cultura da Batata. Embrapa Hortaliças. Organizadores Carlos Alberto Lopes e José Amuri Buso – Brasília: Embrapa Comunicação para Transferência de Tecnologia. 184 p. 16 cm – Coleção Plantar; 42. ISBN 85-7383-0670-0

Marchesan E, Sartori GMS, Avila LA, Machado SLOM, Zanella R, Primel EG, Macedo VRM, Marchezan MG (2010) Resíduos de agrotóxicos na água de rios da depressão central do estado do Rio Grande do Sul, Brasil. Ciênc Rural, Santa Maria 16:123–127

Mattos MLT, Peralba MCR, Dias SLP, Prata F, Camargo L (2002) Monitoramento ambiental do glifosato e do seu metabólito (ácido aminometilfosfônico) na água de lavoura de arroz irrigado. Pesticidas: Revista Ecotoxicologia e Meio Ambiente, Curitiba 12:143–154

Mattos MLT, Martins JF d S, Nunes CDM, Scivittaro WB, Moura F (2007) Monitoramento de agroquímicos em áreas de produção integrada de arroz irrigado no Rio Grande do Sul. Bento Gonçalves. Embrapa Uva e Vinho, Anais

Matthiessen A (1995) Determining the redox capacity of humic substances as a function of pH. Vom Wasser 84:229–235

Melo RF, Brito LT, Miguel AA (2010) Pesticidas e seus impactos no Ambiente. Pesticidas e seus impactos no ambiente. 1ed. 1:1–187

Menezes CC, Fonseca MB, Loro VL, Santi A, Cattaneo R, Clasen B, Pretto A, Morsch VM (2011) Roundup effects on oxidative stress parameters and recovery pattern of Rhamdia quelen. Arch Environ Contam Toxicol 60:665–671

Moraes BS, Clasen B, Pretto A, Cattaneo R, Toni C, Avila LA, Marchezan E, Machado SL, Zanella R, Reimche G (2009) Toxicological responses of Cyprinus carpio after exposure toa commercial herbicide containing imazethapyr and imazapic. Ecotoxicol Environ Saf 72:1172–1178

Murussi CR, Thorstenberg ML, Leitemperger J, Costa M, Clasen B, Santi A, Menezes C, ENGERS VK, Loro VL (2013) Toxic effects of penoxsulam herbicide in two fish species reared in Southern Brazil. Bull Environ Contam Toxicol 8:548

Murussi CR, Horn RC, Santi A, Clasen BE, Reis G, Souza D, Bortolotto JW, Manfio CE, Loro VL (2014) Changes in oxidative markers, endogenous antioxidants and activity of the enzyme acetylcholinesterase in farmers exposed to agricultural pesticides – a pilot study. Ciênc Rural 44:1186–1193

Paschoal AD (1979) Pragas, Praguicidas e a Crise Ambiental: Problemas e Soluções. FGV, Rio de Janeiro, 106p

Primel EG, Zanella R, Kurz MHS, Gonçalves FF, Machado SO, Marchezan E (2005) Poluição das águas por herbicidas utilizados no cultivo do arroz irrigado na região central do estado do Rio Grande do Sul, Brasil: predição teórica e monitoramento. Químa Nova 28:605–609

Sancho E, Villarroel MJ, Fernandez C, Andreu E, Ferrando MD (2010) Short-term exposure to sublethal tebuconazole induces physiological impairment in male zebrafish (*Danio rerio*). Ecotoxicol Environ Saf 73:370–376

Scorza Júnior RP, Névola FA, Ayelo VS (2010) ACHA: avaliação da contaminação hídrica por agrotóxico. Embrapa Agropecuária, Dourados, 31 p

Sequinatto L, Reichert JM, Reinert DJ, Rheinheimer DS, Copetti ACC (2006) Contaminação da água por agrotóxicos numa microbacia cultivada com fumo. Available in: http://www.fisicadosolo.ccr.ufsm.quoos.com.br/downloads/Producao_Resumos/XVIRBMCSA_1.pdf. Accessed in 26 jul 2018

Shweta A, Pandey KC, Gopal K (2007) Biochemical alterations induced by monocrotophos in the blood plasma of fish *Channa punctatus* (Bloch). Pestic Biochem Physiol 88:268–272

Silva DRO, Avila LA, Agostinetto D, Magro TD, Oliveira E, Zanella R, Noldin JA (2009) Monitoramento de agrotóxicos em águas superficiais de regiões orizícolas no sul do Brasil. Ciênc Rural, Santa Maria 39:2283–2389

SINDAG – Sindicato Nacional das Indústrias de Defensivos Agrícolas (2011) Uso de defensivos é intensificado no Brasil. Available in: http://www.sindag.org.br. Accessed in: 09 sep 2015

SINITOX – Sistema Nacional de Informações Tóxico Farmacológicas (2012) Tabela 6. Casos Registrados de Intoxicação Humana por Agente Tóxico e Sexo. Região Sul

Sissino CL, Oliveira-Filho EC (2013) Princípios de toxicologia ambiental: conceitos e aplicações. Interciência, Rio de Janeiro, 216p

Soso AB, Barcellos LJG, Ranzani-Paiva MJ, Kreutz LC, Quevedo RM, Anziliero D, Lima M, Silva LB, Ritter F, Bedin AC, Finco JA (2007) Chronic exposure to sub-lethal concentration of glyphosatebased herbicide alters hormone profiles and effects reproduction of female jundiá (*Rhamdia quelen*). Environ Toxicol Pharmacol 23:308–313

Spacie A, Hamelink JL (1985) Bioacumulation. In: Rand GM, Petrocelli SR (eds) Fundamentals of aquatic toxicology: methods and applications. Hemisphere, New York, pp 495–525

Toni C, Loro VL, Santi A, de Menezes CC, Cattaneo R, Clasen BE, Zanella R (2011) Exposure to tebuconazol in rice field and laboratory conditions induces oxidative stress in carp (Cyprinus carpio). Comp Biochem Physiol C Toxicol Pharmacol 153:128–132

Toni C, Menezes C, Clasen B, Leitemperger J, Pretto A, Adaime MB, Martins LM, Zanella R, Loro VL (2013) Oxidative stress in carp exposed to quinclorac herbicide under rice field condition. Ecotoxicol Environ Saf 92:27–31

USDA – United States Department of Agriculture (2018) Brazil at 2040: customer and competitor. Available in: http://cnas.tamu.edu/ConfPresentations/Brazil%20at%202040%20-%20Customer%20and%20Competitor%20October%202018.pdf. Accessed in: 19 feb 2019

USEPA – United States Environmental Protection Agency. Disponível em: https://www.epa.gov/

Vail DD (2015) Toxicity abounds: new histories on pesticides, environmentalism, and silent spring. Stud Hist Philos Sci Part C: Stud Hist Philos Biol Biomed Sci 53:118–121

Chapter 4
Embryotoxicity and Teratogenicity Induced by Naproxen in *Xenopus laevis*, Species of Ecological Interest in Mexico

Hariz Islas-Flores, Itzayana Pérez-Alvarez, Leobardo Manuel Gómez-Oliván, and Nely San Juan-Reyes

4.1 Introduction

In recent years, the so-called emerging pollutants (or micropollutants) have aroused considerable interest; these compounds are of diverse origin and chemical nature, whose presence and consequences in the environment have gone unnoticed since they are present in waters at low concentrations (from ngL^{-1} to μgL^{-1}); however, they can be harmful to human health and the environment, as they can produce various effects on organisms, such as chronic toxicity, endocrine disruption, and bioaccumulation (Virkutyte et al. 2010). Among the emerging pollutants, those that probably cause the greatest concern and study in recent years are pharmaceuticals, and, among them, one of the groups that have been detected more frequently and at higher concentrations in the environment (Sim et al. 2010) is that of non-steroidal anti-inflammatory drugs (NSAIDs), a heterogeneous group of drugs that are among the most prescribed as analgesics, anti-inflammatories, and antipyretics around the world, and these include acetylsalicylic acid, acetaminophen, ibuprofen, diclofenac, and naproxen (NPX). Its presence in different bodies of water comes not only from excretions, where an important part of the drug is eliminated from the body without being metabolized, but also from the inadequate manufacture and disposal of waste products (Boxall 2004), without forgetting its use in veterinary, agriculture,

H. Islas-Flores (✉) · I. Pérez-Alvarez · L. M. Gómez-Oliván
Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico
e-mail: hislasf@uaemex.mx

N. San Juan-Reyes
Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

Departamento de Farmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_4

livestock, and poultry farming, which has been increasing in recent years (Patiño Menéndez et al. 2014).

NPX is an inhibitor of the synthesis of prostaglandin derived from propionic acid, widely used in current therapy for symptomatic treatment of acute or chronic processes related to pain and inflammation. In the pharmaceutical market, NPX is available in tablets, capsules, suspension, and injectables and is one of the most prescribed pharmaceuticals in the world; although in Mexico there are no official data on its use, it has been reported that NSAIDs are the drugs with the highest consumption in the process of self-medication by users of community pharmacies (Gómez-Oliván et al. 2009), while another study showed that NPX was the most marketed pharmaceutical sold over the counter and the second by prescription in 70 pharmacies located in the state of Hidalgo (Mexico) (Rodríguez-Anaya et al. 2015).

About its presence in bodies of water, NPX has been reported in concentrations ranging from 0.001 to 1,717.31 μg L^{-1} in different countries (Nannou et al. 2015; Guerra et al. 2014; Lolic et al. 2015; Martín et al. 2012; Al Aukidy et al. 2012; Fang et al. 2012; Gonzalez-Rey et al. 2015; Yu et al. 2013; Santos et al. 2013); as regards to Mexico, there are few studies; its presence has been demonstrated in the Mezquital Valley's irrigation system, State of Mexico, at concentrations of 0.60–6.74 μg L^{-1} (Siemens et al. 2008); in the Valley of Tula, Hidalgo, it has concentrations of 7267–13,589 μg L^{-1} (Gibson et al. 2010); in the water supply Lerma-Cutzamala, it was found in groundwater at concentrations of 52–186 ng L^{-1} and in surface water of 1–5 ng L^{-1} (Félix-Cañedo et al. 2013); in the Madín Dam, it has concentrations of 0.21 μL^{-1} (González-González et al. 2014); and in wastewater from a hospital in Toluca, State of Mexico, there are concentrations of 123.5 ng L^{-1} (Neri-Cruz et al. 2015). Pharmaceuticals, like other emerging pollutants, have been reported as inducers of toxic effects in the laboratory of various aquatic organisms; however, there are few reports on NPX, including mortality in T. *platyurus* and *B. calyciflorus* at concentrations of 84.09 mg L^{-1} and 62.48 mg L^{-1}, respectively (Isidori et al. 2005), immobilization in *C. dubia* at 66.37 mg L^{-1} (Isidori et al. 2005), LC50 of 166 mg L^{-1} in *Daphnia magna* (Cleuvers 2004), and inhibition of growth/reproduction in *P. subcapitata*, *B. calyciflorus*, and *C. dubia* in concentrations of 31.82 mg L^{-1}, 0.56 mg L^{-1}, and 0.33 mg L^{-1}, respectively (Isidori et al. 2005; Cleuvers 2004).

The Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX) is an acute trial lasting 96 h in which mortality, morphological alterations, and the minimum concentration capable of inhibiting growth are determined as final parameters. It has been used to make more than 100 scientific contributions focused on the evaluation of physical, chemical substances, mixtures, and environmental samples of water and soil (Prati et al. 2000; Hoke and Ankley 2005). This test is performed using *Xenopus laevis* because of its biological characteristics suitable for captive breeding (short reproductive cycle and fully aquatic habitat) and for being the amphibian most studied and best known in research (Sparling et al. 2000; Robert and Ohta 2009); in addition, the characterization of its embryogenesis has been widely documented at the molecular and cellular levels, providing researchers with a reliable description of the different stages of its embryonic development (Nieuwkoop and Faber 1956).

Due to the above, it is very important to perform toxicological studies to characterize the impact that NPX can produce to the environment and the organisms, so the objective of this work was to evaluate the teratogenesis and the potential of toxicity in the development produced by NPX on *Xenopus laevis* using the FETAX assay, which will allow reliable tests to assess the toxicity of this highly consumed pharmaceutical in Mexico and present in their bodies of water, as well as help determine its possible environmental impact.

4.2 Methods

4.2.1 Test Substance

Naproxen (CAS number 22204-53-1,> 98% purity) $CH_3OC_{10}H_6CH(CH_3)CO_2H$, 230.26 Da, was purchased from Sigma-Aldrich. The stock solution was prepared by dissolving 1 g of NPX in deionized water.

*4.2.2 Test Organisms (*Xenopus laevis*)*

The organisms were obtained from a center of aquaculture located in the State of Querétaro (México); the selection criteria for the specimens were the following: males of 7.5–10 cm in length and 2 years of age and females with length of 10–12.5 cm and 3 years old. The females are identified as having a larger size and the presence of cloacal lips. They were acclimated in natural water, and the following parameters were determined once a month: pH (between 6.5 and 9), TOC (less than 10 mg/L), and alkalinity and hardness by determining $CaCO_3$ (between 16 and 400 mg L^{-1}). They were kept in a room isolated from light sources, having photoperiods 12 h light/12 h dark; males and females were separated and kept in tanks of 60 L at 80% capacity, keeping the walls opaque, maintaining a temperature of 21 ± 3 °C. It was fed three times a week ad libitum with *Chrisotoma sp*. 0.5 ± 0.3 cm in length or with commercial feed.

4.2.3 Evaluation of Teratogenesis (FETAX Assay)

The study was conducted in accordance with the procedures of the standard guide of the American Society for Testing Materials (ASTM (American Society for Testing Materials) 2012).

4.2.3.1 Induction to Ovulation

A pair of male and female was placed in 40 L aquariums, equipped with a mesh, nylon, or plastic, suspended approximately 3 cm from the bottom in which the eggs can be deposited, with the sides of the aquarium opaque and the water temperature maintained at 20 ± 2 °C, with a photoperiod of 12 h light/12 h dark. To induce the amplexus, a subcutaneous injection of 700 IU of human chorionic gonadotropin hormone (HCG, CHORAGON®, Ferring), dissolved in solution 0.9% sterile NaCl to females and 300 IU to males, was applied subcutaneously the night before the test in the dorsal-lymphatic sac, using 1 mL syringes equipped with 26-gauge long needles.

4.2.3.2 Oocyte Selection

The next morning, the laying of the eggs was checked; they were extracted from the fish tank using the nylon mesh and with the help of sterile Pasteur pipettes; they were placed in a separate container; later they were observed using a stereoscope, and those found in the middle blastula stage (Stage 8) were selected.

4.2.3.3 Preparation of the FETAX Medium

The FETAX medium will be prepared by dissolving 625 mg NaCl, 96 mg $NaHCO_3$, 30 mg KCl, 15 mg $CaCl_2$, 60 mg $CaSO_4$ ● $2H_2O$, and 75 mg $MgSO_4$ per liter of deionized water. All reagents were purchased from Sigma-Aldrich (St. Louis). The final pH of the solution was 7.6–7.9.

4.2.3.4 Preparation of NPX Concentrations and Control Group

For the exposed groups, six concentrations (1, 4, 8, 16, 32, and 62.5 mg L^{-1}) of NPX were used and diluted in FETAX medium; the control group was exposed to FETAX medium only. The entire procedure was performed under a laminar flow hood. Subsequently, the mother solution was distributed in amber bottles; the minimum volume of each final concentration was 32 mL of FETAX medium.

4.2.3.5 Treatment and Seeding of Oocytes

In the laminar flow hood, 8 mL of each concentration to be evaluated of NPX were placed in 50 mm Petri dishes previously identified. Additionally, a box with 8 mL of FETAX medium without contaminants was filled as a negative control. With thin tweezers, the eggs having a regular spherical shape and homogeneous cell division were collected, for which each was observed with the Zeiss Stemi 305 stereoscope.

Subsequently, 20 oocytes were added in the middle blastula stage in each Petri dish with the different concentrations for exposed groups and control, at room temperature. Finally, they were kept in the Petri dishes with the lid at 21 ± 2 °C and with dark light in an incubator for 96 h.

4.2.3.6 Assay Monitoring

The FETAX medium of the exposed and control groups was changed daily under the laminar flow hood, for which 8 mL of each prepared concentration was added in new sterile 50 mm Petri dishes previously marked which were kept 1 h 30 min at room temperature to ensure that the FETAX medium was at 20 °C before adding to the eggs. At 24 h, the live larvae were transferred to new Petri dishes using the stereoscope microscope and Moria fine forceps. At 48 and 72 h, the transfer of live larvae was carried out with the aid of a 2 mL sterile Pasteur pipette. A daily report was prepared with observations of the number of dead larvae and precipitates (if any) for each crop.

4.2.3.7 Examination of Larvae

At 96 h it was found that the larvae would swim, otherwise it was reported on a leaf of development parameters in which the malformations present in the larvae were noted; in addition the precipitates were recorded (if they existed), and the number of dead larvae on the observation sheet were removed from the Petri dishes. Euthanasia of the larvae was carried out by placing them in 0.06% MS-222 solution (lethal dose) in a 50 mm Petri dish. Subsequently, each straight larva was measured from the head to the end of the tail using the Zeiss Blue software, and the value was reported on a sheet of development parameters. Subsequently, each larva was examined in the stereoscopic microscope Zeiss Stemi 305 coupled to an Axiocam 5s camera to identify developmental malformations, based on the Atlas of anomalies (Bantle et al. 1998). After the examination, the larvae were eliminated according to internal rules relative to the biological samples.

4.2.3.8 Analysis of Results

Acute toxicity was calculated by determining the median lethal concentration (LC50) and the mean concentration of malformation (EC50) using a probit analysis program included EPA v 1.5. The teratogenic index (IT) was determined by the following relationship: TI = (LC50)/(EC50). To determine the minimum concentration that inhibits growth (CMIC), the head-to-tail measurements of the different concentrations evaluated were compared using a one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test, and the values were considered significant; one $P < 0.05$.

4.3 Results

Table 4.1 shows the results obtained for the number and percentage of deaths and malformations in the exposed embryos, as observed the LC50 for *Xenopus laevis* was 43.486 mg L^{-1} with a confidence interval of 95% (25.141–61.831), whereas for the EC50 of malformations, a result of 8.319 mg L^{-1} was obtained with a confidence interval of 95% (2.420–14.219).

Figure 4.1 shows the malformations presented in the *Xenopus laevi*s embryos exposed to different concentrations of NPX; as can be seen, its severity was higher when increasing the concentration of the NPX.

Among the disorders that appeared most frequently were malformation in the eye, brain, and notochord and abdominal and cardiac edema. Also, in Fig. 4.2 you can see the most representative of each concentration.

On the other hand, the higher concentrations of NPX caused that the size in the larvae was reduced in a significant way. Figure 4.3 shows the relationship observed between the size of the *Xenopus laevis* embryos and the different concentrations of NPX to which they were exposed, a statistically significant difference ($P < 0.05$) was observed with respect to the control group in all concentrations.

The teratogenic index (TI) calculated after exposure to different concentrations of NPX in *Xenopus laevis* was 5.2. TI values greater than 1.5 indicate greater potential for the toxin to cause malformations in embryos (ASTM (American Society for Testing Materials) 2012).

Table 4.1 Lethal and teratogenic effects of *Xenopus laevis* embryos exposed for 96 h at different concentrations of NPX

	Xenopus laevis				
Concentration (mg L^{-1})	Number of embryos exposed	Number of deaths	% mortality	Number of embryos malformed	% malformations
0	60	0	0.0	0	0.0
1	60	18	30	16	26.7
4	60	21	35	27	45.0
8	60	26	43.3	34	56.7
16	60	28	46.6	40	66.7
32	60	29	48.3	47	78.3
62.5	60	32	53.3	52	86.7
LC50	**43.486**				
EC50	**8.319**				
TI	**5.2**				

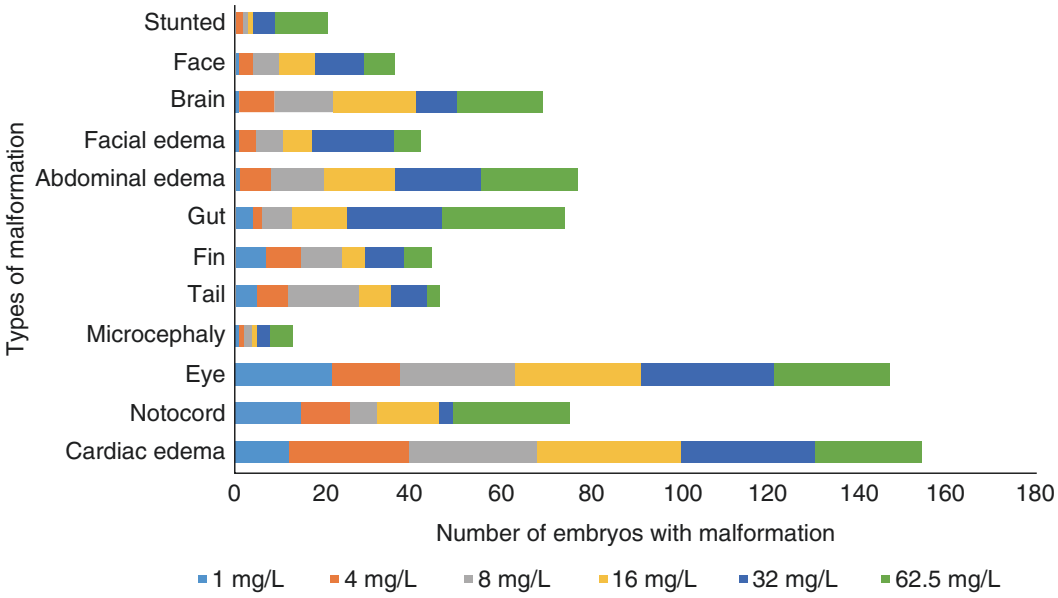

Fig. 4.1 Types and frequency of malformations in *Xenopus laevis* embryos after 96 h of exposure to different concentrations of NPX

4.4 Discussion

Recently, the presence of pharmaceuticals in the environment has attracted the attention of various authorities and research groups due to their ability to cause adverse effects in aquatic organisms and even affect the health of human beings; this is because they were designed to be biologically active at low concentrations and with different mechanisms of action, in addition to having the ability to modify the physiology and possibly the behavior (Boxall 2004), so they represent a threat both for the multiple ways of release to water bodies and for the lack of information on their absorption and effects on aquatic organisms. One of the groups of pharmaceuticals that present a higher incidence in the environment is NSAIDs, and among them of the most found is the NPX due to its low cost and high consumption (e.g., approximately 3000 tons were produced in the year 2003 worldwide (Li et al. 2015)).

In this study, NPX exhibited adverse effects in terms of mortality, malformations, and growth inhibition. When exposing *Xenopus laevis* frog embryos at 6 concentrations (1, 4, 8, 16, 32, 62.5 mg L^{-1}), a LC50 of 43.486 mg L^{-1} was obtained with a 95% confidence interval (25.141–61.831) (Table 4.1). There are several studies in which it is demonstrated that NPX is capable of generating diminution in the degree of survival at low concentrations; for example, when exposing *Oryzias latipes*, changes were observed in the percentage of survival during the juvenile stage, starting from concentrations of 0.5 mg L^{-1} (Kwak et al. 2018). On the other hand, other studies report a LC50 of 269.15 mg L^{-1} obtained after exposing adults of C*yprinus carpio* for 96 h (Gheorghe et al. 2016); however, it is worth highlighting a study conducted by Li et al. (2016) who reports an LC50 of 115.2 mg L^{-1} in zebrafish embryos at a 120 h exposure, considering that these organisms are in early

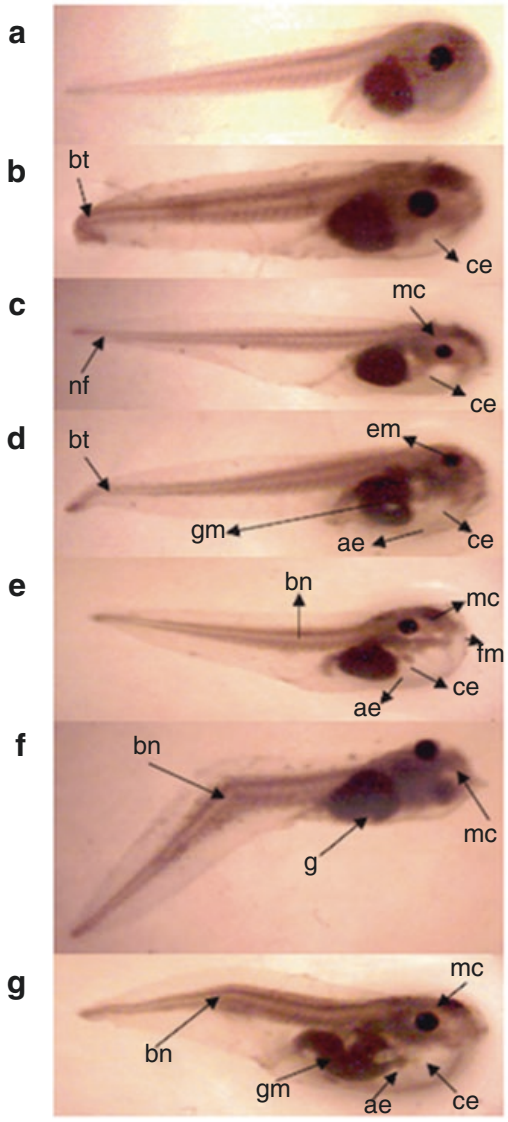

Fig. 4.2 Most representative malformations observed in *Xenopus laevis* embryos exposed to NPX at different concentrations: (**a**) control, (**b**) 1 mg L^{-1}, (**c**) 4 mg L^{-1}, (**d**) 8 mg L^{-1}, (**e**) 16 mg L^{-1}, (**f**) 32 mg L^{-1}, and (**g**) 62.5 mg L^{-1}. Abbreviations: bt bent tail, ce cardiac edema, nf narrow end, mc microcephaly, em eye malformation, ae abdominal edema, gm gut malformation, bn bent notochord, fm face malformation, g gut

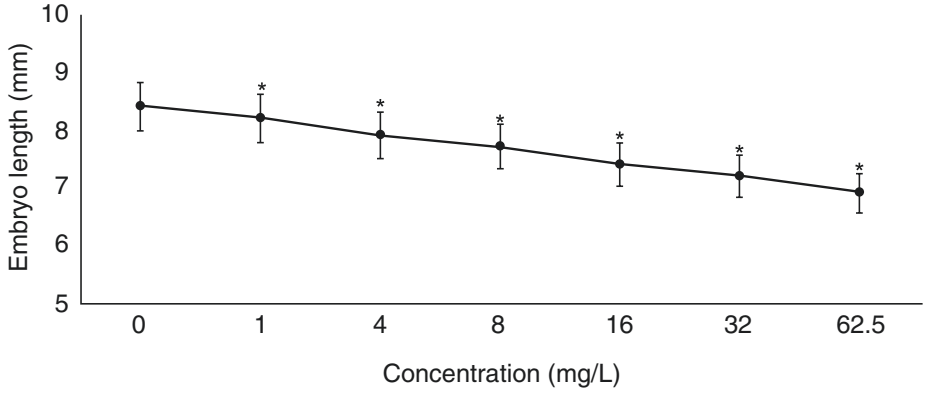

Fig. 4.3 Relationship between NPX concentration and size of the *Xenopus laevis* embryos. ∗Significant difference with respect to its control group, ANOVA and Bonferroni tests ($P < 0.05$)

stages of development, as in this study we can assume that *Xenopus laevis* frog embryos have a greater sensitivity to NPX and possibly other pharmaceuticals with the same mechanism of action that can be found in aquatic environments, highlighting the importance of carrying out acute toxicity studies in amphibians and in the early stages of development since they can manifest more severe toxic effects, due to the fact that organisms go through complex processes of energy exchange and cellular signaling, so it is the phase in which they are most vulnerable. The death of embryos could be explained by the mechanism of action of the NPX, which acts by blocking the enzyme cyclooxygenase (COX), responsible for catalyzing the degradation of arachidonic acid in the production of prostaglandins, which are involved in processes such as neurotransmission, regulation in the circulatory system, vascular permeability, and ion transport through cell membranes (Arkhipova et al. 2005); coupled with this, there is a reduction in leukotriene levels, related to the sending of a cell survival signal (Öhd et al. 2000).

Regarding malformations, Table 4.1 reports an EC50 value of 8.319 mg L^{-1} with a 95% confidence interval (2.420–14.219), the main malformations observed being cardiac and abdominal edema and malformations in the intestine, eye, and brain (Figs. 4.1 and 4.2). A study conducted in *Danio rerio* reports malformations such as pericardial edema from a concentration of 20 mg L^{-1} with an EC50 of 98.3 mg L^{-1} and a concentration-dependent behavior (Li et al. 2016); these results coincide with those obtained in this study since one of the main malformations observed was cardiac edema, which increased in frequency and severity as the concentration of NPX was increased. Other studies report that this pharmaceutical is also capable of altering the hatching time in *Cyprinus carpio* embryos according to Sehonova et al. (Stancova et al. 2015; Stancová et al. 2015) from concentrations of 10 μg L^{-1}, in addition to generating alterations such as hyperpigmentation and malformations in cells of mucous membranes and gills, as well as a decrease in the weight and size of larvae, and it is also capable of generating 100% growth inhibition in *Cymbella sp.* and *Scenedesmus quadricauda* at a concentration of 100 mg L^{-1} in a period of 24 h (Ding et al. 2017); in this study an inhibitory growth concentration (IGC) of 1 mg L^{-1} was obtained (Fig. 4.3) which was the lowest tested concentration; however, it is possible that when exposing *Xenopus laevis* to lower concentrations, adverse effects on the size of the larvae are observed because it is one of the most sensitive parameters of the FETAX assay.

In addition to inhibition of growth, a teratogenic compound causes severe malformations while non-teratogenic ones usually produce death but no malformations (Bantle et al. 1989). In general, values of IT <1.5 indicate a low teratogenic potential, that is, they have little or no separation between concentrations that induce malformations and without embryonic lethality and those concentrations that cause lethal effects (Fort et al. 1992). The value obtained from TI obtained by the exposure of *Xenopus laevis* to NPX was 5.2 (Table 4.1); this value indicates that the test compound has teratogenic potency for this species; this result could be explained by other effects generated by the NPX and that have been described previously: it has been reported that it is able to generate branchial hyperemia, widening of the apex in the lamella, and desquamation of gills at concentrations of 1 μg L^{-1} in *Danio*

rerio adult, as well as harmful effects on the liver, and is related to imbalance in the levels of antioxidant enzymes glutathione peroxidase and catalase (Stancova et al. 2015; Stancová et al. 2015); by generating these, alterations in the levels of antioxidant enzymes can trigger a process of oxidative stress, which generates damage in different biomolecules such as lipids, proteins, and DNA, besides altering multiple signaling pathways (Wells et al. 2009). On the other hand, the ability of NSAIDs to generate toxic effects in various biological processes in aquatic organisms is known, since they may be involved in processes of endocrine disruption, having the ability to alter the processes of steroidogenesis; Kwak et al. (Kwak et al. 2018) suggest that NPX could be related to the alteration in estrogen cycles during early stages of development, generating alterations in vitellogenin levels and in the activation of estrogen receptors; the COX are involved with embryonic development in various processes, mainly COX-1, enzymes necessary for segmentation, production of pro-angiogenic factors, and the development of mesodermal organs, among other biological processes, for which the pharmacological inhibition of COX by the action of NPX can trigger the development of cardiac malformations and the decrease in the size of intersomitic vesicles (Cha et al. 2005, 2006).

4.5 Conclusions

NPX is a teratogen compound during the embryonic development of *Xenopus laevis* and is capable of inducing various malformations, among which are cardiac and abdominal edema and malformations in the intestine, eye, and brain.

References

Al Aukidy M, Verlicchi P, Jelic A et al (2012) Monitoring release of pharmaceutical compounds: occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. Sci Total Environ 438:15–25

Arkhipova O, Grishin S, Sitdikova G, Zefirov A (2005) Presynaptic effects of arachidonic acid and prostaglandin E2 in the frog neuromuscular synapse. Ross Fiziol Zh Im I M Sechenova 91:268–276

ASTM (American Society for Testing Materials) (2012) Standard guide for conducting the frog embryo-teratogenesis assay – *Xenopus*. FETAX:E1439–E1412

Bantle JA, Fort DJ, James BL (1989) Identification of developmental toxicants using the Frog Embryo Teratogenesis Assay-*Xenopus* (FETAX). Hydrobiologia 188/189:577–585

Bantle JA, Dumont JUN, Finch RA, Linder G, Fort DJ (1998) Atlas of abnormalities a guide for the performance of FETAX, 2nd edn. Oklahoma State University, Stillwater

Boxall ABA (2004) The environmental side effects of medication. EMBO Rep 5:1110–1116

Cha YI et al (2005) Cyclooxygenase-1 signaling is required for vascular tube formation during development. Dev Biol 282:274–283

Cha YI, Solnica-krezel L, Dubois RN (2006) Fishing for prostanoids: deciphering the developmental functions of cyclooxygenase-derived prostaglandins. Dev Biol 289:263–272

Cleuvers M (2004) Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxicol Environ Saf 59:309–315

Ding T et al (2017) Biodegradation of naproxen by freshwater algae *Cymbella sp.* and *Scenedesmus quadricauda* and the comparative toxicity. Bioresour Technol 238:164

Fang TH, Nan FH, Chin TS et al (2012) The occurrence and distribution of pharmaceutical compounds in the effluents of a major sewage treatment plant in Northern Taiwan and the receiving coastal waters. Mar Pollut Bull 64(7):1435–1444

Félix-Cañedo TE, Durán-Ávarez JC, Jiménez-Cisneros B (2013) The occurrence and distribution of a group of organic micropollutants in Mexico City's water sources. Sci Total Environ 454:109–118

Fort DJ, Rayburn JR, Bantle JA (1992) Evaluation of acetaminophen-induced developmental toxicity using FETAX. Drug Chem Toxicol 15(4):329–350

Gheorghe S, Petre J, Lucaciu I (2016) Risk screening of pharmaceutical compounds in Romanian aquatic environment. Environ Monit Assess 188:379

Gibson R, Durán-Álvarez JC, Estrada KL et al (2010) Accumulation and leaching potential of some pharmaceuticals and potential endocrine disruptors in soils irrigated with wastewater in the Tula Valley, Mexico. Chemosphere 81(11):1437–1445

Gómez-Oliván LM, Galar-Martínez M, Téllez AM, Carmona FA, Amaya A (2009) Estudio de automedicación en una farmacia comunitaria de la ciudad de Toluca. Rev Mex Cienc Farm 40(1):5–11

González-González ED, Gómez-Oliván LM, Galar-Martínez M et al (2014) Metals and nonsteroidal anti-inflammatory pharmaceuticals drugs present in water from Madín Reservoir (Mexico) induce oxidative stress in gill, blood, and muscle of common carp (*Cyprinus carpio*). Arch Environ Contam Toxicol 67(2):281–295

Gonzalez-Rey M, Tapie N, Le Menach K et al (2015) Occurrence of pharmaceutical compounds and pesticides in aquatic systems. Mar Pollut Bull 96(1):384–400

Guerra P, Kim M, Shah A et al (2014) Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes. Sci Total Environ 473:235–243

Hoke RA, Ankley GT (2005) Application of frog embryo teratogenesis assay-Xenopus to ecological risk assessment. Environ Toxicol Chem 24:2677–2690

Isidori M, Lavorgna M, Nardelli A, Parrella A, Previtera L, Rubino M (2005) Ecotoxicity of naproxen and its phototransformation products. Sci Total Environ 348:93–101

Kwak K et al (2018) Chemosphere Chronic toxicity and endocrine disruption of naproxen in freshwater water fleas and fish, and steroidogenic alteration using H295R cell assay. Chemosphere 204:156–162

Li Z, Sobek A, Radke M (2015) Flume experiments to investigate the environmental fate of pharmaceuticals and their transformation products in streams. Environ Sci Technol 49:6009–6017

Li Q et al (2016) Acute toxicity and histopathological effects of naproxen in zebrafish (*Danio rerio*) early life stages. Environ Sci Pollut Res 23:18832–18841

Lolic A, Paíga P, Santos LH et al (2015) Assessment of non-steroidal anti-inflammatory and analgesic pharmaceuticals in seawaters of North of Portugal: occurrence and environmental risk. Sci Total Environ 508:240–250

Martín J, Camacho-Muñoz D, Santos JL et al (2012) Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: removal and ecotoxicological impact of wastewater discharges and sludge disposal. J Hazard Mater 239:40–47

Nannou CI, Kosma CI, Albanis TA (2015) Occurrence of pharmaceuticals in surface waters: analytical method development and environmental risk assessment. Int J Environ Anal Chem 95(13):1242–1262

Neri-Cruz N, Gómez-Oliván LM, Galar-Martínez M et al (2015) Oxidative stress in *Cyprinus carpio* induced by hospital wastewater in Mexico. Ecotoxicology 24(1):181–193

Nieuwkoop PD, Faber J (1956) Normal table of *Xenopus laevis* (Daudin). A systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis, vol 22. North-Holland Publishing Company, Amsterdam

Öhd JF, Wikström K, Sjölander A (2000) Leukotrienes induce cell-survival signaling in intestinal epithelial cells. Gastroenterology 119:1007–1018

Patiño Menéndez Y, Díaz Fernández E, Ordóñez GS (2014) Microcontaminantes emergentes en aguas: tipos y sistemas de tratamiento. Avances en ciencias e ingeniería 5:2

Prati M, Biganzoli E, Boracchi P, Tesauro M, Monetti C, Bernardini G (2000) Ecotoxicological soil evaluation by FETAX. Chemosphere 41:1621–1628

Robert J, Ohta Y (2009) Comparative and developmental study of the immune system in Xenopus. Dev Dyn 238:1249–1270

Rodríguez-Anaya A, Octavio-Aguilar P, Gaytán-Oyarzún JC (2015) Consumo de fármacos antiinflamatorios no esteroideos en Pachuca, Hidalgo. Salud Publica Mex 57(5):365–366

Santos LH, Gros M, Rodriguez-Mozaz S et al (2013) Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: identification of ecologically relevant pharmaceuticals. Sci Total Environ 461:302–316

Siemens J, Huschek G, Siebe C et al (2008) Concentrations and mobility of human pharmaceuticals in the world's largest wastewater irrigation system, Mexico City-Mezquital Valley. Water Res 42(8):2124–2134

Sim WJ, Lee JW, Oh JE (2010) Occurrence and fate of pharmaceuticals in wastewater treatment plants and rivers in Korea. Environ Pollut 158:1938–1947

Sparling D, Bishop C, Linder G (2000) The current status of amphibian and reptile ecotoxicological research. Society of Environmental Toxicology and Chemistry, Pensacola

Stancova V et al (2015) Oxidative stress indices and histopathological effects of the nonsteroidal anti-inflammatory drug naproxen in adult zebrafish (*Danio rerio*). Neuro Endocrinol Lett Sweden 36 Suppl 1:73–78

Stancová V et al (2015) Effects of the non-steroidal anti-inflammatory drug (NSAID) naproxen on gene expression of antioxidant enzymes in zebrafish (Danio rerio). Environ Toxicol Pharmacol Elsevier BV 40(2):343–348

Virkutyte J, Varma RS, Jegatheesan V (2010) Treatment of micropollutants in water and wastewater. IWA Publishing, London

Wells PG, Mccallum GP, Chen CS, Henderson JT, Lee CJ, Perstin J, Preston TJ, Wiley MJ, Wong AW (2009) Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. Toxicol Sci 108:4–18

Yu Y, Wu L, Chang AC (2013) Seasonal variation of endocrine disrupting compounds, pharmaceuticals and personal care products in wastewater treatment plants. Sci Total Environ 442:310–316

Chapter 5
Differential Responses of Biochemical and Behavioral Parameters in the Native Gastropod *Chilina gibbosa* Exposed Subchronically to Environmental Concentrations of Two Insecticides Used in Argentina

Beverly Boburg, Lucila Thomsett Herbert, Paula Cossi, and Gisela Kristoff

Abbreviations

AcSCh	Acetylthiocholine iodide
AZM	Azinphos-methyl
CAR	Carbaryl
CE	Carboxylesterase
ChE	Cholinesterase
DTNB	5,5-dithio-2-bis-nitrobenzoate
OP	Organophosphate insecticide
p-NPA	p-nitrophenyl acetate
p-NPB	p-nitrophenyl butyrate
SC	Solvent control

B. Boburg
Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Ecotoxicología Acuática: Invertebrados Nativos77, Buenos Aires, Argentina

L. T. Herbert · P. Cossi · G. Kristoff (✉)
Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Laboratorio de Ecotoxicología Acuática: Invertebrados Nativos, Buenos Aires, Argentina

CONICET-Universidad de Buenos Aires, Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN), Buenos Aires, Argentina

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_5

5.1 Introduction

Chilina gibbosa (Sowerby 1841) is a freshwater gastropod from the Chilinidae (pulmonate) family which is endemic to South America, especially abundant in southern Chile and Argentina. In Argentina, it is found in lakes and rivers of Río Negro and Neuquén provinces, North Patagonia (Bosnia et al. 1990; Rumi et al. 2008; Gutiérrez Gregoric 2010; Valdovinos Zarges 2006). It has an important role in the ecosystem as it is a food source for birds and fishes, some of which have commercial value such as the native silverside *Odontesthes hatcheri* and the rainbow trout *Oncorhynchus mykiss* (Bosnia et al. 1990). *C. gibbosa* has several points of interest as model organism for ecotoxicology studies: (1) it is easy to collect and handle, as individuals can usually be found in shallow waters, in an aggregated dispersion pattern (Bosnia et al. 1990), and (2) their limited mobility and ability to excrete pollutants may result in several negative effects at low environmental concentrations of toxicants (Oehlmann and Schulte-Oehlmann 2003), which ensures its effective exposure to any pollutant present in the environment.

The use of native species in ecotoxicological studies has been suggested by US EPA (1976). They have been recommended by several authors as advantageous because the organisms are already acclimated to environmental conditions and are possibly more susceptible to contaminants than invasive species, and results could be considered more ecologically relevant (Baird et al. 2007; Buikema et al. 1982; Krull et al. 2012; Gagneten et al. 2012).

In the Upper Valley of Río Negro and Neuquén (North Patagonia, Argentina), agriculture represents the second most important economic activity (Loewy et al. 2011). Irrigation and pest control are common practices required for agricultural development in this region. Pesticides are applied by ground-based spraying equipment, and a substantial fraction of them reaches surface drainage water and soil (Loewy et al. 1999, 2011). The organophosphate (OP) azinphos-methyl (AZM) and the carbamate carbaryl (CAR) have been two of the main insecticides used in this region. The recommended concentrations in water of AZM and CAR for aquatic life protection in Argentina are ≤ 0.02 μg L^{-1} and 0.05 μg L^{-1}, respectively. Nevertheless, Loewy et al. (1999, 2011) detected a maximum concentration of 79.30 μg L^{-1} of AZM and 45.7 μg L^{-1} of CAR in surface and subsurface waters of the Upper Valley of Río Negro and Neuquén region. AZM and CAR share the same mechanism of action: they are inhibitors of cholinesterase activity (ChE), which can cause neurotoxicity and eventually death. They can also cause other effects such as the inhibition of carboxylesterase activity (CE) (Sanchez-Hernandez 2007; Timbrell 2000).

Inhibition of ChE and CE by AZM and CAR has been previously reported in other aquatic gastropods (Cacciatore et al. 2013; Kristoff et al. 2006, 2010). In *C. gibbosa*, our group has studied different toxic effects of the acute (48 h) exposure to AZM. We reported that environmental concentrations of AZM caused neurotoxicity, inhibition of ChE in whole organism soft tissue, and immunotoxicity, causing a decrease in hemocyte viability and phagocytic activity (Bianco et al. 2013; Cossi et al. 2015; Herbert et al. 2018). However, subchronic exposure had not been studied

yet. Subchronic and chronic assays provide valuable information because they represent a more realistic picture of the impact of contaminants in the environment (Cossi et al. 2018).

The aim of this study was to assess effects on different parameters (lethality, neurotoxicity, and ChE and CE activity) in *C. gibbosa* after 7 and 14 days of exposure to environmental concentrations of AZM and CAR. Our hypothesis were that (1) a subchronic exposure to AZM produces more severe effects than an acute one and that (2) the exposure to CAR causes similar effects on *C. gibbosa* than the ones caused by AZM.

5.2 Methodology

5.2.1 Chemicals

Acetylthiocholine iodide (AcSCh), p-nitrophenyl acetate (p-NPA), p-nitrophenyl butyrate (p-NPB), 5,5-dithio-2-bis-nitrobenzoate (DTNB), azinphos-methyl (AZM) PESTANAL®, and carbaryl (CAR) PESTANAL® were purchased from Sigma-Aldrich. All other chemicals used were also of analytical reagent grade.

5.2.2 Organisms

C. gibbosa snails were collected by hand from the vegetated bank of the river Chimehuin (39°54′57.15″S 71°06′23″W; province of Neuquén, Argentina) at a depth of 5–70 cm. The river Chimehuin originates 20 km upstream of the collection site, from the glacial lake Huechulafquen, located within the Lanín National Park, Neuquén. The collection site can be considered free from agrochemical pollution because agricultural exploitation is banned upstream from it. The snails were then transported to the Laboratorio de Ecotoxicología Acuática: Invertebrados Nativos (EAIN), Ciudad Autónoma de Buenos Aires (CABA), Buenos Aires, Argentina, where bioassays were carried out after at least 20 days of acclimatization in aerated glass aquaria (10 L) at 12 ± 2 °C, under a 12:12 h (L:D) artificial photoperiod regime and with ad libitum goldfish flakes (TetraFin) as food. Adult snails of similar size, 1.6 ± 0.4 mm of shell length, and weight, 0.27 ± 0.08 g, were selected for all the bioassays.

5.2.3 Bioassays

All bioassays were carried out at 12 ± 2 °C under a photoperiod of 12:12 h (L:D). Tap water dechlorinated passively during 72 h was used for the bioassays. Insecticide stock solutions were prepared by dissolving the insecticides in acetone due to their

low solubility in water. The concentrations of AZM and CAR used for the bioassays were obtained by diluting the stock solutions with dechlorinated tap water. Solutions were renewed every 96 h for AZM and daily for CAR according to previous stability studies (Cacciatore et al. 2013, 2018). The molar concentration of AZM and CAR used in the bioassays was 0.063 nM, which corresponds to 20 μg L^{-1} AZM and 13 μg L^{-1}CAR. Both of these concentrations are environmental concentrations found in freshwater of the region of the Upper Valley of Río Negro and Neuquén, Argentina (Loewy et al. 1999, 2011).

Two 14-day subchronic bioassays were carried out, one for each insecticide, by exposing 7 snails per glass vessel containing either 0.05% acetone in dechlorinated tap water as solvent control (SC) or the corresponding concentration of insecticide in dechlorinated tap water (20 μg L^{-1} for AZM and 13 μg L^{-1} for CAR). Six aerated vessels were used for each treatment. Snails were fed TetraFin goldfish flakes every 96 h after solution renewal. At 7 and 14 days, mortality and neurotoxicity signs (adherence and conspicuous protrusion of the head-foot region) were recorded, and homogenates of one snail per vessel were then carried out for measurement of enzymatic activities and protein content. Whole tissue homogenates were carried according to Cossi et al. (2015) after anesthetizing snails on ice during 6–8 minutes, wiping them clean and dry and gently removing their shells.

5.2.4 Enzymatic Activity Assays

Protein content was determined in order to express enzyme activity results as μmol of substrate hydrolyzed per min per mg of protein by following the method of Lowry et al. (1951), using bovine serum albumin as standard. Protein content was expressed as $mg_{protein}$ $ml_{homogenate}^{-1}$.

ChE activity was measured following the method of Ellman et al. (1961), previously adapted for this species (Bianco et al. 2013), using 100 mM phosphate buffer pH 8, 0.2 mM DTNB, 1.5 mM AcSCh as substrate and 200 μL of the supernatant fraction. Specific activity was calculated using 13.6 mM^{-1} cm^{-1} as the molar extinction coefficient.

CE activity was determined by measuring the hydrolysis of p-NPA and p-NPB following the method of Kristoff et al. (2010), adapted for this species by Bianco et al. (2013), using 2.5 mL 100 mM phosphate buffer pH 8.0 containing 5% acetone, 1.5 mM p-NPA or p-NPB, and 150 μL of the supernatant fraction. Specific activity was calculated using 18.6 mM^{-1} cm^{-1}as the molar extinction coefficient for p-nitrophenol.

5.2.5 Data Analysis

Differences in neurotoxic responses in SC and insecticide-exposed snails for each day were tested using Fisher's exact test. Differences in enzyme activity in SC and insecticide-exposed snails between days were tested using two-way

ANOVA. Assumptions of normality and homogeneity of variances were tested by Shapiro-Wilk's normality test and Levene's test, respectively. A log transformation was applied for ChE activity of AZM-exposed snails and CE (p-NPA and p-NPB) activity of CAR-exposed snails in order to meet assumptions. Tukey tests were used to perform post hoc comparisons, and Fisher's Least Significant Difference (LSD) test was applied when interaction between factors resulted significant. The level of significance used was set at 0.05 for all analyses. Statistical analyses were performed using GraphPad Prism and Statistica 7 software.

5.3 Results

Mortality in both bioassays was not significant for SC snails nor for insecticide-exposed snails. Only one snail died after 14 days of AZM exposure.

Exposure to AZM for 7 days reduced the ability of the snails to adhere to the walls of the vessels to 10% adherence. After 14 days, there was no adherence at all (Table 5.1; Fisher's exact test, $P < 0.0001$). AZM also had a strong neurotoxic effect, causing the protrusion of the head-foot region in 100% and 97% of the snails after 7 and 14 days of exposure, respectively (Table 5.1; Fisher's exact test, $P < 0.0001$). In the case of CAR, subchronic exposure had no effect on adherence, and head-foot protrusion was not observed (Table 5.1; Fisher's exact test, $P < 0.0001$).

In the case of AZM, protein content was negatively affected by AZM exposure both after 7 and 14 days, decreasing 16% and 9%, respectively, compared to SC snails (two-way ANOVA; main effect insecticide: $F_{1,20} = 10.3888$, $P = 0.0043$). Protein content increased between 7 and 14 days in both groups (two-way ANOVA; main effect time: $F_{1,20} = 6.2744$, $P = 0.0210$). In the case of CAR, protein content did not vary between SC and exposed snails (two-way ANOVA; main effect insecticide: $F_{1,20} = 0.0099$, $P = 0.6578$) nor between 7 and 14 days of exposure (two-way ANOVA; main effect time: $F_{1,20} = 0.6317$, $P = 0.4361$).

Exposure to AZM caused 87% inhibition of ChE activity after 7 days and 91% after 14 days with respect to SC snails, and overall ChE activity decreased after 14 days with respect to the activity after 7 days (Fig. 5.1; two-way ANOVA; main effect insecticide, $F_{1,20} = 0.6317$, $P = 0.0001$; main effect time, $F_{1,20} = 5.1295$, $P = 0.0348$).

Table 5.1 Summary of lethality and neurotoxic responses of *Chilina gibbosa* after 7 and 14 days of subchronic exposure to either 20 μg L^{-1} azinphos-methyl (AZM) or 13 μg L^{-1} carbaryl (CAR)

	Mortality		Adherence		Head-foot protrusion	
	7 days	14 days	7 days	14 days	7 days	14 days
SC	0%	0%	100%	100%	0%	0%
AZM 20 μg L^{-1}	0%	3%	10%****	0%****	100%****	97%****
SC	0%	0%	100%	100%	0%	0%
CAR 13 μg L^{-1}	0%	0%	100%	100%	0%	0%

CAR concentration was chosen to be the molar equivalent of 20 μg L^{-1}AZM
SC = solvent control (0.05% acetone)
****$P \leq 0.0001$ with respect to SC for each day; Fisher's exact test

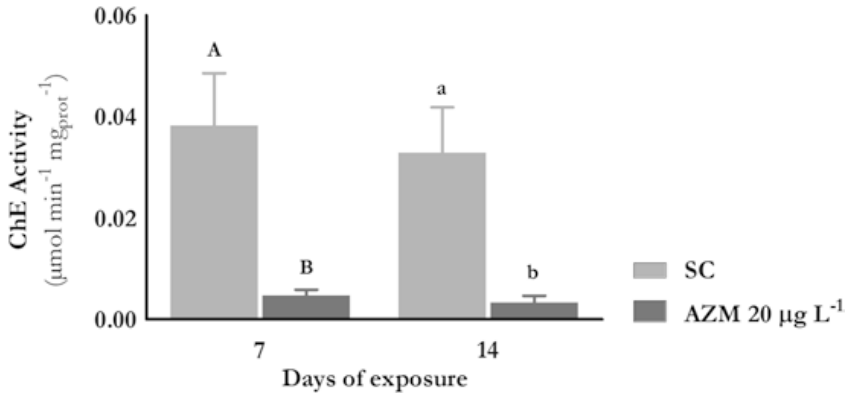

Fig. 5.1 *Chilina gibbosa* cholinesterase (ChE) activity after 7 and 14 days of a subchronic exposure to 20 µg L^{-1} azinphos-methyl (AZM). SC = solvent control (0.05% acetone). Data are expressed as mean ± SD. Different letters indicate significant differences between SC and AZM; different casing (upper or lower case) indicates differences between days (two-way ANOVA; $P < 0.05$)

CE activity, measured using p-NPA as substrate, was not affected by a 7-day exposure to AZM. Nevertheless, after 14 days, CE activity of snails exposed to AZM decreased 40% with respect to SC snails and 34% compared to snails exposed for 7 days (Fig. 5.2a; two-way ANOVA; interaction insecticide x time, $F_{1,20} = 6.1334$, $P = 0.0223$; Fisher's LSD test for the interaction insecticide x time, df = 20, 14 days SC vs. 14 days AZM $P = 0.0062$, 7 days AZM vs. 14 days AZM $P = 0.0259$). In addition, CE activity measured using p-NPB as substrate was affected by AZM exposure only after 14 days of exposure, causing 30% inhibition (Fig. 5.2b; two-way ANOVA; interaction insecticide x time, $F_{1,20} = 4.8823$, $P = 0.0390$; Fisher's LSD test for the interaction insecticide x time, df = 20, 14 days SC vs. 14 days AZM $P = 0.0142$).

Contrastingly, exposure to CAR did not have a significant effect on ChE activity, even though the activity of this enzyme tended to decrease in CAR-exposed snails (25 and 35% after 7 and 14 days of exposure) (Fig. 5.3; two-way ANOVA; main effect insecticide, $F_{1,20} = 4.2082$, $P = 0.0536$; main effect time, $F_{1,20} = 0.0581$, $P = 0.8119$).

Exposure to CAR did not inhibit CE activity measured using p-NPA. Activity increased between 7 and 14 days, regardless of snails having been exposed to CAR or not. (Fig. 5.4a; two-way ANOVA; main effect insecticide, $F_{1,20} = 1.4541$, $P = 0.2419$; main effect time, $F_{1,20} = 20.4203$, $P = 0.0002$). Overall CE activity measured using p-NPB also increased after 14 days (Fig. 5.4b; two-way ANOVA; main effect time: $F_{1,20} = 20.2461$, $P = 0.0002$). However, in this case, CAR caused 60% inhibition at 7 days and 14 days of exposure with respect to SC group. (Fig. 5.4b; two-way ANOVA; main effect insecticide: $F_{1,20} = 51.3028$, $P < 0.0001$).

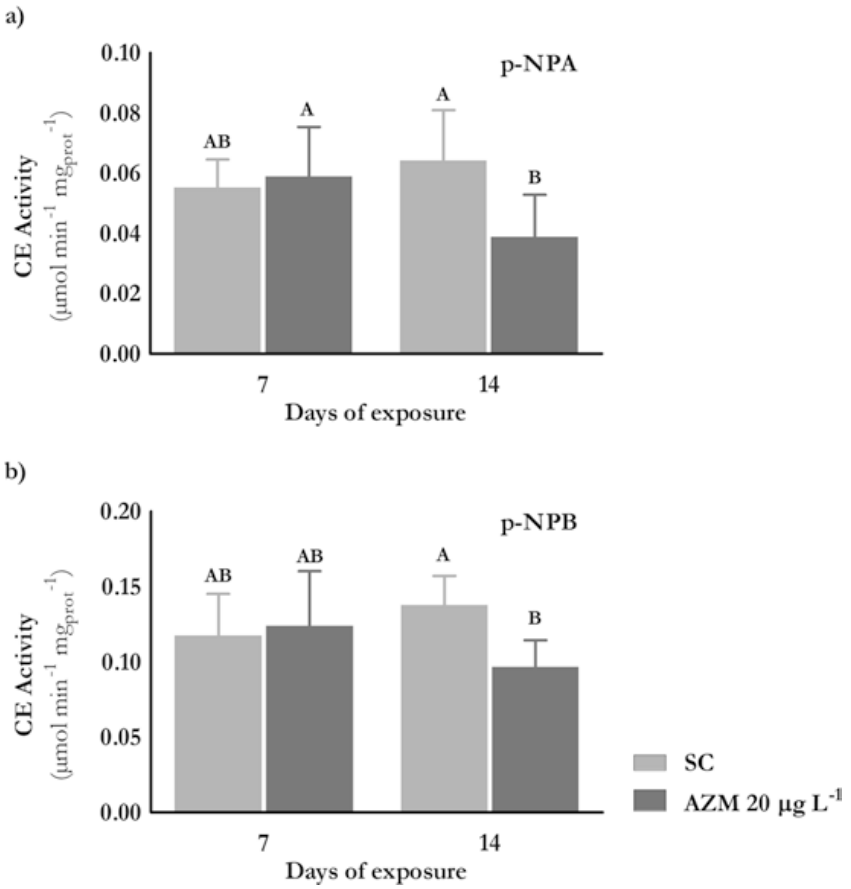

Fig. 5.2 *Chilina gibbosa* carboxylesterase (CE) activity after 7 and 14 days of a subchronic exposure to 20 µg L^{-1} azinphos-methyl (AZM). CE activity was determined using (**a**) p-nitrophenyl acetate (p-NPA) or (**b**) p-nitrophenyl butyrate (p-NPB) as substrates. SC = solvent control (0.05% acetone). Data are expressed as mean ± SD. Different letters indicate significant differences between SC and AZM (two-way ANOVA; $P < 0.05$)

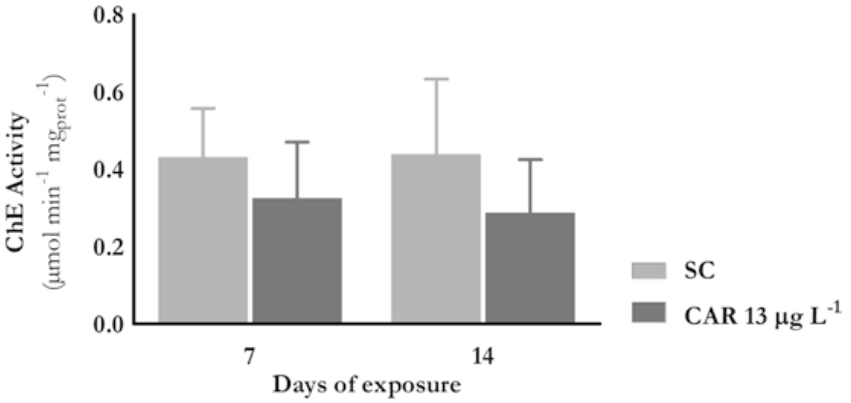

Fig. 5.3 *Chilina gibbosa* cholinesterase (ChE) activity after 7 and 14 days of a subchronic exposure to 13 µg L^{-1} carbaryl (CAR). CAR concentration was chosen to be the molar equivalent of 20 µg L^{-1}AZM. SC = solvent control (0.05% acetone). Data are expressed as mean ± SD

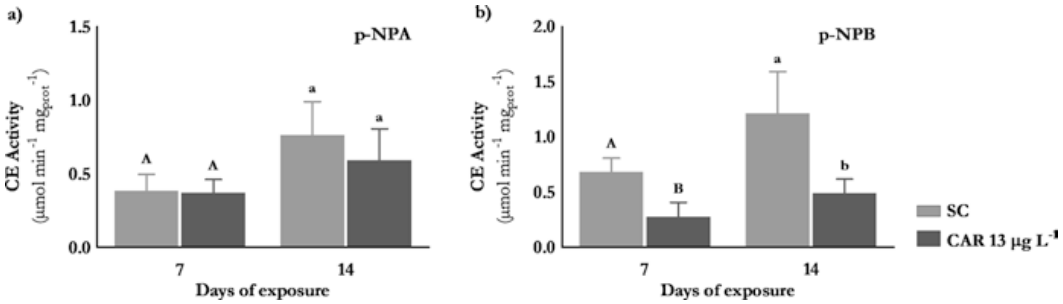

Fig. 5.4 *Chilina gibbosa* carboxylesterase (CE) activity after 7 and 14 days of a subchronic exposure to 13 µg L^{-1} carbaryl (CAR). CE activity was determined using (**a**) p-nitrophenyl acetate (p-NPA) or (**b**) p-nitrophenyl butyrate (p-NPB) as substrates. CAR concentration was chosen to be the molar equivalent of 20 µg L^{-1}AZM. SC = solvent control (0.05% acetone). Data are expressed as mean ± SD. Different letters indicate significant differences between SC and AZM; different casing (upper or lower case) indicates differences between days (two-way ANOVA; $P < 0.05$)

5.4 Discussion

Subchronic exposure to environmental concentrations of AZM and CAR caused sublethal toxic effects in the native gastropod *C. gibbosa*. However, differential responses on biochemical and behavioral biomarkers between insecticides were observed.

AZM caused severe signs of neurotoxicity and decreased protein content and ChE and CE activity. Our original hypothesis was that both insecticides could inhibit ChE because this enzyme is the main mechanism of action of these insecticides. However, CAR did not significantly decrease ChE activity. CAR only inhibited CE activity measured with p-NPB after 14 days of exposure. Other authors have also reported a higher toxicity of AZM than CAR (Kristoff et al. 2006; Kristoff 2010; Ferrari et al. 2004). However, in these cases, significant inhibition of ChE after the exposure to the carbamate was observed.

In *C. gibbosa* exposed to AZM, ChE activity was more sensitive than CE activity, while in organisms exposed to CAR, CE was more sensitive than ChE. CE has been frequently reported as more sensitive to OPs and carbamates than ChE in invertebrate species (Kristoff et al. 2010; Bianco et al. 2014; Wheelock et al. 2008; Agrelo et al. 2019). However, CE is not directly involved in the acute toxicity of OP and carbamates insecticides. CE can protect ChE by removing a significant amount of OPs and carbamates by two main mechanisms: by detoxification through the hydrolysis of ester bonds in some of these insecticides and by providing alternative binding sites (Sanchez-Hernandez 2007; Jokanović 2001).

Several authors have associated a greater sensitivity of CE with the absence of neurotoxic signs (Cossi et al. 2018; Anguiano et al. 2014; Otero and Kristoff 2016) and a greater sensitivity of ChE with neurotoxicity (Kristoff et al. 2006). Consistently, in our study, severe signs of neurotoxicity (lack of adherence and presence of abnormal head-foot protrusion) were observed only when organisms were exposed to AZM which produced a strong inhibition of ChE activity.

A 14-day exposure to AZM increased the toxicity previously reported after an acute (48 h) exposure in *C. gibbosa* (Bianco et al. 2013). At the same concentration of AZM (20 μg L^{-1}), CE activity and protein content were not decreased after an acute exposure, but both parameters were affected after a subchronic one. In concordance with our results, Bianco et al. (2014) observed a decrease in the protein content in the gastropod *Biomphalaria straminea* after 21 days of exposure to AZM, and Rivadeneira et al. (2013) observed inhibition of CE in the gastropod *Planorbarius corneus* exposed 14 days to the OP chlorpyrifos, which had not occurred due to acute exposure (48 h). Some responses to toxicants can appear only after several days of exposure to low concentrations of pollutants, showing the relevance of studying subchronic and chronic effects of contaminants in exposed organism.

The family Chilinidae is considered vulnerable due to its restricted geographic distribution, reduction of habitat availability, aquatic contamination, presence of invasive species, and climate change (Valdovinos Zarges 2006). *C. gibbosa* proved to be a sensitive species when it was exposed in laboratory conditions to insecticides used in Argentina. This could imply that natural populations of this species are threatened in contaminated regions of our country. Since *C. gibbosa* is an important item in aquatic food chains, these effects could represent a risk at higher trophic levels in the ecosystem.

5.5 Conclusions

Insecticides applied in Argentina can cause toxic effects in the native freshwater gastropod *C. gibbosa*, with AZM being more toxic than CAR. The presence of these insecticides in water bodies could put this species at risk, negatively disturbing the environment.

Acknowledgments This research was supported by grants of Universidad de Buenos Aires, PIP-CONICET and AGENCIA PICT. We would like to thank QB (Departamento de Química Biológica, FCEyN, UBA), DBBE (Departamento de Biodiversidad y Biología Experimental, FCEyN, UBA), IQUIBICEN (Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales), and CEAN (Centro de Ecología Aplicada del Neuquén). We are also grateful to L. Culasso Prinos for the support with statistical analysis.

References

Agrelo M, Rivadeneira PR, Cossi PF et al (2019) Azinphos-methyl causes in *Planorbarius corneus* toxic effects on reproduction, offspring survival and B-esterases depending on the exposure time. Comp Biochem Physiol C Toxicol Pharmacol 217:114–121

Anguiano OL, Castro C, Venturino A, Ferrari A (2014) Acute toxicity and biochemical effects of azinphos methyl in the amphipod *Hyalella curvispina*. Environ Toxicol 29:1043–1053

Baird DJ, Brown SS, Lagadic L et al (2007) In situ-based effects measures: determining the ecological relevance of measured responses. Integr Environ Assess Manag 3:259–267

Bianco K, Yusseppone MS, Otero S et al (2013) Cholinesterases and neurotoxicity as highly sensitive biomarkers for an organophosphate insecticide in a freshwater gastropod (*Chilina gibbosa*) with low sensitivity carboxylesterases. Aquat Toxicol 144–145:26–35

Bianco K, Otero S, Oliver AB et al (2014) Resistance in cholinesterase activity after an acute and subchronic exposure to azinphos-methyl in the freshwater gastropod *Biomphalaria straminea*. Ecotoxicol Environ Saf 109:85–92

Bosnia AS, Kaisin FJ, Tablado A (1990) Population dynamics and production of the freshwater snail *Chilina gibbosa* Sowerby 1841 (Chilinidae, Pulmonata) in a North-Patagonian reservoir. Hydrobiologia 190:97–110

Buikema AL, Niederlehner BR, Cairns J (1982) Biological monitoring part IV-toxicity testing. Water Res 16:239–262

Cacciatore LC, Verrengia Guerrero NR, Cochón AC (2013) Cholinesterase and carboxylesterase inhibition in Planorbarius corneus exposed to binary mixtures of azinphos-methyl and chlorpyrifos. Aquat Toxicol 128–129:124–134

Cacciatore LC, Verrengia Guerrero NR, Cochón AC (2018) Toxicokinetic and toxicodynamic studies of carbaryl alone or in binary mixtures with azinphos methyl in the freshwater gastropod Planorbarius corneus. Aquat Toxicol 199:276–284

Cossi PF, Boburg B, Luquet CM, Kristoff G (2015) Recovery study of cholinesterases and neurotoxic signs in the non-target freshwater invertebrate *Chilina gibbosa* after an acute exposure to an environmental concentration of azinphos-methyl. Aquat Toxicol 167:248–256

Cossi PF, Herbert LT, Yusseppone MS et al (2018) Environmental concentrations of azinphos-methyl cause different toxic effects without affecting the main target (cholinesterases) in the freshwater gastropod *Biomphalaria straminea*. Ecotoxicol Environ Saf 162:287–295

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Ferrari A, Anguiano OL, Soleño J et al (2004) Different susceptibility of two aquatic vertebrates (*Oncorhynchus mykiss* and *Bufo arenarum*) to azinphos methyl and carbaryl. Comp Biochem Physiol C Toxicol Pharmacol 139:239–243

Gagneten AM, Tumini G, Imhof A, Gervasio S (2012) Comparative study of lead accumulation in different organs of the freshwater crab *Zilchiopsis oronensis*. Water Air Soil Pollut 223:617–624

Gutiérrez Gregoric DE (2010) Redescription of two endemic species of Chilinidae (Gastropoda: Hygrophila) from Del Plata basin (South America). J Conchol 40:321–332

Herbert LT, Castro JM, Bianchi VA et al (2018) Effects of azinphos-methyl on enzymatic activity and cellular immune response in the hemolymph of the freshwater snail *Chilina gibbosa*. Pestic Biochem Physiol 150:71–77

Jokanović M (2001) Biotransformation of organophosphorus compounds. Toxicology 166:139–160

Kristoff G (2010) Estudio comparativo de biomarcadores en los invertebrados acuáticos *Biomphalaria glabrata y Lumbriculus variegatus* expuestos a pesticidas de relevancia ambiental. Doctoral Thesis, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires

Kristoff G, Verrengia Guerrero N, de D'Angelo AMP, Cochón AC (2006) Inhibition of cholinesterase activity by azinphos-methyl in two freshwater invertebrates: *Biomphalaria glabrata* and *Lumbriculus variegatus*. Toxicology 222:185–194

Kristoff G, Guerrero NRV, Cochón AC (2010) Inhibition of cholinesterases and carboxylesterases of two invertebrate species, Biomphalaria glabrata and Lumbriculus variegatus, by the carbamate pesticide carbaryl. Aquat Toxicol 96:115–123

Krull M, Barros F, Barros K (2012) Key issues in aquatic ecotoxicology in Brazil: a critical review. J Braz Soc Ecotoxicol 7027:57–66

Loewy M, Kirs V, Carvajal G et al (1999) Groundwater contamination by azinphos methyl in the Northern Patagonic Region (Argentina). Sci Total Environ 225:211–218

Loewy RM, Monza LB, Kirs VE, Savini MC (2011) Pesticide distribution in an agricultural environment in Argentina. J Environ Sci Health B 46:662–670

Lowry OH, Rosebrough NJ, Lewis Farr A, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Oehlmann J, Schulte-Oehlmann U (2003) Bioindicators & Biomonitors - principles, concepts and applications. Trace Met other Contam Environ 6:577–635

Otero S, Kristoff G (2016) *In vitro* and *in vivo* studies of cholinesterases and carboxylesterases in *Planorbarius corneus* exposed to a phosphorodithioate insecticide: finding the most sensitive combination of enzymes, substrates, tissues and recovery capacity. Aquat Toxicol 180:186–195

Rivadeneira P, Agrelo M, Otero S, Kristoff G (2013) Different effects of subchronic exposure to low concentrations of the organophosphate insecticide chlorpyrifos in a freshwater gastropod. Ecotoxicol Environ Saf 90:82–88

Rumi A, Gutiérrez Gregoric DE, Núñez V, Darrigran GA (2008) Malacología Latinoamericana. Moluscos de agua dulce de Argentina. Rev Biol Trop 56:77–111

Sanchez-Hernandez JC (2007). Ecotoxicological perspectives of b-esterases in the assessment. In: Plattenberg R (ed). Environmental pollution: New research (pp 1–45). Nova Science Publishers, Inc, Nueva York, NY

Timbrell J (2000) Principles of biochemical toxicology. Taylor and Francis, London

US EPA (1976) Quality criteria for water, Washington, DC. (EPA-440/9-76-023)

Valdovinos Zarges C (2006) Estado de conocimiento de los Gastrópodos dulceacuícolas de Chile. Gayana 70:88–95

Wheelock CE, Phillips BM, Anderson BS et al (2008) Applications of carboxylesterase activity in environmental monitoring and toxicity identification evaluations (TIEs). In: Whitacre DM (ed) Reviews of environmental contamination and toxicology (pp 117–178). Springer, New York, NY

Chapter 6
Oxidative Stress Induced by Water from a Hospital Effluent of the City of Toluca, Mexico, on *Hyalella azteca*

Leobardo Manuel Gómez-Oliván, Hariz Islas-Flores, Nely San Juan-Reyes, Marcela Galar-Martínez, Sandra García-Medina, and Armando Elizalde-Velázquez

6.1 Introduction

In 2012, in the State of Mexico, the average daily availability of water for each of the more than 14 million inhabitants of the state was less than 270 liters/person (National Water Commission). Anthropogenic activities have led to an increase in their consumption and consequently serious pollution problems in ecosystems (Ansari and Matondkar 2014). In addition, more dangerous pollutants have been identified in aquatic ecosystems due to their persistence, since, unlike other xenobiotics, they can be biotransformed, but they can accumulate in the different compartments of the water column, favoring in some cases their availability and their entrance to the different hydrobionts (Islas-Flores et al. 2013; Islas-Flores et al. 2014; Saucedo-Vence et al. 2015; González-González et al. 2014; Sanjuan-Reyes et al. 2013; Gómez-Oliván et al. 2014a; Gómez-Oliván et al. 2014b).

Part of the daily demand for water is concentrated in public places. In this sense, it has been observed that hospitals consume a significant daily volume of water, which ranges from 400 to 1200 liters/bed (Zhou et al., 2014).

The consumption of water in hospitals in turn generates significant volumes of wastewater loaded with toxic chemical compounds, organic matter, drug residues, heavy metals, organohalogen compounds, antiseptics, detergents, solvents, medi-

L. M. Gómez-Oliván (✉) · H. Islas-Flores · N. San Juan-Reyes · A. Elizalde-Velázquez
Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico
e-mail: lmgomezo@uaemex.mx

M. Galar-Martínez · S. García-Medina
Laboratorio de Toxicología Acuática, Departamento de Farmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, Mexico City, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_6

cines, excreta, and human secretions, in addition to pathogenic microorganisms (enterobacteria, fecal coliforms, among others) (Neri-Cruz et al. 2015; Pérez-Alvarez et al. 2018; Luja-Mondragón et al. 2019).

The main problem with hospital effluents is that they are discharged, without previous treatment to the municipal network, along with domestic wastewater, and this can generate risks for the organisms that are present in the bodies of water, as well as alterations to their ecological balance (Gupta et al. 2009).

These hospital effluents are a complex mixture, whose toxic effects will depend on the synergistic and antagonistic interactions between their components, for which, they are capable of generating serious environmental problems and can be 50–150 times more toxic than domestic effluents, constituting a potential risk for the human being and the environment, which generates an impact on public health (Emmanuel et al. 2005; Verlicchi et al. 2010; Oliveira et al. 2017; Verlicchi and Zambello 2016). The magnitude of the impact that pollutants have on hospital effluents has begun to be evaluated in recent years in scientific and governmental areas (Emmanuel et al. 2005; Verlicchi and Zambello 2016; Magdaleno et al. 2014).

Currently, the toxic effects of various chemical substances such as drugs, disinfectants, dyes, and solvents on aquatic organisms are known, of which several are present in the residual hospital effluents, constituting a potential risk for the natural biological balance of aquatic ecosystems (Martínez-Viveros et al. 2018; Martínez-Rodríguez et al. 2018; Cortes-Diaz et al. 2017; Cardoso-Vera et al. 2017; Gómez-Oliván et al. 2017; Elizalde-Velázquez et al. 2017).

The wastewater from hospitals can contain different chemical compounds that indirectly affect aquatic ecosystems, as described by different authors. Therefore, the complex mixture of compounds can have toxic effects on the environment, although these compounds are in small amounts (Gupta et al. 2009; Santos et al. 2013; Jolibois and Guerbet 2005).

In agreement with these studies, we can assume that the toxicity of a very complex mixture is governed not only for the specific mode of action of a single component. At the concentrations at which acute toxicity usually occurs, the toxicity of a single pharmaceutical will be predominantly due to the specific mode of toxic action. However, in mixtures the concentration of each single component decreases, while the number of components with various different specific modes of toxic action increases. The toxicity of chemical mixtures is relatively well understood through the concepts of concentration addition and independent action, with synergism being acknowledged as only a rare occurrence. In accordance with international guides, prospective risk assessment studies must take account the contribution of the "cocktail effects" of chemical mixtures at very low dose of the single substance in order to understand and to define effective measures or improve ecological status (Gómez-Oliván et al. 2014b).

Several studies have shown the ability to generate various toxic effects such as cytotoxicity, genotoxicity, embryotoxicity, and teratogenicity, using species such as *Cyprinus carpio*, *Xenopus laevis*, and *Lithobates catesbeianus*, using various biomarkers (Neri-Cruz et al. 2015; Olvera-Néstor et al. 2016; Pérez-Alvarez et al. 2018; Luja-Mondragón et al. 2019).

As previously mentioned, hospital effluents can generate different biological responses in different bioindicator organisms such as crustaceans, amphipods, frogs, and fish. One of the toxic responses that these effluents can generate is oxidative stress.

The potential of oxygen free radicals and other reactive oxygen species (ROS) to damage tissues and cellular components is called oxidative stress; in biological systems, their determination can be used to measure the toxic effects produced by environmental conditions, including exposure to pollutants, which especially induce a variety of toxic mechanisms such as oxidative damage to membrane lipids and changes in antioxidant proteins (Perez-Coyotl et al. 2017; Novoa-Luna et al. 2016; Saucedo-Vence et al. 2017; Apak et al. 2016; Schieber and Chandel 2014; Gómez-Oliván et al. 2012).

The aim of this work was to determine the oxidative stress induced by a hospital effluent on different organs and the blood of *Cyprinus carpio*.

6.2 Methods

Unless otherwise stated, reagents in this section were obtained from Sigma-Aldrich (St. Louis MO).

6.2.1 Sampling of Hospital Effluent

Twenty liters of untreated wastewater sample from a hospital in the State of Mexico (Fig. 6.1) were taken (April, 2018), based on the Mexican Standard NMX-AA-003-1980 (Sampling of Wastewater) (Procuraduría Federal de Protección al Ambiente DO de la F 25 M 1980, 1980). The samples were collected in dark-colored polyethylene bottles, previously washed with non-ionic detergent and rinsed with deionized water before sampling and hermetically sealed. After the collection, the samples were stored at 40 °C.

6.2.2 Pharmaceutical Determination

In order to carry out the quantification in the hospital effluent, the pharmaceuticals present in their schedule of basic medications were taken into account, and those that represented the greatest consumption according to the hospital's activities were selected; of these, five main groups were selected: beta-blockers and antidiabetics (first and second cause of death, respectively, in the State of Mexico (Instituto de Información e Investigación Geográfica, Estadística y Catastral del Estado de México IGECEM 2019)), beta-lactams (best-selling pharmaceuticals in Mexico),

Fig. 6.1 Map of the hospital effluent studied

hormones, and NSAIDs (highly consumed pharmaceuticals and of which two are considered to be from the watch list of the European Union: 17-b estradiol and diclofenac (Decisión de Ejecución (UE) 2019)).

The methodology described by López-Serna and Petrović (2012) was carried out; the chromatographic separation was performed using an Acquity UPLC system and a reversed-phase BEH C18 column (2.1 mm × 100 mm, 1.7 mm particle size), both from Waters Corporation.

The compounds analyzed in positive ionization (PI) mode were eluted using a mobile phase consisting of acetonitrile (ACN) and 0.1% aqueous formic acid. The elution started at 10% ACN, was linearly increased to 7.5 min over 6.2 min, was further increased to 100% ACN over 0.35 min, and was kept isocratic for 0.5 min. Total running time for the analysis was 8 min. In the negative ionization (NI) mode, compounds were eluted with ACN: MeOH (1:1, v/v) and 10 mM ammonium acetate. The elution was linearly increased from 20% ACN to 80% ACN over 1.3 min, further increased to 90% ACN over 0.7 min, and then kept isocratic for 2 min. The total running time was 5 min. The flow rate in both cases (PI and NI) was 0.4 mL/min with injection volume 20 mL. Samples were maintained at 15 °C in the injection station and the column at 30 °C.

For most compounds, two selected reaction monitoring transitions between the precursor ion and the most abundant fragment ions were monitored. The limit of detection (LOD), estimated as the concentration of analyte that produces a signal-to-noise ratio (S/N) of 3, represents the mean of the LODs for each analyte. The limit of quantification (LOQ) was estimated as the concentration of analyte producing a signal-to-noise ratio (S/N) of 10.

6.2.3 *Metal Determination*

Quantification of eight metals was carried out (As, Cd, Cu, Cr, Hg, Ni, Pb, and Zn), which were selected because their concentration in water is regulated by Mexican laws (NOM-001-SEMARNAT 1996; NOM-002-SEMARNAT 1996). Metals were quantified using the method proposed by Eaton et al. (1995) (Baird et al. 2019). To 0.5 mL of effluent was added 2 mL concentrated nitric acid. The samples were placed in an autoclave at 120 °C and 15 lb. pressure for 1 h. They were then filtered, diluted with deionized water, and read on a Varian AA1475 atomic absorption spectrophotometer (Melbourne, Australia). Results were obtained by interpolation on a type curve with the standard atomic absorption solution for each metal (1 mg/L).

6.2.4 *Test Organisms (*Cyprinus carpio*)*

Test organisms were obtained from the Tiacaque carpicola center, State of Mexico, and they were transferred to the Environmental Toxicology Laboratory of the Faculty of Chemistry of the Autonomous University of the State of Mexico. For the acclimation and maintenance of the test, organisms were distributed in glass tanks of 160 L capacity in a synthetic medium (174 mg/L of $NaHCO_3$, 120 mg/L of $MgSO_4$, 8 mg/L of KCl, and 120 mg/L of $CaSO_4.H_2O$) of pH 7.5–8.5, at room temperature, with constant aeration and light/dark photoperiods 12:12 and fed every third day with Purina ™ for fish.

6.2.5 *Microbiological and Physicochemical Properties of Wastewater*

Microbial analysis consisted in total coliforms (TC) and fecal coliforms (FC) determination using the most probable number (MPN) method (Baird et al. 2019). In the presumptive test for coliforms, three 10 mL, three 1 mL, and three 0.1 mL volumes of the appropriate dilution of the water sample were inoculated in nine fermentation tubes with a Durham vial in MacConkey broth. The inoculated tubes were incubated for 48 h at 37 °C, and those presenting gas and acid were confirmed in Levine eosin methylene blue agar at 37 °C for TC and in MacConkey broth with a Durham vial at 44 °C for 24 h for FC. The physicochemical tests included the determination of pH in NMX-AA-008-SCFI-2011 (NMX-AA-008-SCFI 2000) total hardness, total chloride, and fluoride content (ppm., ion selective electrode) and residual free chlorine in agreement with the Standard Methods for Examination of Water and Wastewater (Baird et al. 2019).

6.2.6 Sublethal Toxicity for Oxidative Stress Biomarkers

For the sublethal study, it was used in LOAEL value, that is to say 0.54%, and to this proportion of the hospital effluent, there were five test systems with six fish each. The exposure time was 12, 24, 48, 72, and 96 h. A free hospital effluent test system was established for each exposure time. The sublethal study was done in triplicate. In total, 180 organisms were used for this study. At the end of each exposure time, the organisms were removed from the systems and placed in a tank containing 50 mg/L of clove oil, in order to anesthetize the organisms. The blood, gill, liver, and brain samples were obtained. The blood samples were taken in the caudal vein with a hypodermic syringe of 1 mL previously heparinized, and for each 100 μL blood, 400 μL of phosphate-buffered saline (PBS) pH 7.4 was added, and to obtain the supernatant, the remaining samples (gills, liver, brain) were dissected in an ice bath, then weighed and homogenized in 2 mL of PBS pH 7.4, and centrifuged at 12500 rpm at −4 °C for 15 min. All biomarkers of oxidative stress were performed with the supernatant.

6.2.6.1 Oxidative Stress Biomarkers

Determination of Hydroperoxide Content

The determination was made following the method of Jiang et al. (1992). A 100 μL of sample (previously deproteinized with 10% trichloroacetic acid) was taken, and 900 μL of the reaction mixture [0.25 mM FeSO4, 25 mM H2SO4] was added [0.1 mM xyleneol orange and 4 mM butylated hydroxytoluene in 90% (v/v) methanol]. The mixture was incubated for 60 min at room temperature, and the absorbance at 560 nm was determined against a blank that contained only the reaction mixture. The results were extrapolated in a standard curve and expressed in nM HPC (cumene hydroperoxide)/mg protein.

Determination of Lipoperoxidation Level

The determination of lipoperoxidation level was carried out following the method of Büege and Aust (1978). To 100 μL of the sample (without centrifugation) was added buffer solution Tris-HCl 150 mM pH 7.4 to complete 1 mL. The sample was incubated at 37 °C for 30 min, and then 2 mL of TCA-TBA [thiobarbituric acid at 0.375% in trichloroacetic acid at 15%] was added, after which a thermal shock (with the help of a water bath) in boiling water for 45 min. At the end of this time, it was centrifuged at 3000 rpm for 10 min, and the absorbance at 535 nm was determined. The results were expressed in mM of malondialdehyde (MDA)/mg of protein, using the molar extinction coefficient (CEM) of 1.56 × 105 M/cm.

Determination of Carbonylated Proteins

The determination was made following the methodology of Levine et al. (1994) modified by Parvez and Raisuddin (2005) and Burcham (2007). To 100 µL of the supernatant was added 150 µL of 10 mM DNPH in HCl 2 M. It was incubated at room temperature for 1 h in the dark. After incubation, 500 µL of 20% trichloroacetic acid was added and allowed to stand for 15 min at 4 °C. The precipitate was centrifuged at 11,000 rpm for 5 min. The button was washed several times with ethanol-ethyl acetate 1:1 and then dissolved in 1 mL of a 6 M solution of guanidine pH 2.3 and incubated 37 °C for 30 min. The absorbance was determined at 366 nm. The results were expressed in µM reactive carbonyls (C=O)/mg protein, using the CEM of 21,000 M/cm.

Determination of Superoxide Dismutase Activity

It was determined by developing the method of Misra and Fridovich (1972). A 40 µL of the homogenate was placed in a quartz cell, and 260 µL of carbonate buffer [sodium carbonate 50 mM and EDTA 0.1 mM] was added at pH 10.2. Subsequently, 200 µL of 30 mM adrenaline was added, and the absorbance was determined at 480 nm at 30 s and 5 min. Enzymatic activity was determined using the SOD CEM (21 M/m). The results were expressed as IU/mg protein.

Determination of Catalase Activity

It was carried out following the method of Radi et al. (1991). A 20 µL of the supernatant were placed in a quartz cell, and 1 mL of buffer solution [sucrose 0.3 M, EDTA 1 mM, HEPES 5 mM, and KH_2PO_4] was added [5 mM] and 0.2 mL of a 20 mM H_2O_2 solution. Subsequently, the absorbance readings were taken at 240 nm, at 0 and 60 s. The results were obtained substituting the absorbance value obtained for each of the times in the formula – concentration of CAT = [(A0-A60)/CEM], where the CEM of H_2O_2 is 0.043 mM/cm – and were expressed as µM H_2O_2/mg protein.

Determination of Glutathione Peroxidase

It was determined by the method of Stephensen et al. (2000). A 100 µL of the supernatant was placed in a quartz cell and 10 µL of glutathione reductase (2 U glutathione reductase), in addition to 290 µL of the reaction buffer [K_2HPO_4 50 mM, KH_2PO_4 were added. 50 mM pH 7.0, reduced glutathione 3.5 mM, sodium azide 1 mM, NADPH 0.12 mM)] and 100 µL of H_2O_2 0.8 mM. Subsequently absorbance was determined at 340 nm, at 0 and 60 s. The results were obtained using the following equation: GPx concentration = [(A0-A60)/CEM], where the NADPH CEM is 6.2 mM/cm. The results were expressed as mM NADPH/mg protein.

Determination of Protein Concentration

It was determined by the method of Bradford (1976). To 25 μL of the supernatant were added 75 μL of deionized water and 2.5 mL of the Bradford reagent (0.05 g of Coomassie blue, 25 mL of 96% ethanol, and 50 mL of H3PO4 in 500 mL of deionized water). The tubes were shaken and allowed to stand for 5 min, and then the absorbance at 595 nm was determined. The results were extrapolated in a standard curve made with albumin.

6.3 Results

6.3.1 Physicochemical Characterization of Hospital Effluent

Table 6.1 shows the results of the physicochemical parameters determined in the hospital effluent under study. These results were contrasted with the Mexican regulatory framework, especially with the Mexican official norms NOM-001-SEMARNAT 1996, and NOM-073-ECOL 1994. Some of the physicochemical parameters do not exceed the values of the mentioned norms as temperature, pH, chlorides, fluorides, hardness, total suspended solids, total phosphorus, total nitrogen, and BOD. Dissolved oxygen was 14.8 mg/L; conductivity, 116.9 μS/cm; ammonia, 0.94 mg/L; and NaClO, 1.7 mg/L; these parameters are not considered in either of these norms.

Table 6.1 Physicochemical characteristics of the hospital effluent

Physicochemical parameter	Hospital effluent evaluated
Temperature (°C)	16.1 ± 0.5
Oxygen dissolved (mg/L)	14.8 ± 04
Conductivity (μS/cm)	116.9 ± 0.5
pH	7.4 ± 0.2
Chlorides (mg/L)	215 ± 3.7
Fluorides (mg/L)	7.4 ± 0.8
Hardness (mg/L)	3.36 ± 0.2
Ammonia (mg/L)	0.94 ± 0.3
Total suspended solids (mg/L)	39 ± 1.3
Total phosphorus (mg/L)	9.1 ± 0.9
Total nitrogen (mg/L)	19 ± 0.5
Biochemical oxygen demand (mg/L)	39 ± 0.4
NaClO (mg/L)	1.7 ± 0.3

6.3.2 Pharmaceutical Concentrations in Hospital Effluent

The concentrations of β-lactam antibiotics, NSAIDs, hormones, antidiabetics, and β-blockers are shown in Table 6.2. As can be highlighted, Penicillin G, acetaminophen, metoprolol, naproxen, and glibenclamide were the drugs found in higher concentrations in hospital effluent. Estos fármacos estuvieron presentes en concentraciones de μg/L. These drugs were present in concentrations of μg/L.

6.3.3 Metal Determination in Hospital Effluent

Table 6.3 shows the main metals identified in the hospital effluent. As can be seen, the metals that were found in the highest concentration were Ni, Cr, Pb, Zn, and Cu in mg/L concentrations. The metals Pb and Hg exceed the permissible limits for the protection of aquatic life, proposed in the Mexican regulations.

Table 6.2 Pharmaceutical concentration present in hospital effluent

Pharmaceutical group	Drug	Concentration (μg/L)
β-lactam antibiotics	Penicillin G	4.12 ± 0.31
	Penicillin V	0.53 ± 0.08
Nonsteroidal anti-inflammatory drugs	Acetaminophen	2.71 ± 0.05
	Diclofenac	0.62 ± 0.09
	Ibuprofen	0.74 ± 0.05
	Naproxen	1.85 ± 0.32
Hormones	17-β-estradiol	0.09 ± 0.001
Antidiabetics	Glibenclamide	1.84 ± 0.03
	Metformin	1.24 ± 0.04
β-blockers	Atenolol	0.24 ± 0.002
	Metoprolol	2.15 ± 0.28

Table 6.3 Metals detected in the hospital effluent

Metals	Concentration (mg/L)
As	0.019 ± 0.001
Cd	0.064 ± 0.001
Cu	0.381 ± 0.001
Cr	0.647 ± 0.002
Hg	0.0419 ± 0.001
Ni	0.841 ± 0.001
Pb	0.483 ± 0.040
Zn	0.523 ± 0.037

Table 6.4 Oxidative stress biomarkers

Time	Fluid or organ			
	Gill	Brain	Liver	Blood
Hydroperoxide content (nM CHP/mg protein)				
12	0.153 (↑3.4)	0.048 (↑6.7)	0.098 (↑18.1)	0.183 (↑2.2)
24	0.238 (↑60.8)	0.101 (↑124.4)	0.0.85 (↑2.4)	0.210 (↑17.3)
48	0.327 (↑121)	0.115 (↑155.6)	0.133 (↑3.4)	0.278 (↑55.3)
72	0.378 (↑155.4)	0.126 (↑180)	0.145 (↑36.1)	0.302 (↑ 68.7)
96	0.421 (↑184.5)	0.145 (↑222.2)	0.151 (↑81.9)	0.426 ↑ (138)
Lipid peroxidation level (mM MDA/mg protein)				
12	31.3 (↑7.9)	12.3 (↑2.5)	15.9 (↑9.6)	8.43 (↑20.4)
24	39.7(↑36.9)	12.9 (↑7.5)	17.3 (↑19.3)	12.5 (↑78.6)
48	41.8 (↑44.1)	13.3 (↑10.8)	21.6 (↑48.9)	19.7 (↑181.4)
72	42.3 (↑45.8)	14.5 (↑20.9)	24.9 (↑71.7)	21.8 (↑190.7)
96	44.7 (↑54.1)	17.9 (↑49.2)	28.7 (↑97.8)	25.6 (↑241.3)
Protein carbonyl content (μM reactive carbonyls/mg protein)				
12	3.83×10^{-9} (↑2.3)	4.61×10^{-9} (↑2.4)	7.39×10^{-9} (↑5.6)	4.42×10^{-9} (↑10.5)
24	3.91×10^{-9} (↑4.2)	5.37×10^{-9} (↑19.3)	8.27×10^{-9} (↑18.4)	5.23×10^{-9} (↑30.8)
48	4.12×10^{-9} (↑7.9)	5.91×10^{-9} (↑31.3)	8.46×10^{-9} (↑20.9)	5.18×10^{-9} (↑29.5)
72	4.83×10^{-9} (↑9.9)	6.14×10^{-9} (↑36.5)	9.39×10^{-9} (↑34.1)	6.35×10^{-9} (↑58.7)
96	4.92×10^{-9} (↑31.2)	6.83×10^{-9} (↑51.7)	10.25×10^{-9} (↑46.4)	6.73×10^{-9} (↑68.3)
SOD activity (IU/mg protein)				
12	7.23 (↑20.5)	4.55 (↑13.8)	8.14 (↑35.7)	5.16 (↑14.6)
24	8.14 (↑35.7)	6.17 (↑54.3)	8.96 (↑49.3)	6.23 (↑38.4)
48	8.33 (↑38.8)	7.13 (↑78.2)	9.18 (↑53)	7.61 (↑69.1)
72	9.15 (↑52.5)	7.95 (↑98.8)	10.7 (↑78.3)	8.23 (↑82.9)
96	10.11 (↑68.5)	8.16 (↑104)	11.23 (↑87.1)	8.92 (↑98.2)
CAT activity (μM H_2O_2/mg protein)				
12	3721 (↑6.1)	2032 (↑35.4)	3974 (↑32.5)	623 (↑13.3)
24	4123 (↑17.8)	3127 (↑108.5)	4017 (↑33.9)	718 (↑30.5)
48	4839 (↑38.3)	3978 (↑165.2)	4978 (↑65.8)	893 (↑62.4)
72	5023 (↑43.5)	4169 (↑177.9)	5127 (↑70.9)	974 (↑77.1)
96	6078 (↑73.7)	5029 (↑235.3)	5689 (↑89.6)	998 (↑81.5)
GPx activity (mM NADPH/mg protein)				
12	0.023 (↑27.8)	0.008 (↑33.3)	0.012 (↑20)	0.007 (↑40)
24	0.032 (↑77.7)	0.012 (↑100)	0.035 (↑250)	0.013 (↑160)
48	0.045 (↑150)	0.021 (↑250)	0.051 (↑410)	0.021 (↑320)
72	0.047 (↑161.2)	0.046(↑666.7)	0.048 (↑380)	0.037 (↑640)
96	0.078 (↑333.4)	0.057 (↑850)	0.063 (↑530)	0.041 (↑720)

6.3.4 Oxidative Stress Evaluation

The results of the biomarkers of oxidative stress are shown in Table 6.4. As can be seen, all cell oxidation biomarkers had statistically significant increases compared to the control group. In the case of HPC, blood and gills had the highest values of

this biomarker. For LPX, the gills and liver had the highest values and for PCC were the liver and brain. In the case of biomarkers of antioxidation, the highest values were presented in the gill and liver for the activity of SOD, CAT, and GPx.

6.4 Discussion

Some current studies have shown the toxicity produced by hospital wastewater. This situation occurs because large volumes of wastewater are generated in hospitals that contain substances such as drugs, detergents, chemical agents, diagnostic agents, and pathogens, among others (Boillot et al. 2008; Orias and Perrodin 2013; Laffite et al. 2016). These waters are generally released to the municipal sewage system without previous treatment, or with treatments that are not very efficient to remove these compounds. As a result, the presence of these contaminants in water bodies is imminent, and this can generate deleterious effects on organisms that are present in aquatic ecosystems.

The effects and the ecotoxicological repercussions of the different types of chemical substances, as well as their concentration contained in the hospital effluents, are unknown, as well as the possible degradation of these compounds by abiotic or biotic conditions of the system. Turbidity, water shading, and water depth, as well as the seasonal changes in sunlight exposure, have a substantial impact on compound in surface waters. Also, these substances may be sorbed by sediments and hence be no longer amenable to photochemical degradation (Kümmerer 2008; K'oreje et al. 2016).

Due to the above, the physicochemical properties are determinant in the toxicity of an effluent. In the hospital under study, the properties were determined as can be seen in Table 6.1; these were contrasted with the official Mexican norms [the maximum permissible limits in the official Mexican norms for wastewater discharges into national waters and resources (NOM-001-SEMARNAT-1996) or municipal and urban sewage systems (NOM-002-SEMARNAT-1996)]. In the effluents of this hospital, most of the physicochemical parameters are within the limits established in the Mexican norms. However, it is important to note that some of the parameters measured are not referred to in these standards. For example, NaClO was presented at a concentration of 1.7 mg/L. This compound has been associated with toxic effects in aquatic organisms such as *Cyprinus carpio* generating genotoxicity in erythrocytes of this organisms (Buschini et al. 2004); likewise this compound has been related to hepatic alterations of common carp (Elia et al. 2006).

On the other hand, pollutants such as metals and drugs that have been associated with different toxic responses were quantified. However, it is important to mention that one of the answers that these compounds can generate is oxidative stress. For example, metals such as Hg and Zn induced oxidative stress in *Cyprinus carpio* (González-González et al. 2014). The metals found in the effluent under study have also been associated with genotoxicity, cytotoxicity, embryotoxicity, and teratogenicity in species such as *Cyprinus carpio, Xenopus laevis,* and

Lithobates catesbeianus (González-González et al. 2014; Pérez-Alvarez et al. 2018; Luja-Mondragón et al. 2019; Pérez-Coyotl et al. 2017).

On the other hand, emerging contaminants drug-type identified in the hospital effluent studied have been associated with different toxic effects. For example, NSAIDs acetaminophen, diclofenac, ibuprofen, and naproxen have shown that in concentrations of ng/L to mg/L they can generate oxidative stress in species such as *Daphnia magna, Hyalella azteca*, and *Cyprinus carpio* (Islas-Flores et al. 2013; Islas-Flores et al. 2014; Gómez-Oliván et al. 2014b; Oviedo-Gómez et al. 2010; Nava-Álvarez et al. 2014; Saucedo-Vence et al. 2015), as well as cytotoxicity and genotoxicity in *D. Magna* and *Cyprinus carpio* (Gómez-Oliván et al. 2014a; Islas-Flores et al. 2017) and embryotoxicity in amphibian species such as *Xenopus laevis* and *Lithobates catesbeianus* (Cardoso-Vera et al. 2017). On the other hand, several studies report that 17-β-estradiol is capable of inducing oxidative stress, genotoxicity, and cytotoxicity in *Cyprinus carpio* (Gutiérrez-gómez et al. 2016; Orozco-Hernández et al. 2018). Also, glibenclamide which is an antidiabetic and metoprolol a beta-blocker are inducers of oxidative stress in species such as *Cyprinus carpio* and alterations in embryos of *Danio rerio* (Martínez-Viveros et al. 2018; Martínez-Rodríguez et al. 2018; Bittner et al. 2018; Sun et al. 2014).

The dangerous thing, with hospital effluents, is that they not only have a contaminant but generally have mixtures of these, which can generate interactions of additivity, synergism, and potentiation, increasing the toxicity of the effluent. Likewise, the abiotic characteristics of the system may have an influence on toxicity, i.e., the light can produce photodegradation of the compounds generating degradation products that can be more toxic than the original compounds.

The increase in the biomarkers of cellular oxidation (HPC, LPX, PCC) in the carps exposed to the hospital effluent can be explained by the biotransformation that drugs can have in *C carpio*, through the system of mixed oxidase function. When drugs are passed through phase I reactions through cytochrome P_{450}, intermediates can be generated in cycle reactions that produce highly oxidizing substances such as H_2O_2 and O_2* (Islas-Flores et al. 2013; González-González et al. 2014), which are responsible for oxidizing membrane lipids, producing lipoperoxidation, in addition to increasing the content of hydroperoxides and promote the carbonylation of proteins (Cortes-Diaz et al. 2017; Luja-Mondragón et al. 2019; Pérez-Coyotl et al. 2017).

On the other hand, the results obtained in the antioxidant activity, which also show a statistically significant increase, can be explained because the production of hydrogen peroxide and the radical superoxide anion can favor the increase of antioxidant enzymes such as SOD and CAT (Gómez-Oliván et al. 2014b; Vlahogianni et al. 2007; Novoa-Luna et al. 2016; Wilhelm Filho 1996).

The oxidizing metabolites of some of the drugs found in the effluent, as well as the metals identified, may also be responsible for the increase in the biomarkers of cellular oxidation and antioxidation found in this study.

6.5 Conclusions

With the obtained results, we can conclude that the hospital effluent under study was able to induce oxidative stress in the organs, tissues, and fluids of *Cyprinus carpio*. This response may be due to the presence of contaminants such as metals and drugs, as well as mixtures. Also, the physicochemical characteristics of the effluent under study can be associated with the toxic response identified in these fish.

References

Ansari ZA, Matondkar SGP (2014) Anthropogenic activities including pollution and contamination of coastal marine environment. J Ecophysiol Occup Health 14

Apak R, Özyürek M, Güçlü K, Çapanoğlu E (2016) Antioxidant activity/capacity measurement. 3. Reactive oxygen and nitrogen species (ROS/RNS) scavenging assays, oxidative stress biomarkers, and chromatographic/chemometric assays. J Agric Food Chem 64:1046–1070

Baird R, Eaton AD, Rice EW, Bridgewater L American Public Health Association, American Water Works Association, Water Environment Federation. *Standard Methods for the Examination of Water and Wastewater*. [cited 10 March 2019]. Available from https://www.casadellibro.com/libro-standard-methods-for-the-examination-of-water-and-wastewater/9780875532875/6219860

Bittner L, Teixido E, Seiwert B, Escher BI, Klüver N (2018) Influence of pH on the uptake and toxicity of β-blockers in embryos of zebrafish, Danio rerio. Aquat Toxicol 201:129–137

Boillot C, Bazin C, Tissot-Guerraz F, Droguet J, Perraud M, Cetre JC, Trepo D, Perrodin Y (2008) Daily physicochemical, microbiological and ecotoxicological fluctuations of a hospital effluent according to technical and care activities. Sci Total Environ 403:113–129

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Buege JA, Aust SD (1978) Microsomal Lipid Peroxidation. Methods Enzymol 52:302–310

Burcham PC (2007) Modified protein carbonyl assay detects oxidised membrane proteins: a new tool for assessing drug- and chemically-induced oxidative cell injury. J Pharmacol Toxicol Methods 56:18–22

Buschini A, Martino A, Gustavino B, Monfrinotti M, Poli P, Rossi C, Santoro M, Dörr AJM, Rizzoni M (2004) Comet assay and micronucleus test in circulating erythrocytes of Cyprinus carpio specimens exposed in situ to lake waters treated with disinfectants for potabilization. Mutat Res Toxicol Environ Mutagen 557:119–129

Cardoso-Vera JD, Islas-Flores H, SanJuan-Reyes N, Montero-Castro EI, Galar-Martínez M, García-Medina S, Elizalde-Velázquez A, Dublán-García O, Gómez-Oliván LM (2017) Comparative study of diclofenac-induced embryotoxicity and teratogenesis in Xenopus laevis and Lithobates catesbeianus, using the frog embryo teratogenesis assay: Xenopus (FETAX). Sci Total Environ 574:467–475

Cortes-Diaz MJA, Rodríguez-Flores J, Castañeda-Peñalvo G, Galar-Martínez M, Islas-Flores H, Dublán-García O, Gómez-Oliván LM (2017) Sublethal effects induced by captopril on Cyprinus carpio as determined by oxidative stress biomarkers. Sci Total Environ 605–606:811–823

Decisión de Ejecución (UE) 2015/495 de la Comisión, de 20 de marzo de 2015, por la que se establece una lista de seguimiento de sustancias para el seguimiento a nivel de la Unión en el ámbito de la política del agua de conformidad con la Directiva 2008/105/CE del Parlamento Europeo y del Consejo (notificada en el documento C (2015) 1756) Texto con relevancia EEE.

[cited 10 March 2019]. Available from https://publications.europa.eu/en/publication-detail/-/publication/a90868de-d1f9-11e4-9de8-01aa75ed71a1/language-en

Eaton DA, Clesceri LS, Greenberg AE (eds) (1995) Standard methods for the examination of water and wastewater, 19th edn. American Public Health Association, Washington, DC, pp 8–90

Elia AC, Anastasi V, Dörr AJM (2006) Hepatic antioxidant enzymes and total glutathione of Cyprinus carpio exposed to three disinfectants, chlorine dioxide, sodium hypochlorite and peracetic acid, for superficial water potabilization. Chemosphere 64:1633–1641

Elizalde-Velázquez A, Martínez-Rodríguez H, Galar-Martínez M, Dublán-García O, Islas-Flores H, Rodríguez-Flores J, Castañeda-Peñalvo G, Lizcano-Sanz I, Gómez-Oliván LM (2017) Effect of amoxicillin exposure on brain, gill, liver, and kidney of common carp (*Cyprinus carpio*): The role of amoxicilloic acid. Environ Toxicol 32:1102–1120

Emmanuel E, Perrodin Y, Keck G, Blanchard J-M, Vermande P (2005) Ecotoxicological risk assessment of hospital wastewater: a proposed framework for raw effluents discharging into urban sewer network. J Hazard Mater 117:1–11

Gómez-Oliván LM, Neri-Cruz N, Galar-Martínez M, Vieyra-Reyes P, García-Medina S, Razo-Estrada C, Dublán-García O, Corral-Avitia AY (2012) Assessing the oxidative stress induced by paracetamol spiked in artificial sediment on hyalella azteca. Water Air Soil Pollut 223:5097–5104

Gómez-Oliván LM, Galar-Martínez M, García-Medina S, Valdés-Alanís A, Islas-Flores H, Neri-Cruz N (2014a) Genotoxic response and oxidative stress induced by diclofenac, ibuprofen and naproxen in Daphnia magna. Drug Chem Toxicol 37:391–399

Gómez-Oliván LM, Neri-Cruz N, Galar-Martínez M, Islas-Flores H, García-Medina S (2014b) Binary mixtures of diclofenac with paracetamol, ibuprofen, naproxen, and acetylsalicylic acid and these pharmaceuticals in isolated form induce oxidative stress on Hyalella azteca. Environ Monit Assess 186:7259–7271

Gómez-Oliván LM, Mendoza-Zenil YP, SanJuan-Reyes N, Galar-Martínez M, Ramírez-Durán N, Rodríguez Martín-Doimeadios RDC, Rodríguez-Fariñas N, Islas-Flores H, Elizalde-Velázquez A, García-Medina S, Pérez-Pastén Borja R (2017) Geno- and cytotoxicity induced on Cyprinus carpio by aluminum, iron, mercury and mixture thereof. Ecotoxicol Environ Saf 135:98–105

González-González ED, Gómez-Oliván LM, Galar-Martínez M, Vieyra-Reyes P, Islas-Flores H, García-Medina S, Jiménez-Vargas JM, Razo-Estrada C, Pérez-Pastén R (2014) Metals and nonsteroidal anti-inflammatory pharmaceuticals drugs present in water from Madín reservoir (Mexico) induce oxidative stress in gill, blood, and muscle of common carp (Cyprinus carpio). Arch Environ Contam Toxicol 67:281–295

Gupta P, Mathur N, Bhatnagar P, Nagar P, Srivastava S (2009) Genotoxicity evaluation of hospital wastewaters. Ecotoxicol Environ Saf 72:1925–1932

Gutiérrez-Gómez AA, SanJuan-Reyes N, Galar-Martínez M, Dublán-García O, Islas-Flores H, Pérez-Alvárez I, Gómez-Oliván LM (2016) 17 β-Estradiol induced oxidative stress in gill, brain, liver, kidney and blood of common carp (Cyprinus carpio). Electron J Biol 12:53–63

Instituto de Información e Investigación Geográfica, Estadística y Catastral del Estado de México IGECEM (2019) Centro de Colaboracio_n Geoespacial: Gobierno del Estado de Me_xico, IGECEM, INEGI (CCG) [cited 10 March 2019]. Available from http://igecem.edomex.gob.mx/

Islas-Flores H, Gómez-Oliván LM, Galar-Martínez M, Colín-Cruz A, Neri-Cruz N, García-Medina S (2013) Diclofenac-induced oxidative stress in brain, liver, gill and blood of common carp (Cyprinus carpio). Ecotoxicol Environ Saf 92:32–38

Islas-Flores H, Gómez-Oliván LM, Galar-Martínez M, García-Medina S, Neri-Cruz N, Dublán-García O (2014) Effect of ibuprofen exposure on blood, gill, liver, and brain on common carp (Cyprinus carpio) using oxidative stress biomarkers. Environ Sci Pollut Res 21:5157–5166

Islas-Flores H, Manuel Gómez-Oliván L, Galar-Martínez M, Michelle Sánchez-Ocampo E, SanJuan-Reyes N, Ortíz-Reynoso M, Dublán-García O (2017) Cyto-genotoxicity and oxidative stress in common carp (Cyprinus carpio) exposed to a mixture of ibuprofen and diclofenac. Environ Toxicol 32:1637–1650

Jiang ZY, Hunt JV, Wolff SP (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 202:384–389

Jolibois B, Guerbet M (2005) Evaluation of industrial, hospital and domestic wastewater genotoxicity with the Salmonella fluctuation test and the SOS chromotest. Mutat Res 565:151–162

K'oreje KO, Vergeynst L, Ombaka D, De Wispelaere P, Okoth M, Van Langenhove H, Demeestere K (2016) Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. Chemosphere 149:238–244

Kümmerer K (2008) Pharmaceuticals in the environment : sources, fate, effects, and risks. Springer, Berlin

Laffite A, Kilunga PI, Kayembe JM, Devarajan N, Mulaji CK, Giuliani G, Slaveykova VI, Poté J (2016) Hospital effluents are one of several sources of metal, antibiotic resistance genes, and bacterial markers disseminated in Sub-Saharan Urban Rivers. Front Microbiol 7:1128

Levine RL, Williams JA, Stadtman EP, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–357

López-Serna R, Petrović M (2012) Occurrence and distribution of multi-class pharmaceuticals and their active metabolites and transformation products in the Ebro River basin (NE Spain). Sci Total Environ 440:280–289

Luja-Mondragón M, Gómez-Oliván LM, SanJuan-Reyes N, Islas-Flores H, Orozco-Hernández JM, Heredia-García G, Galar-Martínez M, Dublán-García O (2019) Alterations to embryonic development and teratogenic effects induced by a hospital effluent on Cyprinus carpio oocytes. Sci Total Environ 660:751–764

Magdaleno A, Juárez AB, Dragani V, Saenz ME, Paz M, Moretton J (2014) Ecotoxicological and genotoxic evaluation of Buenos Aires city (Argentina) hospital wastewater. J Toxicol 2014:248461

Martínez-Rodríguez H, Donkor K, Brewer S, Martínez M, SanJuan-Reyes N, Islas-Flores H, Sánchez-Aceves L, Elizalde Velazquez A, Gómez-Oliván L (2018) Metoprolol induces oxidative damage in common carp (Cyprinus carpio). Aquat Toxicol 197:122

Martínez-Viveros EM, Islas-Flores H, Dublán-García O, Galar-Martínez M, SanJuan-Reyes N, García-Medina S, Hernández-Navarro MD, Gómez-Oliván LM (2018) Environmentally relevant concentrations of glibenclamide induce oxidative stress in common carp (Cyprinus carpio). Chemosphere 197:105

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Nava-Álvarez R, Razo-Estrada AC, García-Medina S, Gómez-Olivan LM, Galar-Martínez M (2014) Oxidative stress induced by mixture of diclofenac and acetaminophen on common carp (Cyprinus carpio). Water Air Soil Pollut 225

Neri-Cruz N, Gómez-Oliván LM, Galar-Martínez M, del Socorro R-FM, Islas-Flores H, García-Medina S, Jiménez-Vargas JM, SanJuan-Reyes N (2015) Oxidative stress in Cyprinus carpio induced by hospital wastewater in Mexico. Ecotoxicology 24:181–193

NMX-AA-008-SCFI (2000) Análisis de agua - determinación del pH - Metodo de prueba. [cited 10 March 2019]. Available from http://elaguacbtis162.blogspot.com/2012/05/112-nmx-aa-008-scfi-2000-analisis-de.html

NOM-001-SEMARNAT (1996) Norma oficial mexicana que establece los límites máximos permisibles de contamiantes en las descargas de aguas residuales en aguas y bienes nacionales. 1996. *Procuraduría Federal de Protección al Ambiente, Diario Oficial de la Federación 30 October* 1996. [cited 18 September 2018]. Available from http://www.profepa.gob.mx/innovaportal/file/3290/1/nom-001-semarnat-1996.pdf

NOM-002-SEMARNAT (1996) Que establece los límites máximos permisibles de contaminantes en las descargas de aguas residuales. | SIA-BZF. [cited 10 March 2019]. Available from https://www.estudiosambientales.com.mx/blog/NOM/NOM-002-SEMARNAT-1996-aguas-residuales-resumen.html

Novoa-Luna KA, Mendoza-Zepeda A, Natividad R, Romero R, Galar-Martínez M, Gómez-Oliván LM (2016) Biological hazard evaluation of a pharmaceutical effluent before and after a photo-Fenton treatment. Sci Total Environ 569–570:830–840

Oliveira TS, Al Aukidy M, Verlicchi P (2017) Occurrence of common pollutants and pharmaceuticals in hospital effluents. Springer, Cham, pp 17–32. https://doi.org/10.1007/698_2017_9

Olvera-Néstor CG, Morales-Avila E, Gómez-Olivan LM, Galár-Martínez M, García-Medina S, Neri-Cruz N (2016) Biomarkers of cytotoxic, genotoxic and apoptotic effects in Cyprinus carpio exposed to complex mixture of contaminants from hospital effluents. Bull Environ Contam Toxicol 96:326–332

Orias F, Perrodin Y (2013) Characterisation of the ecotoxicity of hospital effluents: a review. Sci Total Environ 454–455:250–276

Orozco-Hernández L, Gutiérrez-Gómez AA, SanJuan-Reyes N, Islas-Flores H, García-Medina S, Galar-Martínez M, Dublán-García O, Natividad R, Gómez-Oliván LM (2018) 17B-Estradiol induces cyto-genotoxicity on blood cells of common carp (Cyprinus carpio). Chemosphere 191:118–127

Oviedo-Gómez DGC, Galar-Martínez M, García-Medina S, Razo-Estrada C, Gómez-Oliván LM (2010) Diclofenac-enriched artificial sediment induces oxidative stress in Hyalella azteca. Environ Toxicol Pharmacol 29:39–43

Parvez S, Raisuddin S (2005) Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish Channa punctata (Bloch). Environ Toxicol Pharmacol 20:112–117

Pérez-Alvarez I, Islas-Flores H, Gómez-Oliván LM, Barceló D, López De Alda M, Pérez Solsona S, Sánchez-Aceves L, SanJuan-Reyes N, Galar-Martínez M (2018) Determination of metals and pharmaceutical compounds released in hospital wastewater from Toluca, Mexico, and evaluation of their toxic impact. Environ Pollut 240:330–341

Perez-Coyotl I, Martinez-Vieyra C, Galar-Martinez M, Gomez-Olivan LM, Garcia-Medina S, Islas-Flores H, Perez-Pasten Borja R, Gasca-Perez E, Novoa-Luna KA, Dublan-Garcia O (2017) DNA damage and cytotoxicity induced on common carp by pollutants in water from an urban reservoir. Madin reservoir, a case study. Chemosphere 185:789–797

Pérez-Coyotl I, Martínez-Vieyra C, Galar-Martínez M, Gómez-Oliván LM, García-Medina S, Islas-Flores H, Pérez-Pasten Borja R, Gasca-Pérez E, Novoa-Luna KA, Dublán-García O (2017) DNA damage and cytotoxicity induced on common carp by pollutants in water from an urban reservoir. Madín reservoir, a case study. Chemosphere 185:789–797

Procuraduría Federal de Protección al Ambiente DO de la F 25 M 1980 (1980) *NMX-AA-003,* 1980. *Wastewater sampling.* [cited 18 September 2018]. Available from https://www.gob.mx/cms/uploads/attachment/file/166762/NMX-AA-003-1980.pdf

Radi R, Turrens JF, Chang LY, Bush KM, Crapo JD, Freeman BA (1991) Detection of catalase in rat heart mitochondria. J Biol Chem 266:22028–22034

Sanjuan-Reyes N, Gómez-Oliván LM, Galar-Martínez M, Vieyra-Reyes P, García-Medina S, Islas-Flores H, Neri-Cruz N (2013) Effluent from an NSAID-manufacturing plant in Mexico induces oxidative stress on Cyprinus Carpio. Water Air Soil Pollut 224

Santos LHMLM, Gros M, Rodriguez-Mozaz S, Delerue-Matos C, Pena A, Barceló D, Montenegro MCBSM (2013) Contribution of hospital effluents to the load of pharmaceuticals in urban wastewaters: identification of ecologically relevant pharmaceuticals. Sci Total Environ 461–462:302–316

Saucedo-Vence K, Dublán-García O, López-Martínez LX, Morachis-Valdes G, Galar-Martínez M, Islas-Flores H, Gómez-Oliván LM (2015) Short and long-term exposure to diclofenac alter oxidative stress status in common carp Cyprinus carpio. Ecotoxicology 24:527–539

Saucedo-Vence K, Elizalde-Velázquez A, Dublán-García O, Galar-Martínez M, Islas-Flores H, SanJuan-Reyes N, García-Medina S, Hernández-Navarro MD, Gómez-Oliván LM (2017) Toxicological hazard induced by sucralose to environmentally relevant concentrations in common carp (Cyprinus carpio). Sci Total Environ 575:347–357

Schieber M, Chandel NS (2014) ROS function in redox signaling and oxidative stress. Curr Biol 24:R453–R462

Stephensen E, Svavarsson J, Sturve J, Ericson G, Adolfsson-Erici M, Förlin L (2000) Biochemical indicators of pollution exposure in shorthorn sculpin (Myoxocephalus scorpius), caught in four harbours on the southwest coast of Iceland. Aquat Toxicol 48:431–442

Sun L, Xin L, Peng Z, Jin R, Jin Y, Qian H, Fu Z (2014) Toxicity and enantiospecific differences of two β-blockers, propranolol and metoprolol, in the embryos and larvae of zebrafish (*Danio rerio*). Environ Toxicol 29:1367–1378

Verlicchi P, Zambello E (2016) Predicted and measured concentrations of pharmaceuticals in hospital effluents. Examination of the strengths and weaknesses of the two approaches through the analysis of a case study. Sci Total Environ 565:82–94

Verlicchi P, Galletti A, Petrovic M, Barceló D (2010) Hospital effluents as a source of emerging pollutants: an overview of micropollutants and sustainable treatment options. J Hydrol 389:416–428

Vlahogianni T, Dassenakis M, Scoullos MJ, Valavanidis A (2007) Integrated use of biomarkers (superoxide dismutase, catalase and lipid peroxidation) in mussels Mytilus galloprovincialis for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. Mar Pollut Bull 54:1361–1371

Wilhelm Filho D (1996) Fish antioxidant defenses--a comparative approach. Braz J Med Biol Res 29:1735–1742

Zhou H, Huang J, Yuan Y, Tang B (2014) Prediction of water consumption in hospitals based on a modified Grey GM (0, 1|sin) model of oscillation sequence: the example of Wuhan City. J Appl Math 2014:1–7

Chapter 7
Evaluation of the Toxicity of Municipal Effluents from a Locality in the State of Mexico Using *Hyalella azteca* as a Bioindicator

Leopoldo Islas-Flores, Karen Adriana Novoa-Luna, Hariz Islas-Flores, Nely San Juan-Reyes, and Leobardo Manuel Gómez-Oliván

7.1 Introduction

The United Nations has estimated that by 2050, the world population will be 9.7 billion people and the vast majority of this growth were in developing countries; therefore, according to FAO's forecasts, 80% of the increase in food production needed to cope with this population growth will be obtained by an increase in yield of crops and annual crops in the same soil, therefore, not coming from the expansion of the crops. The pesticides will continue to be used in order to avoid important losses, so their effects on people and the environment are a permanent concern (Organización Mundial de la Salud 2018).

With respect to Mexico, according to data from the Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food, the share of agriculture in the national gross domestic product is 4%, but its impact on the economic and social development of the country is greater, since practically all food production origi-

L. Islas-Flores
Facultad de Planeación Urbana y Regional de la Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

K. A. Novoa-Luna · H. Islas-Flores (✉) · L. M. Gómez-Oliván
Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico
e-mail: hislasf@uaemex.mx

N. San Juan-Reyes
Laboratorio de Toxicología Ambiental, Facultad de Química, Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico

Departamento de Farmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_7

nates in this sector, being fundamental in food security, the cost of living, and the real income of the population as a whole (INEGI – SAGARPA 2014). On the other hand, the cultivation of ornamental plants is also important for the agricultural sector in terms of national and international trade, of which, only four states register 87.2% of total production and the first place is occupied by the State of Mexico with 61.3% of the national total. The most important municipalities of the State of Mexico in which the floricultural activity takes place are Villa Guerrero, Coatepec Harinas, Tenancingo, Zumpahuacán, Malinalco, and Ixtapan de la Sal, in which 90% of the state production is concentrated and 80% of the the national production of the main flower crops (Gobierno del Estado de México, Comisión Estatal de Parque Naturales y de la Fauna 2016).

The ecological, recreational, and tourist state park called "Hermenegildo Galeana" (HGP) is located in the municipality of Tenancingo, State of Mexico, and was established by the presidential decree published in the Official Gazette of the Federation on April 3, 1980. It is located geographically to the northwest of the Municipality of Tenancingo, State of Mexico, at an altitude that oscillates between 2060 and 2490 m above sea level, at the geographic coordinates 18° 57′ 05″ and 19° 02′ 25″ north latitude and 99° 35′ 45″ and 99° 38′ 37″ west longitude, with an area of 340.37 hectares (Fig. 7.1). There are about 30 different species of plants, ferns, mosses, and different fungi, as well as housing species such as lynx, jaguarundi, coyote, tuza, Peter squirrel,

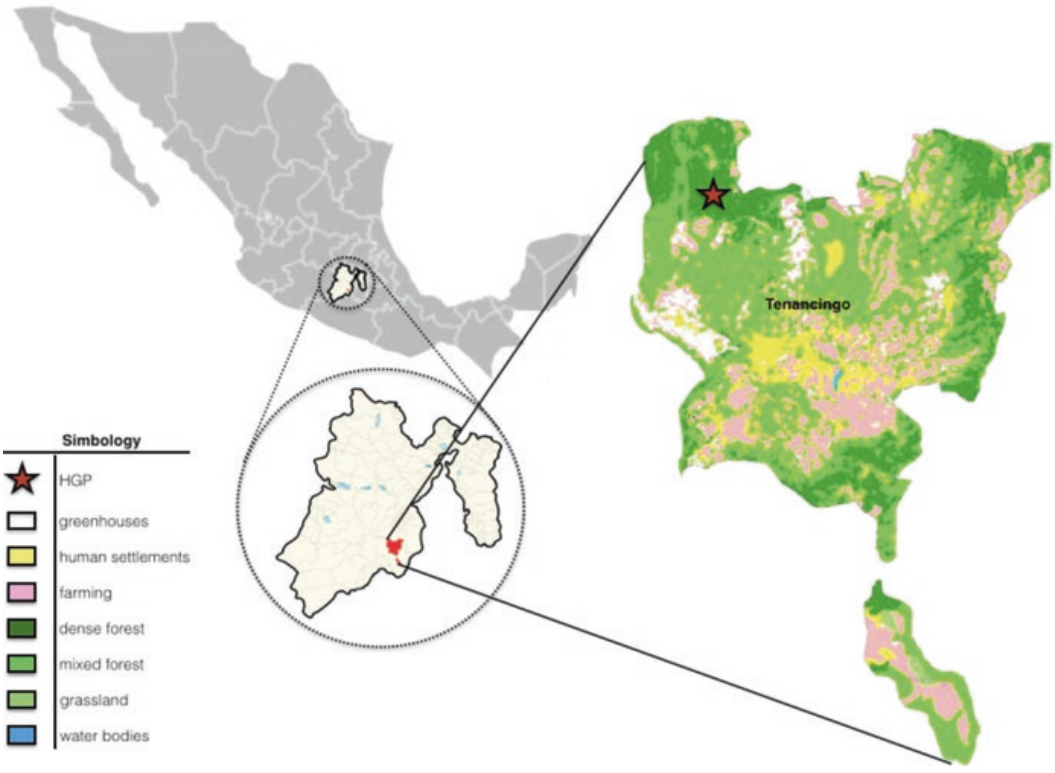

Fig. 7.1 Location map of the Hermenegildo Galeana Park (HGP) in the municipality of Tenancingo, State of Mexico and land use (Islas Flores and Ceballos 2018)

skunk, rabbits, opossum, Cozumel badger, armadillo, fox, buzzard, red-tailed hawk of Tres Marías, carpenter, hummingbird, sparrow (finch of Guadalupe), swallow, quail, heron, lizard, snake, salamander, and viper. It includes part of the hydrological region of the raft, and two perennial fluvial currents are distinguished, one corresponds to "La Fábrica," located at the east end of the Park, and another is located westward called "Puentecillas"; the rest of the fluvial network that makes up the HGP are intermittent. The localities included in the HGP and its area of influence correspond to San Antonio Agua Bendita and Monte de Pozo, representing the central communities, and Agua Bendita and Cruz Vidriada, as influential populations. It is important to mention that although these localities as such are not immersed in the park, they have an immediate location, connectivity, and accessibility; their main food product for self-consumption is corn, while products such as oats, beans, and vegetables are destined both for consumption of the population and for commercialization of the same (Gobierno del Estado de México, Comisión Estatal de Parque Naturales y de la Fauna 2016); on the other hand, in the municipalities of Tenancingo, Villa Guerrero, and Coatepec Harinas, 4378 hectares are destined for floriculture (Sánchez and Pacheco 2009). To carry out, the said activities use various chemical products (agrochemicals) such as fertilizers, insecticides, fungicides, and herbicides, which serve to nourish the plant and the soil, eliminate pests, and accelerate growth, as well as the flowering and ripening of the fruits; and with this they assure the quality and quantity of the production during the different periods of the year, without taking into account the contamination of soil, water, and air, in addition to the risk they generate to health, not only to people but also to biota who lives around (Riccioppo 2011).

Some biomarkers at the biochemical level include studies in which the objective is to evaluate oxidative stress through damage to macromolecules induced by reactive oxygen species (ROS) as a general route of toxicity induced by some chemicals in the redox cycle. These ROS include the radical superoxide anion, hydrogen peroxide, and the highly reactive hydroxyl radical (Barata et al. 2005). In this study, it was decided to use oxidative stress as a biomarker of toxicity, which is caused by an imbalance between ROS and the body's antioxidant systems. The most important antioxidant enzymes are superoxide dismutase (SOD, dismutes O^{2-} to H_2O_2), catalase (CAT, reduces H_2O_2 to water), and glutathione peroxidase (GPX, detoxifies H_2O_2 or organic hydroxyperoxides produced, e.g. by lipoperoxidation) (Barata et al. 2005). Organisms can adapt to an increase in ROS production through an increase in the regulation of antioxidant defenses, such as the activity of antioxidant enzymes (Livingstone 2003). Failures in antioxidant defense to detoxify excess ROS production can lead to significant oxidative damage including enzyme inactivation, protein degradation, DNA damage, and lipoperoxidation (Halliwell and Gutteridge 1999).

Hyalella azteca are organisms that have a small body (3–8 mm, and males are larger than females). The second section of the males is much larger and wider than the female. The color is variable (white, green, or brown); they feed mainly on filamentous algae and diatoms, but they can also eat decomposing organic matter (Espina and Vanegas 1996). Due to its advantages as a biological indicator, they are used as an indicator of environmental quality of water, as well as sediments due to their high sensitivity to various pollutants, since they are facultative benthic organisms, being able to live both in the water column and in the superficial sediments (Brun et al. 2006).

Due to this, the objective of this work was to evaluate the toxicity induced by effluents from the HGP in a sentinel organism, *Hyalella azteca*, using biomarkers of oxidative stress as hydroperoxide content, lipoperoxidation degree, and carbonylated protein content, in addition to the activity of antioxidant enzymes superoxide dismutas and catalase.

7.2 Methods

7.2.1 Sampling of the HGP Effluent

The effluent samples were obtained from HGP (Fig. 7.1), located in the municipality of Tenancingo, 36 km south of the city of Toluca, capital of the State of Mexico (Islas Flores and Ceballos 2018). Sampling was carried out using the procedure stipulated in NMX-AA-003-1980. The samples were collected in 20 L polyethylene containers, previously washed with 30% nitric acid and then with deionized water; the samples were covered, identified, and protected from light, immediately transferred to the laboratory and stored at 4 °C.

7.2.2 Test Organism

The test organisms (*Hyalella azteca*) (Fig. 7.2) were collected from their natural habitat in the Laguna de San Miguel de Almaya, municipality of Capulhuac, State of Mexico, and were transported to the laboratory in plastic bags with constant aeration. For their cultivation, they were kept in reconstituted water (174 mg L^{-1} $NaHCO_3$, 120 mg L^{-1} $MgSO_4$, 8 mg L^{-1} KCl y 120 mg L^{-1} $CaSO_4 \cdot 2H_2O$) with a range of pH 7.5–8.5, room temperature (with constant oxygenation 6.4–6.6 mg L^{-1}

Fig. 7.2 *Hyalella azteca*

O_2) and photoperiod of 16 h light/8 h dark, fed ad libitum with ground lettuce. The organisms used in the toxicity tests were neonates of the third generation by sexual reproduction of a culture of 4 months.

7.2.3 Preparation of Artificial Sediment

The composition of the artificial sediment was 70% sand, 20% kaolinite, and 10% organic matter. It was used as a source of organic matter compost of lamb, which was inactivated by dry heat in a temperature range of 55–60 °C for a period of 3 days. The artificial sediment was sterilized in an autoclave for three cycles of 15 min at 121 °C and 15 lbs of pressure, with intervals of 1 h apart. And the pH was measured with a potentiometer when the systems were mounted.

7.2.4 Toxicity Tests

7.2.4.1 Acute Toxicity

The test systems were mounted in polyethylene containers of 150 mL capacity, containing reconstituted water and artificial sediment in a ratio of 3:1, provided with continuous oxygenation, photoperiod of 16 h light/8 h dark, and room temperature. The exposure systems were static and no food was provided to the organisms during the exposure periods. In order to establish the concentration intended for the evaluation of oxidative stress, the mean lethal concentration (LC50) was determined, for which ten systems were used, with different proportions of the effluent obtained from the HGP (1, 3, 5, 7, 9, 20, 40, 60, 80, and 100%), as well as a free control group of the effluent, in which ten test organisms were placed (2 days old at start of test). The exposure time was 72 h; every 24 h the count of the dead (immobile) organisms was done, which were removed from the systems. The study was done in triplicate.

7.2.4.2 Sublethal Toxicity Assays

Sublethal toxicity assays were conducted in order to determine the oxidative stress in the effluent of the HGP (100%), in four test systems with 150 mg wet tissue of *Hyalella azteca*. The assessed exposure time was 72 h. An effluent-free control system with 150 mg wet tissue of *Hyalella azteca* (entire organisms were used) was set up and sublethal assays were performed by triplicate. After exposure, specimens were removed and homogenized in 1 mL Tris buffer solution pH 7. The supernatant was centrifuged at 12, 500 rpm for 15 min at −4 °C. Total protein content was determined and was used to express the results of the oxidative stress biomarkers evaluated. All biochemical assays were done on the supernatant, except for LPX assessment in which the bud was used.

Determination of HPC

HPC was determined by the Jiang et al. method (Jiang et al. 1992). To 100 µL of supernatant (previously deproteinized with 10% trichloroacetic acid) was added 900 µL of the reaction mixture (0.25 mM $FeSO_4$, 25 mM H_2SO_4, 0.1 mM xylenol orange, and 4 mM butyl hydroxytoluene in 90% (v/v) methanol). The mixture was incubated for 60 min at room temperature and absorbance was read at 560 nm against a blank containing only reaction mixture. Results were interpolated on a type curve and expressed as nM CHP (cumene hydroperoxide) mg protein^{-1}.

Determination of LPX

LPX was determined using the thiobarbituric acid-reactive substances method (Büege and Aust 1978). To 100 ml of supernatant was added Tris-HCl buffer solution pH 7.4 until a 1 ml volume was attained. Samples were incubated at 37 °C for 30 min; 2 ml TBA-TCA reagent [0.375% thiobarbituric acid in 15% trichloroacetic acid] was added and samples were shaken in a vortex. They were then heated to boiling for 45 min and allowed to cool, and the precipitate removed by centrifugation at 3000 rpm for 10 min. Absorbance was read at 535 nm against a reaction blank. Malondialdehyde (MDA) content was calculated using the molar extinction coefficient (MEC) of MDA (1.56 × 105 M cm^{-1}). Results were expressed as mM MDA protein^{-1}.

Determination of PCC

PCC was determined using the method of Levine et al. (Levine et al. 1994) as modified by Parvez and Raisuddin (Parvez and Raisuddin 2005) and Burcham (Burcham 2007). To 100 µl of supernatant was added 150 µl of 10 mM DNPH in 2 M HCl and the resulting solution was incubated at room temperature for 1 h in the dark. Next, 500 µl of 20% trichloroacetic acid was added and the solution was allowed to rest for 15 min at 4 °C. The precipitate was centrifuged at 11, 000 × g for 5 min. The bud was washed several times with 1:1 ethanol/ethyl acetate, then dissolved in 1 ml of 6 M guanidine solution (pH 2.3), and incubated at 37 °C for 30 min. Absorbance was read at 366 nm. Results were expressed as nM reactive carbonyls formed (C=O)/mg protein, using the MEC of 21,000 M cm^{-1}.

Determination of SOD Activity

SOD activity was determined by the Misra and Fridovich method (Misra and Fridovich 1972). To 40 µl of supernatant in a 1-cm cuvette was added 260 µl carbonate buffer solution (50 mM sodium carbonate and 0.1 mM EDTA) pH 10.2, plus

200 µl adrenaline (30 mM). Absorbance was read at 480 nm after 30 s and 5 min. Enzyme activity was determined using the MEC of SOD (21 M cm^{-1}). Results were expressed as IU SOD mg $protein^{-1}$.

Determination of CAT Activity

CAT activity was determined by the Radi et al. method (Radi et al. 1991). To 20 mL of supernatant was added 1 mL isolation buffer solution [0.3 M saccharose, 1 ml EDTA, 5 mM HEPES, and 5 mM KH_2PO_4], plus 0.2 mL of a hydrogen peroxide solution (20 mM). Absorbance was read at 240 nm after 0 and 60 s. Results were derived by substituting the absorbance value obtained for each of these times in the formula: CAT concentration = $(A_0 - A_{60})$/MEC) where the MEC of H_2O_2 is 0.043 mM/cm and was expressed as µM H_2O_2 mg $protein^{-1}$.

Determination of Protein Content

To 25 µl of supernatant was added 75 µl deionized water and 2.5 mL Bradford's reagent (0.05 g Coomassie blue dye, 25 mL of 96% ethanol, and 50 mL H_3PO_4, in 500 mL deionized water). The test tubes were shaken and allowed to rest for 5 min prior to reading of absorbance at 595 nm and interpolation on a bovine albumin curve (Bradford 1976).

7.2.5 Statistical Analysis

In the sublethal toxicity assays, statistical evaluation of results was done with one-way analysis of variance (ANOVA) and differences between means were compared using the Tukey-Kramer multiple comparisons test, with P set at 0.05. Statistical determinations were made with the SPSS v10 software package.

7.3 Results

Table 7.1 lists the results obtained from the exposure of *Hyalella azteca* for 72 h at different concentrations of the HGP effluent, as can be observed even though the organisms were exposed only to the effluent (100%); the death of 6.7% was obtained, so it was impossible to calculate the LC50.

HPC results are shown in Fig. 7.3. In *H. azteca*, significant differences with respect to the control group were not observed with the HGP effluent; an increase of 6.7% with respect to the control was observed.

Table 7.1 Mortality in *Hyalella azteca* exposed to different concentrations of effluents of HGP

Water proportion of the HGP (%)	Exposed organisms	Dead organisms	% Mortality
1	30	0	0.0
3	30	0	0.0
5	30	0	0.0
7	30	0	0.0
9	30	0	0.0
20	30	1	3.3
40	30	0	0.0
60	30	0	0.0
80	30	2	6.7
100	30	2	6.7

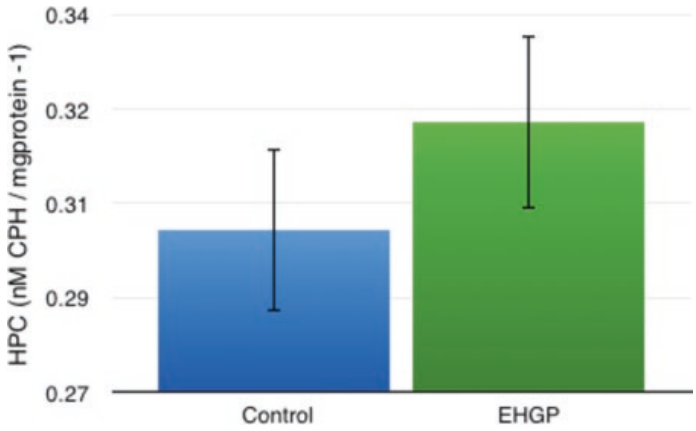

Fig. 7.3 Hydroperoxide content (HPC) in *H. azteca* exposed to effluent of HGP for 72 h

LPX results are shown in Fig. 7.4; as can be seen, there was a 5% decrease with respect to the control.

Figure 7.5 shows the results of PCC determination. There was no significant difference between the groups, only an increase of 3.8% with respect to the control.

Figure 7.6 shows the results of SOD activity. As can be seen, there was an increase in the activity of the SOD by 2.9% with respect to the control.

Figure 7.7 shows the results of determination of CAT activity; no significant difference was observed with respect to the control group, and as can be seen an increase of 26.7% was presented.

7.4 Discussion

One of the objectives proposed in this work was to determine the LC50 of the HGP effluent in *H. azteca*, after 72 h of exposure; however it could not be done because although it was exposed to 100% of the effluent, it was not obtained

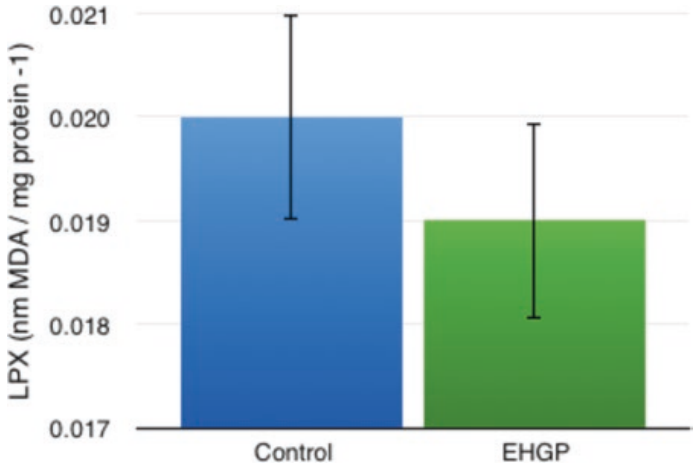

Fig. 7.4 Lipid peroxidation (LPX) in *H. azteca* exposed to effluent of HGP for 72 h

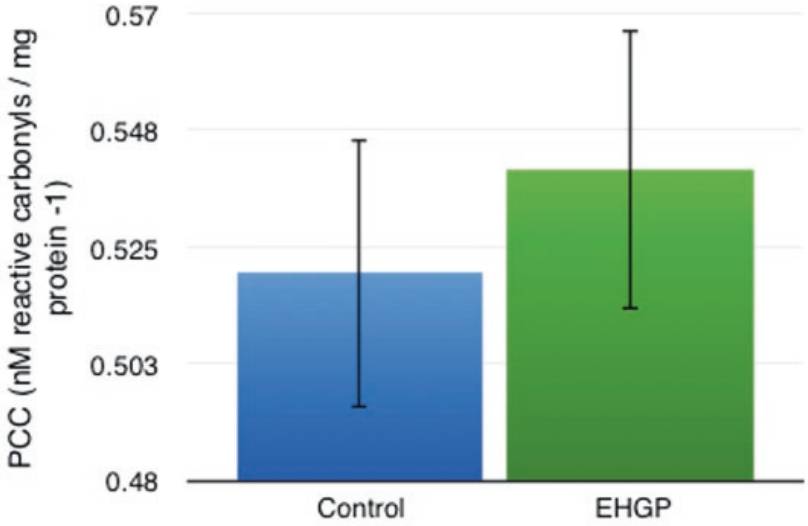

Fig. 7.5 Protein carbonyl content (PCC) in *H. azteca* exposed to effluent of HGP for 72 h

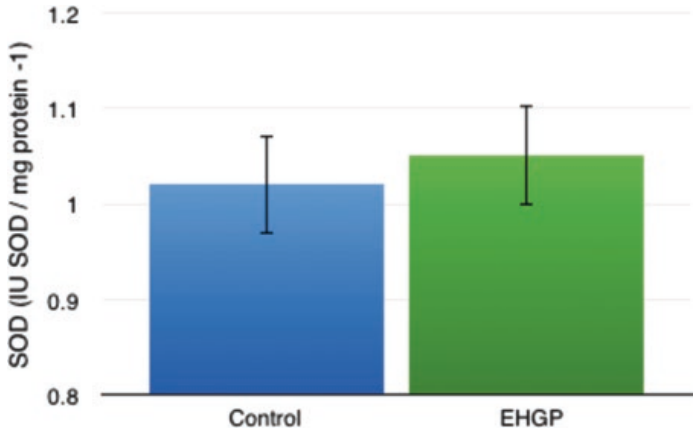

Fig. 7.6 Superoxide dismutase activity in *H. azteca* exposed to effluent of HGP for 72 h

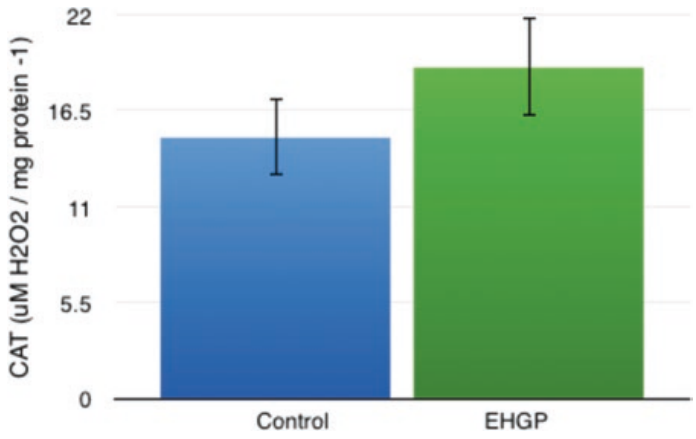

Fig. 7.7 Catalase activity in *H. azteca* exposed to effluent of HGP for 72 h

more than the 6.7% mortality. Subsequently, the sublethal study was carried out using 100% of the HGP effluent, through the determination of oxidative stress biomarkers. The content of hydroperoxides (HPC) was determined; an increase of 6.7% was observed compared to the control group, while the value of lipid peroxidation (LPX) showed a 5% decrease. These processes are considered the first mechanism of cell destruction, since an increase in HPC and LPX and decrease in antioxidant protection generate epoxides that can react spontaneously with nucleophilic centers in the cell, and, in this way, they are covalently bound to DNA, RNA, and proteins (Barata et al. 2005; Livingstone 2003; Halliwell and Gutteridge 1999). Regarding the determination of carbonylated proteins (CPC), an increase of 3.8% was also obtained. Dröge (Dröge 2002) mentions that micromolar concentrations of between 20 and 400 superoxide anion or hydrogen peroxide increase proteolysis up to 11 times in relation to baseline; on the contrary, micromolar concentrations inhibit the process and lead to the intracellular accumulation of oxidized proteins.

ROS are produced in cells as a result of the metabolic processes that carry out (Vlahogianni et al. 2007). To minimize oxidative damage to cellular components, organisms have developed antioxidant defenses (Barata et al. 2005). In invertebrate species, SOD and CAT are considered to play an antioxidant role greater than GPX, whereas for vertebrates it is the opposite (Halliwell and Gutteridge 1999). The first line of enzymatic antioxidant defense is superoxide dismutase that catalyzes the conversion of superoxide anion to hydrogen peroxide. In the determination of this enzyme, an increase of 2.9% was observed with respect to the control. The CAT is the second line of defense against oxidation. In the determination of CAT activity after 72 h of exposure to the HGP effluent in *H. azteca*, an increase of 26.7% was observed. The activity of SOD and CAT is similar or greater in invertebrates, indicating its important role in the antioxidant protection of aquatic invertebrates (Livingstone 2003). As it can be observed with the previous results, it can be said that the HGP effluent is not toxic for *Hyalella azteca*

since there were no significant differences with respect to the control in the different biomarkers evaluated; however it is known that the HGP is located within the floricultural zone of the State of Mexico, being the main economic activity, with the largest number of greenhouses, and that contributes with 80% of the flowers exported to North America and Europe (Martínez et al. 2014); in addition in the floricultural activity prevails a high use of pesticides, both in the field (open system) and in greenhouses (closed systems), and this has been associated with risks to the health of exposed persons, especially in children and pregnant women, who support fumigation, cutting, and carrying activities of the flowers (Oviedo-Zúñiga et al. 2003).

Among the most commonly used pesticides in this municipality are the Rovral, Sencor, glyphosate, Benomilo, Manzate, captan, Furadan, Tamaron, Nuvacron, and Pentaclor 600F, some of which have been restricted in their application by the European Union and the Environmental Protection Agency (Red de Agricultura Sostenible 2011; Torres Morales 2016), due to its high levels of toxicity and its impact on human health, since overexposure to Furadan, Tamaron, and Nuvacron causes headache, dizziness, respiratory distress, abdominal spotting, nausea, salivation, blurred vision, dilated pupils, wet nose and mouth, bruising of the skin, seizures, tremor, coma, and death, as a result of the inhibition of acetylcholinesterase or erythrocyte acetylcholinesterase. On the other hand, on Pentaclor 600F, an organochlorine pesticide, it is highly persistent in the environment and its risk to human health has not yet been sufficiently studied (Yucra et al. 2008; Otieno et al. 2010). It should be noted that although the existing information on the health damage caused by these pesticides is acknowledged, it has not restricted its use in Mexico (Comision Intersecretarial para el Control del Proceso y Uso de Plaguicidas, Fertilizantes y Sustancias Toxicas (CICOPLAFEST) 2004). For example, a study conducted by Castillo-Cadena et al. (Castillo-Cadena et al. 2017) determined the frequency and etiology of congenital malformations by monitoring 18-month newborns of a flower community of Tenancingo and compared it with an urban community; their results showed that in Tenancingo, 20% of newborns had some type of malformations congenital, while in the urban area, 6% had some malformation, and in addition congenital malformations occurred more frequently in the area of floriculture and that due to the percentage of multifactorial etiology is higher, it is likely that there is an association with exposure to pesticides. Other studies have shown the harmful effects of pesticides on germ cells, specifically affecting the parameters of male sperm, so they may be less likely to fertilize (Abell et al. 2000), a fact compatible with other studies that found a direct relationship between a greater environmental exposure to pesticides and lower mobility, concentration and percentage of sperm (Hauser 2008). In a study conducted by Abell et al. (Abell et al. 2000) showed a significant difference in the parameters of sperm quality, decreasing sperm count, mobility, as well as the number of normal and abnormal sperm and an increase in structural abnormalities of sperm

among the occupationally exposed group to pesticides in the municipality of Tenancingo in front of a group not exposed. In spermatogenesis, whether the pesticides damage the immature cell, this can cause the damaged cell to be eliminated, so that only those without apparent damage can mature and eventually stop ejaculation, which explains the decrease in sperm count (Recio et al. 2008; Spira and Multigner 1998). Added to this, Alonso Zotea (Alonso Zotea 2015) evaluated 126 children of both sexes from 6 to 12 years of age, 63 children belonging to a primary school surrounded by greenhouses no more than 100 m away (exposed to pesticides) from the floricultural zone of the State of Mexico and 63 children from the primary school not exposed; their results showed that the values of micronuclei in exposed and unexposed children were found within normal parameters, while the nuclear abnormalities of the oral mucosa in the exposed group suggest genotoxic damage due to exposure to pesticides, due to the continuous exposure of children working in greenhouses, to indirect exposure and lack of protection measures.

As expected, an issue of great concern is the environmental impact generated by agriculture and floriculture due to the use of agrochemicals in some cases in high quantities. Being of great importance, the contamination of soil and water with toxic substances, such as heavy metals. Dotor-López et al. (2017) demonstrated by atomic absorption spectrophotometry the presence and concentration of heavy metals: Al, As, Cr, Pb, Cu and Zn in strawberry fruits (*FragariaXananassa* Duch.) Var. festival, as well as, in samples of soil and water used in its production. in the municipalities of Tenancingo and Villa Guerrero, their results show that the Al and Zn were the elements present in greater quantity (mg kg^{-1}) both in strawberry fruits, as in soil and water, while the Cr was detected in Villa Guerrero in fruit and water, and Pb in fruits from Tenancingo. On the effects of exposure to soil samples, Tecuapetla conducted a bioassay using *Eisenia andrei* as a biomarker, exposed for 14 days to soil from greenhouses and a control group used as control obtained from HGP soils (without specifying the actual location), because both soils presented similar characteristics (pH, organic carbon, real and apparent density), the cholinesterase activity of the exposed worms presented the same behavior as the samples from the greenhouses under study, concluding that HGP's soil it turned out to be toxic for the organisms tested (Tecuapetla 2014). As it can be seen, the aforementioned results do not match what was obtained in this study, and although this could be considered good because as mentioned above, in this park there is a great diversity of flora and fauna that could be affected by the presence of pesticides and metals in the environment, it could not be concluded that there is no toxicity for them, since a more exhaustive study should be carried out, taking into account other sampling sites, different matrices (soil and water) in different seasons (rain and drought) and the proximity to the possible sources of emission of pollutants; as well as the use of different bioindicators within trophic chains.

7.5 Conclusions

Within the agricultural and floricultural production process there is excessive use of agrochemicals which generate different effects on health such as endocrine disruption, cancer, genetic malformations, sexual cell abnormalities, kidney and liver damage, as well as development. The HGP effluents evaluated in this study were not shown to be toxic in *Hyalella azteca* exposed for 72 h, however, due to the reports of the different toxic effects found previously in the same study area, it is suggested to carry out more studies taking into account other sampling sites of the park, as well as different matrices (soil and water), in different seasons of the year and in locations that suggest an impact due to anthropogenic activities.

References

Abell A, Ernst E, Bonde JP (2000) Semen quality and sexual hormones in greenhouse workers. Scand J Work Environ Health 26(6):492–500

Alonso Zotea A (2015) Identificación de micronucleos y anormalidades nucleares en mucosa bucal de niños expuestos y no expuestos a plaguicidas. Tesis para obtener grado de Maestría. Universidad Autonoma del Estado de Mexico

Barata C, Varo I, Navarro JC, Arun S, Porte C (2005) Antioxidant enzyme activities and lípid peroxidation in the freshwater cladoceran Daphnia magna exposed to redox cycling compounds. Comp Biochem Physiol 140(C):175–186

Bradford M (1976) A rapid and sensitive method for the quantitation of microorganism quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248–254

Brun L, Bernier M, Losier R, Doe K, Jackman P, Lee H (2006) Pharmaceutically active compounds in Atlantic Canadian sewage treatment plant effluents and receiving waters and potential for environmental effects as measured by acute and chronic aquatic toxicity. Environ Toxicol Chem 26(Suppl 8):2163–2176

Büege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310

Burcham PC (2007) Modified protein carbonyl assay detects oxidised membrane proteins: a new tool for assessing drug- and chemically-induced oxidative cell injury. J Pharmacol Toxicol Methods 56:18–22

Castillo-Cadena J, Mejia-Sanchez F, López-Arriaga JA (2017) Congenital malformations according to etiology in newborns from the floricultural zone of Mexico state. Environ Sci Pollut Res 24:7662–7667

Comision Intersecretarial para el Control del Proceso y Uso de Plaguicidas, Fertilizantes y Sustancias Toxicas (CICOPLAFEST) (2004) Catálogo oficial de plaguicidas. Comision Intersecretarial para el Control del Proceso y Uso de Plaguicidas, Fertilizantes y Sustancias Toxicas, México

Dotor-López GI, Zuñiga-Cruz A, Cruz-Monterrosa RG, Díaz-Ramírez M, Rayas-Amor AA (2017) Cuantificación de metales pesados en el cultivo de la fresa (Fragariaxananassa Duch.var. festival) en Tenancingo y Villa Guerrero, Estado de México. Agroproductividad 10:29–33

Dröge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95

Espina S, Vanegas C (1996) Ecotoxicología y Contaminación. In: Botello AV, Rojas Galaviz JL, Benítez y J, Zárate Lomelí D (eds) Golfo de México, Contaminación e Impacto Ambiental: Diagnóstico y Tendencias. EPOMEX, Campeche, pp 69–106

Gobierno del Estado de México, Comisión Estatal de Parque Naturales y de la Fauna (2016) Programa de manejo del Parque Estatal ecológico, recreativo y turístico "Hermenegildo Galeana"

Halliwell B, Gutteridge JMC (1999) Free radicals in biology and medicine. Oxford University Press, Oxford

Hauser R (2008) Urinary phthalate metabolites and semen quality: a review of a potential biomarker of susceptibility. Int J Androl 31(2):112–116

INEGI – SAGARPA (2014) Encuesta Nacional Agropecuaria, Reporte del CEDRSSA, México, septiembre de 2015, Centro de Estudios para el Desarrollo Rural Sustentable y la Soberanía Alimentaria (CEDRSSA)

Islas Flores L, Ceballos G (2018) Depredación de un cincuate (*Pituophis lineaticollis*) y una ardilla arborícola (*Sciurus aureogaster*) por un gato montés (*Lynx rufus escuinapae*) en el parque estatal hermenegildo galeana, Estado de México. Rev Mex Mastozool 1(8):1–7

Jiang ZY, Hunt JV, Wolff SP (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 202(2):384–389

Levine RL, Williams JA, Stadtman ER, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–357

Livingstone DR (2003) Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Rev Med Vet 154:427–430

Martínez LG, Mejia SF, Serment GJH, Castillo CJ (2014) Quality spermatic alterations in floriculturists exposed to pesticides in Villa Guerrero, State of Mexico. Am J Agric For 2(6):284–288

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Organización Mundial de la Salud (2018) Residuos de plaguicidas en los alimentos. Available in: https://www.who.int/es/news-room/fact-sheets/detail/pesticide-residues-in-food. Acceso: 5/03/2019

Otieno OP, Lalah OJ, Virani M, Jondiko OI, Schramm KW (2010) Carbofuran and its toxic metabolites provide forensic evidence for furadan exposure in vultures (*Gyps africanus*) in Kenya. Bull Environ Contam Toxicol 84(5):536–544

Oviedo-Zúñiga AM, Karam-Calderón Miguel A, Rodríguez GC (2003) Percepción de riesgo por el uso de plaguicidas en niños escolares, Villa Guerrero, Estado de México. Revista de Toxicología en línea 11:23–46

Parvez S, Raisuddin S (2005) Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish Channa punctata (Bloch). Environ Toxicol Pharmacol 20:112–117

Radi R, Turrens JF, Chang LY, Bush KM, Carpo JD, Freeman BA (1991) Detection of catalase in rat heart mitochondria. J Biol Chem 266:22028–22034

Recio VR, Ocampo GG, Borja AV, Moran MJ, Cebrian GM (2008) Organophosphorus pesticide exposure decreases sperm quality: association between sperm parameters and urinary pesticide levels. J Appl Toxicol 28(5):674–680

Red de Agricultura Sostenible (2011) Lista de Plaguicidas Prohibidos. Red de Agricultura Sostenible (RAS), San José, pp 2–8

Riccioppo R (2011) Agroquímicos: sus efectos en la población medidas de prevención. Comisión de Trabajo Médico, Colegio de Médicos Distrito – VII

Sánchez J, Pacheco F (2009) Floricultura, en La diversidad Biológica del Estado de México. Gobierno del Estado de México, Toluca, pp 291–297

Spira A, Multigner L (1998) The effect of industrial and agricultura pollution on human spermatogenesis. Hum Reprod 13(8):2041–2042

Tecuapetla MG (2014) Ecotoxicidad producida por agroquímicos empleados en el cultivo de Gerbera Jamesonii en invernadero, en Villa Guerrero, Estado de México (Tesis). Universidad Autónoma del Estado de México, Toluca

Torres Morales AV (2016) Repercusiones a la Salud por el uso de agroquímicos en los viveros de Tenancingo (2015–2016). Tesis para obtener título de licenciatura. Universidad Autónoma del Estado de México

Vlahogianni T, Dassenakis M, Scoullos MJ, Valavanidis A (2007) Integrated use of biomarkers (superoxide dismutase, catalase and lipid peroxidation) in mussels Mytilus galloprovincialis for assessing heavy metals' pollution in coastal areas from the Saronikos Gulf of Greece. Mar Pollut Bull 54:1361–1371

Yucra S, Gasco M, Rubio J, Gonzales FG (2008) Exposición ocupacional a plomo y pesticidas órganofosforados: efecto sobre la salud reproductiva masculina. Rev Peru Med Exp Salud Publica 25(4):394–402

Chapter 8
Analysis of Heavy Metals Present on Air Through the Toxicity Analysis in Water by the Gas Washer Method, Using the Organism *Daphnia magna*

Tamila Kleine, Therezinha Maria Novais de Oliveira, and Sandra Helena Westrupp Medeiros

8.1 Introduction

Air quality is the term normally used to translate the degree of pollution into the atmospheric environment. Air pollution occurs when gaseous waste changes the aesthetic aspect, composition, or shape of the physical environment (Filho 2010).

The atmosphere of an urban center consists of gases and particles that can become polluting when they are above the natural concentrations of the atmosphere. These gases and particles can be emitted from natural sources such as soil, pollen, or volcanoes or from anthropogenic activities such as burning of fuels, industry, or agricultural activity (Seinfeld and Pandis 1998).

The World Health Organization (WHO), as long ago as 2002, gave high priority to air pollution and estimated that it accounts for 1.4% of all deaths and a decrease of 0.8% in the total years of life globally. In 2012, WHO estimated that around seven million deaths worldwide stem from air pollution from urban and rural sources (WHO 2018). In addition, based on data from the Urban Air Quality Guide, updated in 2016, nine out of ten people worldwide breathe contaminated air, and it is estimated that 4.2 million people died from air pollution. If indoor air pollution is also considered, the estimate increases to seven million people (WHO 2018, web).

T. Kleine (✉)
PhD in Health and Environment from Universidade da Região de Joinville, Joinville, Brazil

T. M. N. de Oliveira
Department of Research and Graduate Studies at the Universidade da Região de Joinville, Joinville, Brazil

S. H. W. Medeiros
Department of Sanitary and Environmental Engineering, Universidade da Região de Joinville, Joinville, Brazil

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_8

Regarding heavy metals, Amaral (2016) classified metals according to abundance and toxicity, taking into account three criteria:

- Noncritical metals (Na, K, Ca, Mg, Al, and Fe)
- Rare, insoluble, and toxic metals (W, Ti, Zr, and Ba)
- Very toxic and relatively available metals (Ni, Cu, Zn, As, Cd, Hg, and Pb)

Metals such as Pb, Cr, and Hg have no biological function and can be highly toxic to living organisms and the environment. Other metals such as Fe, Cu, and Zn are essential to living beings, as they act on relevant metabolic routes; in high concentrations, however, metals also become harmful (Ferreira 2003).

Volatile organic compounds (VOCs) comprise a large number of pollutants that are present in the atmospheric air (Hoshi et al. 2008). The composition and presence of these compounds are directly related to the use of vehicular fuels (Martins et al. 2007). Benzene, ethylbenzene, toluene, and xylenes represent a large fraction of these compounds emitted in urban environments (Singh et al. 1992).

The WHO proposes guidelines only for BTEX and the USEPA does not propose any standards; only the US Occupational Safety and Health Agency defines some standards (Han and Naeher 2006).

The costs involved in reducing the emission of pollutants are mostly quite high. Therefore quantification of the effects of pollution and the determination of acceptable limits for the levels of various environmental pollutants are key issues and deserve special attention in developing countries, living not only with high levels of pollution but also with few resources for investments in prevention and control measures (Daumas et al. 2004). Investments in both equipment and research are few, and in the twenty-first century, the picture has not been greatly modified, and even worsened in some cases, making the health situation serious as a consequence of air pollution.

Environmental toxicological assessments are an effective way to evidence effects of combinations of factors present in the ecosystem. Through its realization, the knowledge obtained in chemical analysis is maximized, generally not useful for the development of actions.

Ecotoxicology can be characterized as the science for which ecology and toxicology converge with the aim of managing the risk of exposure of organisms to harmful substances present in the environment, enabling the establishment of safety measures to prevent their harmful effects (Zagatto 2014).

It is impracticable for a laboratory to perform trials with all species of an ecosystem. As a result, in the 1950s, in several countries, such as Germany, the United States, France, and England, certain organisms were selected by criteria that considered an ecosystemic viewpoint to represent the most important groups among those that make up the functioning of an ecosystem. For this, many researches were carried out with several species of plants and animals, in order to indicate the organisms that were representative of each ecosystem. It was considered that, even within the same taxonomic group, each species has a differentiated sensitivity (Knie and Lopes 2004).

For the analysis of atmospheric pollution of heavy metals, lichens, fungi, and higher plants are presented in the literature as the main bioindicator organisms. Carneiro (2004) identified 112 plant species used as atmospheric pollution

bioindicators, 22 lichens, and 15 mosses. There are also other organisms that can be used for this purpose. Silva et al., for example, used in 2007 the mollusk *Cantareus aspersus* for the evaluation of genotoxicity induced by atmospheric pollutants.

In Brazil, tests to assess acute or chronic toxicity in freshwater species are consolidated and standardized, for example, acute *Daphnia magna*, and chronic toxicity tests with *Ceriodaphnia dubia* (Costa et al. 2008). But there are no standardized bodies for the analysis of atmospheric pollution.

The genus *Daphnia* is widely used in research in biology and ecology, due to the fact that they are key species in the food chain of freshwater organisms (Artal et al. 2013; Becker et al. 2014); are the main consumers of algae, bacteria, and protozoa; and are the first food of fish, making them interesting as a genus indicative of the disturbance of aquatic ecosystems (Covich et al. 2010; Dodson et al. 2010). A key species is a species whose presence controls populations at lower or higher trophic levels (Campos 2015).

Considering that atmospheric pollution has as a mechanism of natural removal of the pollutants, the dry and humid deposition (e.g., rain and mist), these pollutants are transferred directly to rivers, lakes, and seas and thus can affect sweet species, so that there is interconnection between pollution of water bodies with the pollution of atmospheric air.

It is known that gases are solubilized in liquids with different magnitudes. For example, the solubility of xenon in n-octane is 470 times greater than the solubility of helium in water. This incredible characteristic of the gases to be solubilized in different amounts in the liquids caused the studies to intensify, and, consequently, several applications arose (Galvão 2011), thus allowing the use of a gas scrubber to perform toxicological tests for environmental pollutants.

8.2 Testing Organism

Daphnia magna are freshwater microcrustaceans belonging to the order Cladocera (class Branchiopoda). Their populations are abundant in lentic habitats (standing water) (Antunes and Castro 2017).

They are organisms representative of plankton (a group of organisms that do not have the swimming movements that would enable it to swim against currents) and feed on fine particles of suspended organic matter, including yeasts, bacteria, and microalgae (phytoplankton) (Antunes and Castro 2017).

It serves as food for a huge variety of invertebrates and vertebrates (Antunes and Castro 2017), which makes this organism a key species in the food chain, a characteristic that increases its relevance as an indicator of environmental quality and makes it quite appropriate for the performance of toxicity tests.

Morphologically, they have a bivalve cuticular shell (exoskeleton) that involves only their body, not the head, and use the second pair of antennas as the main organ of locomotion. They are characterized by having leaf-shaped thoracic appendages that constitute the main respiratory surface and, at the same time, form part of the particulate filtration apparatus (Antunes and Castro 2017). Its anatomy can be seen in Fig. 8.1.

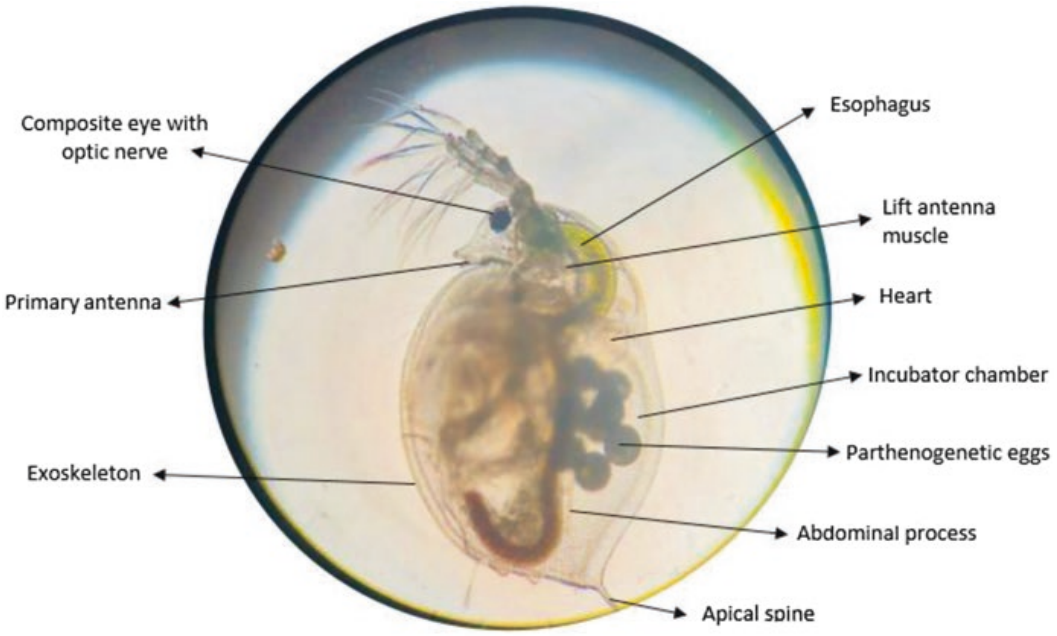

Fig. 8.1 Simplified anatomy of *Daphnia magna.* (Source: The Author)

Asexual reproduction may occur under favorable environmental conditions: a female may give rise to genetically identical juvenile females (parthenogenesis reproduction) or, when exposed to adverse environmental conditions (decrease in water level, overpopulation, and low temperatures, among others), females can produce males; and sexually, in the presence of males, some females produce sexed eggs (that have suffered meiosis) that can be fertilized. The fertilized eggs do not develop. These eggs are enveloped by a protective membrane, forming a "patty-like" structure called ephippium (Fig. 8.2) (Antunes and Castro 2017).

Daphnia are organisms that are characterized by having a short life cycle when compared to humans or other vertebrates, high fertility rates, and great sensitivity to many different stressors. For this reason, they are of great scientific interest (Loureiro et al. 2013). Furthermore, the ease of its maintenance in the laboratory and its mode of reproduction, which allows to control the genetic variability of the organisms, make its use more and more indicated (Antunes and Castro 2017).

They have an average life expectancy of 60 days. Its development comprises four phases: egg, juvenile, adolescent, and adults. At an early stage of development, it is possible to observe the existence of a structure – nauplii – in which the larvae are protected inside the egg. After hatching, juveniles are born, morphologically identical to adults, which will be released about 2 days after this event. The absence of external larval stages and the production of juveniles identical to adults indicate that it is a direct development (Covich et al. 2010).

The duration and quality of the life cycle of *Daphnia* are deeply related to environmental conditions but are closely dependent on food as well. When the mother is well fed, the eggs receive a large amount of lipids. If the feed is a limiting factor, the progenitor can mature to a smaller size than normal, producing smaller than

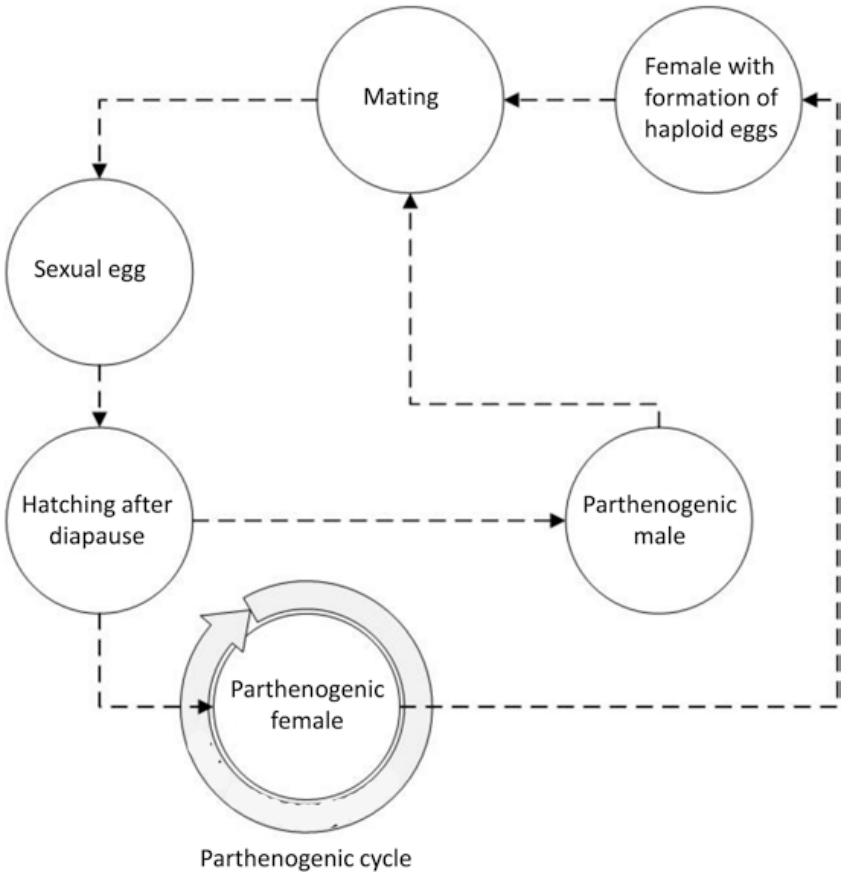

Fig. 8.2 (A) *Daphnia magna* life cycle. Representation of both reproductive processes: parthenogenesis and sexual reproduction. (B) Female with parthenogenic eggs in their incubator chamber. (C) Female with formed ephippium. (Source: Ebert 2005)

expected offspring (Enserink et al. 1990; Gabsi et al. 2014). On the other hand, if the food is abundant, the initial size influences the age at which the female reaches maturity (Gabsi et al. 2014).

In relation to the cultivation of these organisms, norms (ABNT 2009; OECD 2012) allow some flexibility, as long as they guarantee the well-being of the organisms, which makes crop success highly dependent on the knowledge and experience of the responsible technician and does not encourage the construction of comparative bases, which prevents comparisons between research laboratories (Knie and Lopes 2004).

8.3 Collection of Atmospheric Pollutants Through a Gas Scrubber

In order to perform the air collection, a device similar to a small volume sampler (APV) is set up to determine the concentration of pollutant gas in the air by the absorption process, as can be seen in Fig. 8.3, in order to perform the capture of

Fig. 8.3 Equipment for the sampling of air in liquid media. (Source: The Author)

water-soluble fractions of air pollutants into water. This dissolution is possible because it is governed by Henry's law, which states that the solubility of a gas in water depends on the partial pressure of the gas exerted on the liquid. Therefore, the proportionality constant used in this law varies with gas and temperature.

The use of the suction pump (vacuum pump with oil-free piston – BMC B-390), represented by the number 1 in Fig. 8.3, causes the water to be used for the analysis and preparation of toxicological tests to have no contact with no internal part of the engine, eliminating the possibility of possible contamination. Thus, the water to be used for the analysis and preparation of the toxicity tests is influenced only by air.

The vacuum pump used has an air displacement of 39 l/min, with a power of 100 W.

In order to control the flow, a gasometer (represented in Fig. 8.3 by number 2) is installed next to the engine, so it is possible to know the volume of air that came into contact with the water in the time determined for the collection of water to be analyzed.

Represented in Fig. 8.3 by number 3, the protective vial is intended to prevent the water used in the bubbler from reaching the gasometer or the suction pump.

The bubbler (no. 4) is where the water to be used for toxicological tests or physical/chemical tests is stored. It has, in its upper part, an air inlet (represented by an arrow in Fig. 8.3) composed of a glass tube that has contact with the water of the interior. At its side, another glass straw, disposed in such a way that it does not come into contact with the water inside the bubbler, only with the air, is arranged in such a way that its outlet is connected to the protective bottle.

All four equipment used are interconnected with sterile plastic hoses (changed at each collection) and fences so that there is no external contamination.

The determination of the time of collection can be altered according to the characteristics of the place to be analyzed, based on the possibilities of safety in relation to the equipment and the place of collection.

In order to rule out the possibility of decanting a possible pollutant or microparticle, a manual stirring must be performed before transferring the water from the absorption system to the vial to be sent to the laboratory for certain analyses.

8.4 Toxicity Test

The toxicological tests to be carried out with the sample collected in the gas scrubber may vary between acute, chronic, and transgenerational, and tests with other organisms or even physical and chemical analyses can be performed. This method of analyzing atmospheric pollution through a scrubber is a simpler way of analyzing various pollutants.

In relation to the test organism focus of this chapter, the acute tests consist of the exposure of young individuals to the samples during a period of 48 h. For the accomplishment of the test, the one recommended by the norm NBR 12.713 (ABNT 2009) was followed.

To perform a chronic test, the methodology developed by Brentano (2006) is presented, with some modifications. The method consists in exposing organisms to the sample and controls a representative period of their life cycle for 21 days to evaluate effects on the survival and reproduction of organisms.

The system adopted is the semi-static, in which the organisms are periodically transferred to a new test solution, maintaining the same characteristics of the initial one.

During the first week, the replacement occurs on the 7th day. In the next 2 weeks, twice a week. The objective is to always maintain the toxic potential of the sample, since environmental samples are poorly stable and can be rapidly degraded (Knie and Lopes 2004). At each replacement, new vials are prepared with sample and the individuals are transferred thereto with the aid of a Pasteur pipette with the tip cut off, because that the organisms are already in adolescent/adults phase, thus avoiding any possible damage to their body structure, dilution of the sample and stress on organisms.

To perform the chronic test, 10 plastic cups of 25 ml each are used for the control, plus 10 25 ml plastic cups for each sample. In each vial is added a test organism aged from 2 to 26 h.

The individuals are fed daily with the unicellular seaweed *Desmodesmus subspicatus*, following the same method of feeding the culture, during the first week of the test. In the subsequent 2 weeks, they are fed every 2 days and at the time of renewal of the medium.

The organisms are kept in the incubator chamber, in a photoperiod 16 h light/8 h dark and at a temperature 20 ± 2 ° C during the 21 days of the test. The observations were performed on the 7th day and thereafter two times a week, concomitantly with the renewal of the medium.

The parameters indicated for analysis are survival, primiparous age, and fecundity. Survival is measured as the number of surviving progenitors at the end of the 21-day trial. Primitive age is assessed by observing the age of the progenitors at the time of their first reproduction. The standard for the OECD *Daphnia magna* chemical test also advises the report of primiparous age, among other parameters (OECD 2012). Fertility is translated into the average number of newborns produced per female.

In order to perform the transgenerational test, the test is started in the same way as the chronic test. However, in order to analyze two more generations, starting the 14th day of the test, the specific methodology of transgenerational is started.

On the 14th test day, at the time of reading the test for sample replacement and counting of the organisms, it is necessary to remove five pups (of each solution to be tested, in the case of this research, five of the control and five of the sample), randomly chosen, each from a different mother, for continuity of the transgenerational test. To carry out the transfer, a Pasteur pipette with the cut tip is used, taking care again to avoid the dilution of the sample or to cause stress to the organisms.

The initial generation maintains the same routine of cleaning and counting of the organisms, until completing its 21 days of tests, because it is a chronic transgenerational test for all analyzed generations.

With the withdrawal of the 5 pups, they are individually placed in 50 ml plastic cups, containing 25 mL of sample, at the same concentrations of the initial generation, initiating a chronic test for generation 1, puppies of the initial generation, which will be submitted to the same methodology mentioned above, but with characteristics of an organism generated in a contaminated sample, and that is maintaining its life cycle under the same conditions.

With the completion of the 21-day assay of the initial generation, the organisms are discarded, keeping only generation 1 (puppies of the initial generation). By the time they are completing their 14th test day, 1 pup will be removed from each mother, and the chronic trial for generation 2 (generation 1 pups) will begin.

Thus, three generations of the test organism are chronically analyzed, being the initial generation 1 and 2. Throughout the assay the organisms are exposed to the same concentration of the sample/control solution. Below, Fig. 8.4 illustrates the operation of the assay.

The assays are considered valid in cases where the mortality in the negative control did not exceed 20% (OECD 2012).

For the analysis of the results of the transgenerational test, the parameters of longevity, average of fecundity per organism, and average of fecundity per posture of each generation are evaluated. It is sought to verify the concentration of nonobserved effect (CENO), being analyzed the same parameters of the chronic test, but in three generations.

The results of the generational toxicity tests should be indicative of the long-term effects of exposure to toxic substances. However, adaptation or extinction of populations exposed for several generations may occur (Paumen et al. 2008).

Prenatal exposure to environmental toxins (such as the one used in this trial) may alter the differentiation of primordial germ cells, which is initiated during fetal

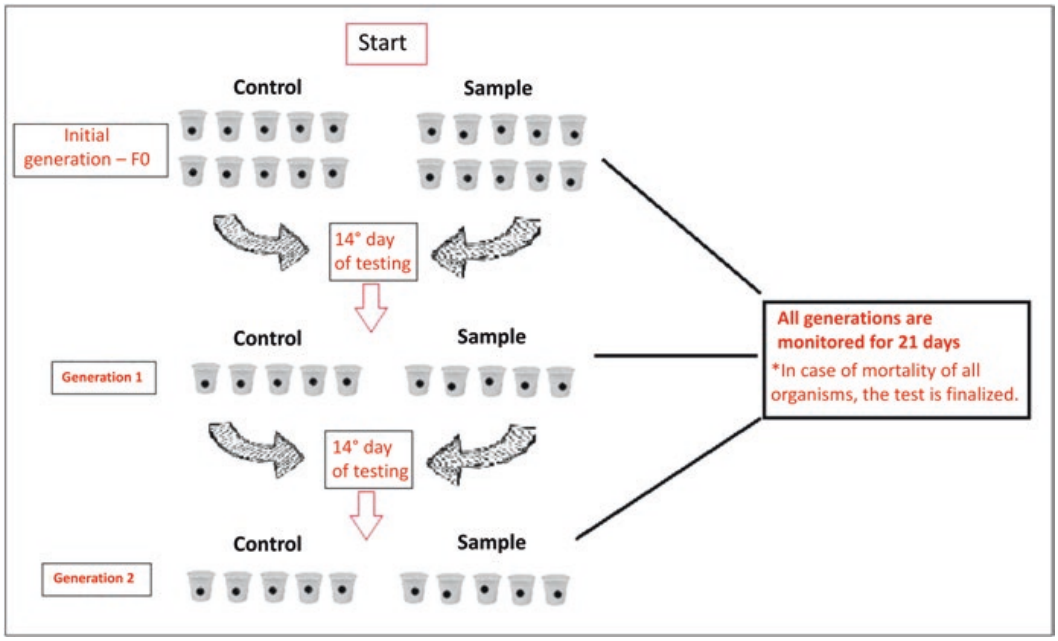

Fig. 8.4 Explanatory diagram of the transgenerational test

development, and induce transgenerational epigenetic disorders (Saitou and Yamaji 2012; Stouder and Paoloni-Giacobino 2010).

8.5 Case Study

The case study included samples and acute, chronic, transgenerational, and chemical analyses carried out in the city of Joinville (Brazil) (Fig. 8.5).

The city of Joinville concentrates a great part of economic and industrial activity, with emphasis on the metalworking, plastic, textile, metallurgical, chemical, and pharmaceutical sectors. The gross domestic product of Joinville is also one of the largest in the country, at around R $ 24,570,851.00 per year (IBGE 2017). With a population of 577,077 inhabitants, it has a per capita GDP of R $ 44,303.65 (IBGE 2017).

For the development of the work, a sampling point located in a private residence at the coordinates 26 ° 17′36.8 "S 48 ° 49'09.2" W was defined. This location was chosen because it is close to the industrial area, located at the top of a hill, thus avoiding physical barriers to the movement of air masses, besides being an easily accessible place to install the equipment, necessary maintenance in the period collection, and availability of electricity.

After collecting with a gas scrubber, as specified in item 3, the sample was sent to a certified laboratory and were analyzed HPA's, some heavy metals, and some pollutants with a high possibility of contamination in the region.

Fig. 8.5 Location of the municipality of Joinville in the State of Santa Catarina and the map of Brazil

In order to analyze the possible accumulation of the chemical elements analyzed, samples were taken on a temporal scale in a period of 24 h.

For the identification of pollutants, the laboratory was asked to analyze 26 compounds. In Table 8.1, these compounds are listed and the analysis method used by the contracted

laboratory is explained, as well as its result and limit of quantification. However, of the 26 compounds analyzed, only mercury and zinc were present in the sample in quantity above the limit of quantification used by the contracted laboratory.

Based on the results of the first collection with chemical analysis, it was decided to change the analyzed compounds, maintaining mercury and zinc, because their results were above the limit of quantification in the initial analysis, and also cadmium and lead. Cadmium is used in the treatment of fungicides, batteries, rubber

Table 8.1 Analytical results of the 26 compounds analyzed in a sample of the atmospheric air absorption system of the Boa Vista neighborhood (continued)

Compound analyzed	Result	Method	LQ (µg/L)
Acenaphthene	<0.01 µg/L	EPA 8270 C/D	0.01
Acenaphthylene	<0.01 µg/L	EPA 8270 C/D	0.01
Anthracene	<0.01 µg/L	EPA 8270 C/D	0.01
Benzene	<0.05 µg/L	POP 384 Ver. 2 e POP 385 Ver. 0	0.5
Benz(a)anthracene	<0.01 µg/L	EPA 8270 C/D	0.01
Benzo(a)pyrene	<0.01 µg/L	EPA 8270 C/D	0.01
Benzo(b)fluoranthene	<0.01 µg/L	EPA 8270 C/D	0.01
Benzo(ghi)perylene	<0.01 µg/L	EPA 8270 C/D	0.01
Benzo(k)fluoranthene	<0.01 µg/L	EPA 8270 C/D	0.01
Chrysene	<0.01 µg/L	EPA 8270 C/D	0.01
Dibenz(a,h)anthracene	<0.01 µg/L	EPA 8270 C/D	0.01
Ethylbenzene	<0.05 µg/L	POP 384 Ver. 2 e POP 385 Ver. 0	0.5
Phenanthrene	<0.01 µg/L	EPA 8270 C/D	0.01
Phenol	<0.01 µg/L	EPA 8270 D:2007 e EPA 3550 D: 2007	0.1
Fluoranthene	<0.01 µg/L	EPA 8270 C/D	0.01
Fluorene	<0.01 µg/L	EPA 8270 C/D	0.01
Indene(1,2,3-c,d)pyrene	<0.01 µg/L	EPA 8270 C/D	0.01
Naphthalene	<0.05 µg/L	POP 384 Ver. 2 e POP 385 Ver. 0	0.5
Pyrene	<0.01 µg/L		0.01
Toluene	<0.05 µg/L	POP 384 Ver. 2 e POP 385 Ver. 0	0.5
Xylenes	<1.5 µg/L	POP 384 Ver. 2 e POP 385 Ver. 0	1.5
Cadmium	<1 µg/L	SMWW, 22° edição. 2012, Method 3120 B	1
Lead	<1 µg/L	SMWW, 22° edition. 2012, Method 3120 B	1
Chrome	<1 µg/L	SMWW, 22° edition. 2012, Method 3120 B	1
Mercury	1.24 µg/L	SMWW, 22° edition. 2012, Method 3120 B	0,75
Zinc	25.3 µg/L	SMWW, 22° edition. 2012, Method 3120 B	1

treatment, pigment production, as well as in the electroplating industries, to give brightness and resistance to corrosion to objects (Moore and Ramamoorthy 1984) and lead (Nriagu 1988) is a toxic element of great environmental contamination due to its large industrial use, such as in the extractive, oil, accumulators, paints and dyes, ceramic, and military industries.

In addition, it was decided to include arsenic analyses, since it is a toxic chemical element and widely distributed in the biosphere (Watanabe and Hirano 2013) and can be found in the atmosphere (Farias et al. 2012) and that, in addition to known toxicity, it is considered a carcinogenic element (Newman and Unger 2002). As for aluminum, it has been considered the third most frequent chemical element in the earth's crust, with a frequency of 7.1% (Lindsay 1979), and an element commonly used by industries in the region.

In Table 8.2, it is possible to observe the results of the analyzed compounds per sample period, which presented a result higher than the limit of quantification of the method of analysis used by the contracted laboratory (Fig. 8.6).

In the 24-h test, concentrations of 31.7 µg.L-1 of aluminum and 18.7 µg.L-1 of zinc were detected in the sample, being rounded to perform this step of the research to 0.02 mg.L-1 and 0.03 mg.L-1, respectively.

From these data, acute, chronic, and transgenerational tests were performed for the test organism *Daphnia magna*.

With the accomplishment of the chemical-physical analyses, it was proved the possibility of capturing certain atmospheric pollutants through the method of analysis using a gas scrubber to collect these pollutants, being passed from air to water.

To perform the toxicological tests in the laboratory, it was necessary to prepare a sample similar to that which was collected using the gas scrubber. For this, the use of zinc chloride and aluminum chloride was chosen due to its high solubility.

However, due to the high toxicity of this mixture, for the transgenerational assay, 1/16 of the average of each compound was used, using in the test the concentrations of 1.25x10–3 mg.L-1 of zinc cole and 1875 × 10–3 mg.L-1 of aluminum chloride.

To perform the tests, pH and salinity were always within the values indicated in the standard, and there was no need to change the sample.

Table 8.2 Quantification of the zinc and aluminum compounds by period at the sampling point

	Concentration µg/L	
Collection duration (h)	Zinc	Aluminum
1	12.5	24.8
3	10.8	24.9
6	13.4	18.3
9	12	21.3
12	17.3	27.3
24	18.7	31.7

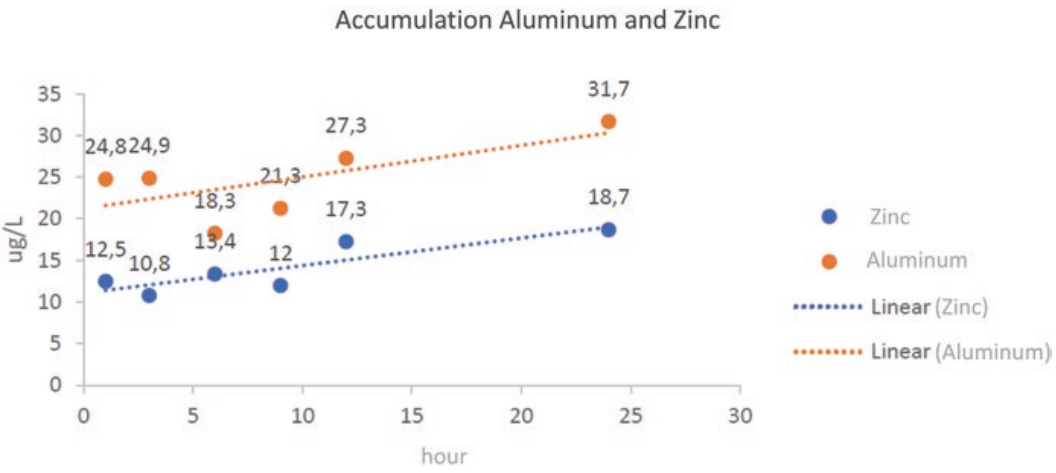

Fig. 8.6 Accumulation of zinc and aluminum in water sample of the absorption system, referring to the collection carried out in the Boa Vista neighborhood

In relation to the acute and conical toxicity test, the above sample did not present toxicity, but in generation 1, pups of the initial generation, at the end of the first week of the test, the control remained alive 100%, while the sample presented 100% mortality.

8.6 Conclusion

The use of the gas scrubber method adapted for the collection and analysis of air pollutants is valid and cost-effective, facilitating many research related to air quality standards.

The use of transgenerational toxicological assays is of great importance, since it is known that various substances do not present acute or chronic toxicity effects for certain compounds but may present transgenerational effects, as presented in this thesis, thus affecting an entire environment.

The choice of test organism to be used for a given test should be made with great care, for which there is no possibility of misinterpretation of possible results.

References

ABNT – Associação Brasileira de Normas Técnicas (2009) NBR 12.713: Ecotoxicidade aquática – Toxicidade aguda – Método de ensaio com *Daphnia* spp (Crustacea, Cladocera). Rio de Janeiro

ABIQUIM (1999) Comissão de Economia. A Competitividade da Indústria Química Brasileira, São Paulo

Amaral BS (2016) Perfil da concentração de poluentes regulamentados e não regulamentados emitidos a partir da combustão de biocombustíveis originários de um motos de ciclo diesel. Tese de Doutorado. Pós-Graduação em Química da PUC-Rio

Antunes SC, Castro BB (2017) Pulgas-de-água (Daphnia spp.). Rev Ciência Elem V5(4):050

Artal MC, Almeida G, Caloto-Oliveira A et al (2013) Toxicity assessment of an effluent derived from an inactivated uranium mine: the Poços de Caldas (Brazil) example. J Braz Soc Ecotoxicol 8:35–40

Becker D, Van Damme K, Turner E et al (2014) The genomics of Cladoceran physiology. In: Physiology of the Cladocera. Academic Press, London, pp 235–258

Brentano DM (2006) Desenvolvimento e aplicação do teste de toxicidade crônica com *Daphnia magna*: avaliação de efluentes tratados de um aterro sanitário. Dissertação (Mestrado em Engenharia e Ambiental) – Universidade Federal de Santa Catarina, Florianópolis

Campos JFFAF (2015) Avaliação da toxicidade das águas do Rio Cubatão (Norte) – Joinville, SC, com base em ensaios ecotoxicológicos com *Daphnia magna*. Dissertação (Mestrado em Ecologia) – Universidade do Minho – Escola de Ciências

Carneiro RMA (2004) Bioindicadores vegetais de poluição atmosférica: uma contribuição para a saúde da comunidade. Dissertação (Enfermagem em saúde pública). Escola de enfermagem de Ribeirão Preto da Universidade de São Paulo

Costa CR, Olivi P, Botta CMR et al (2008) A toxicidade em ambientes aquáticos: discussão e métodos de avaliação. Química Nova 31:1820–1830

Covich AP, Thorp JH, Rogers DC (2010) Introduction to the subphylum Crustacea. In: Thorp JH, Covich AP (eds) Ecology and classification of North American freshwater invertebrates, 3rd edn. ScienceDirect

Daumas RP, Mendonça S, Azevedo G et al (2004) Poluição do ar e mortalidade em idosos no município do Rio de Janeiro: Análise de série temporal. Caderno de Saúde Pública, Rio de Janeiro

Dodson SL, Cáceres CE, Rogers DC (2010) Cladocera and other branchiopods. In: Thorp JH, Covich AP (org). Ecology and classification of North American freshwater invertebrates. 3° edn. Academic Press, pp 774–828. Elsevier

Ebert D (2005) Introduction to Daphnia Biology. In: Ecology, Epidemiology, and Evolution of Parasitism in Daphnia. Bethesda: National Library of Medicine

Enserink L, Luttmer W, Maas-Diepeveen H (1990) Reproductive strategy *of Daphnia magna* affects the sensitivity of its progeny in acute toxicity tests. Aquat Toxicol 17:15–26

Farias JS, Milani MR, Niencheski LFH et al (2012). Especiação Química De Arsênio Inorgânico No Estuário Da Laguna Dos Patos (Rs, Brasil). Química Nova 35(07):1401–1406

Ferreira CJS (2003) Impacto automotivo em populações de *Ctenomys minutus* na planície costeira do RS: avaliação do teor de metais tóxicos e medição de lipoperoxidação**.** Universidade Federal do Rio Grande do Sul

Filho AS (2010) Proposta de um método para avaliação da toxicidade aguda de efluentes gasosos. Trabalho de Conclusão de Curso de Graduação em Engenharia Sanitária e Ambiental. Universidade Federal de Santa Catarina – UFSC, Florianópolis

Gabsi F, Glazier DS, Hammers-Wirtz M et al (2014) How do interactive maternal traits and environmental factors determine offspring size in *Daphnia magna*? Int J Limnol 50(1):9–18

Galvão AC (2011) Estudo experimental da solubilidade do metano e do dióxido de carbono em glicóis a diferentes temperaturas e pressões e modelagem da solubilidade aplicando o potencial químico. Tese de doutorado apresentada à Faculdade de Engenharia Química. Universidade Estadual de Campinas. São Paulo

Han X, Naeher LPA, L P A (2006) Review of traffic-related air pollution exposure assessment studies in the developing world. Environ Int 32(1):106–120

Hoshi J, Amano S, Sasaki Y et al (2008) Investigation and estimation of emission sources of 54 volatile organic compounds in ambient air in Tokyo. Atmospheric Environment 42(10):2383–2393

IBGE – Instituto Brasileiro de Geografia e Estatística (2017) Consulta de área, população e dados básico do município. Disponível em: https://www.ibge.gov.br/

Knie JL, Lopes EWB (2004) Testes ecotoxicológicos: métodos, técnicas e aplicações: FATMA/GTZ. Florianópolis

Lindsay WL (1979) Chemicai equilibria in soils. Wiley Interscience, New York, 499p

Loureiro C, Gonçalves F, Pedrosa MA et al (2013) Efeitos de alterações ambientais em populações de invertebrados: uma abordagem prática. CAPtar 4:42–56

Martins EM, Arbilla G, Bauerfeldt GF et al (2007) Atmospheric levels of aldehydes and BTEX and their relationship with vehicular fleet changes in Rio de Janeiro urban area. Chemosphere 67(10):2096–2103

Moore JW, Ramamoorthy S (1984) Heavy metals in natural waters. Springer, New York, 328p

Newman C, Unger MA (2002) Fundamentals of ecotoxicology, 2nd edn. CRC, Boca Raton

Nriagu JO (1988) A silent epidemic of environmental metal poisoning? Environ Pollut 50:139–161

OCDE – Organização para a Cooperação e Desenvolvimento Econômico (2012) *Daphnia magna* reproduction test. OECD guidelines for the testing of chemicals, vol 211, pp 1–21. OECD Publishing

Paumen ML, Steenbergen E, Kraak MS et al (2008) Multigeneration exposure of the springtail *Folsomia candida* to phenanthrene: from dose−response relationships to threshold concentrations. Environ Sci Technol 42(18):6985–6990, 15 set. American Chemical Society (ACS). https://doi.org/10.1021/es8007744

Saitou M, Yamaji M (2012) Primordial germ cells in mice. Cold Spring Harb Perspect Biol 4(11):1

Seinfeld JH, Pandis SN (1998) Atmospheric chemistry and physics: from air pollution to climate change. Wiley, New York

Silva J, Simioni AA, Groff M et al (2007) O ensaio cometa na avaliação da genotoxicidade induzida por poluentes atmosféricos utilizando como biomonitor o molusco *Cantareus aspersus* (Müller, 1774). J Braz Ecotox 2(1):45–51

Singh HB, Salas L, Viezee W et al (1992) Measurement of volatile organic chemicals at selected sites in California. Atmos Environ 26(16):2929–2946

Stouder C, Paoloni-Giacobino A (2010) Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm. Reproduction 139(2):373–379, 1 fev

Watanabe T, Hirano S (2013) Metabolism of arsenic and its toxicological relevance. Arch Toxicology (87); 969–979

WHO – World Health Organization (2018) 7 million premature deaths annually linked to air pollution. Access in: outubro, Available in: http://www.who.int/mediacentre/news/releases/2014/air-pollution/en

Zagatto PA (2014) Ecotoxicologia. In: Zagatto PA, Bertoletti E (eds) Ecotoxicologia Aquática: Princípios e Aplicações, 2ª edn. RiMa, São Carlos, pp 1–13

Chapter 9
Ecotoxicological Studies of Metal Pollution in Sea Turtles of Latin America

Camila Miguel and Marcelo Renan de Deus Santos

9.1 Introduction

Metals are natural components of rocks and soil. Natural processes like weathering and erosion are responsible for their entering into water bodies. However, rapid urban development and increases in fertilizer and pesticide use, as well as industrial activities such as mining and smelting, fossil fuel use, and many forms of waste disposal, have drastically raised the amount of such elements in the environment (Athar and Vohora 1995). Estuaries and coastal regions generally act as the final receiving body of these substances, and their increased concentrations tend to accumulate, concentrate, and biomagnify through the food chain since the organisms are not able to completely eliminate the metals absorbed (Storelli et al. 2005; Camacho et al. 2013).

Some metals, such as Al, As, Co, Cr, Cu, Se, and Zn, play a crucial role in animal metabolism and growth pathways, but deviations above the normal range result in metal toxicity, while concentrations below the range can also be detrimental to the functioning of the organism (Keller et al. 2006). Other metals (Pb, Hg, Cd, As) have no function in the organism, and their accumulation can pose a threat to the wildlife that interacts with them. This interaction occurs through ingestion, inhalation, and/or absorption. However, the most common route for these elements is from dietary intake (Anan et al. 2001). In sea turtles, the bioaccumulation of heavy metals is

C. Miguel (✉)
Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, RS, Brazil

Instituto Marcos Daniel (IMD), Vitória, ES, Brazil
e-mail: camila.miguel@acad.pucrs.br; www.imd.org.br/

M. R. de Deus Santos
Instituto Marcos Daniel (IMD), Vitória, ES, Brazil

Universidade Vila Velha (UVV), Vitória, ES, Brazil
e-mail: www.imd.org.br/

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_9

critical, due to their long life spans, high daily consumption rates, and extended periods of time spent in coastal foraging grounds near sources of pollution.

Most sea turtle populations around the globe are listed as threatened or endangered (IUCN 2019), facing both natural and anthropogenic stressors linked to dramatic ontogenetic life cycle changes. Although physically robust and able to accommodate severe physical damage, sea turtles appear surprisingly susceptible to biological and chemical insults. The adverse effects include compromised physiology, chronic stress, impaired immune function, and an increase in disease susceptibility, like fibropapillomatosis (Aguirre et al. 1994; Aguirre and Lutz 2004).

On the other hand, sea turtles present several particular advantages as indicators of heavy metal pollution (Lam et al. 2004). Sea turtles have a wide distribution range during their life cycle, which begins in the terrestrial environment, and then moving to a pelagic phase and returning to coastal areas to feed, they may serve as a good proxy for overall ecosystem health (Kampalath et al. 2006). Furthermore, the development within a wide range of environments increases the possibility of interacting with anthropogenic impacts including pollution, fishing, or changing environments (Marcovaldi et al. 2006; Gilman et al. 2007).

In addition to their high mobility throughout different ecosystems, the long life history of sea turtles enables an extended exposure period to contaminants, both spatially and temporally. As they occupy different trophic levels, from herbivorous to carnivorous, they can provide a fairly comprehensive profile of contamination throughout the food chain (Anan et al. 2001). Besides that, levels of metals in sea turtles may reveal a better picture of hazards to humans than measurements taken in the physical environment, plants, or invertebrates (Anan et al. 2002).

Sea turtles are also considered sentinel species (Aguirre and Lutz 2004) since they suffer from disease processes related to environmental conditions as the worldwide distributed fibropapillomatosis, a skin cancer associated with a herpesvirus 5 (ChHV5) which affects especially green turtles.

Thus, sea turtles can be useful as sentinel species and bioindicators for diverse pollutants, and they can help us to understand the risk not only to the species themselves but also to the ecosystem at large.

9.2 Sea Turtles of Latin America

Latin America contains 13 dependencies and 20 countries, which cover an area that stretches from the northern border of Mexico to the southern tip of South America, including the Caribbean. Of the seven species of sea turtles, six are found in the coast of Latin America; these include *Chelonia mydas* (and *Chelonia mydas agassizii*), *Eretmochelys imbricata*, *Caretta caretta*, *Lepidochelys olivacea*, *Lepidochelys kempii*, and *Dermochelys coriacea*.

Many coastal areas are important foraging and nesting sites for these species. However, some of them are exposed to environmental pollution via anthropogenic (industries, mining activities, oil and gas) or natural sources (naturally enriched soil

and sediment, runoff, and atmospheric deposition) which could affect the development, health, reproduction, and survival of these animals.

Sea turtles are also an important food source in many coastal communities, because they can provide an easy-access source of protein and income, especially for low-income families or communities. Moreover, hunting sea turtles is a traditional practice for certain indigenous populations in Guyana, Mexico, and Nicaragua, for whom turtles are culturally important. The collection and sale of eggs and/or adult turtles has, with limitations, been allowed in certain countries, including Costa Rica, Guatemala, and Honduras (Ross et al. 2017). However, little is known about the potential exposure to heavy metal intake through the consumption of sea turtles and their eggs.

Studies regarding the toxicology of heavy metals in sea turtles from Latin America have assessed five species, but *Chelonia mydas* and *Lepidochelys olivacea* are more highlighted (Fig. 9.1). It differs from the studies made all over the world, where the most studied species are *Chelonia mydas* and *Caretta caretta* (Cortés-Gomes et al. 2017). Due to their toxicity risk, cadmium, lead, and mercury are among the most studied metals.

The researchers have identified a range of contaminants in various sea turtle tissues (liver, kidney, muscle, adipose tissue, heart, brain, bone, salt gland), but noninvasive and nonlethal methods of monitoring are crucial to allow a large number of samples, including live and healthy animals. That is why some authors have aimed assessing the use of blood, scutes, and/or eggs as a predictive matrix for the pollutants metabolized by the organism.

9.3 Biomarkers of Ecotoxicological Assessment

The term biomarker refers to body fluids, cells, or tissues and behavioral, physiological, or energetic responses of organisms that may indicate the presence of or exposure to contaminants (Livingstone 1993). In the following section, the biomarkers used in sea turtle toxicological studies are described and the publications about them are pointed out.

9.3.1 Blood

The use of blood in assessing the exposure to contaminants is a good option because it is quick and easy to collect and the animals can return to the environment immediately following venipuncture. Blood has been recognized as an indicator of recent exposure to pollutants in sea turtles, since it is the first means of transport of these elements throughout the body and before targeting organs (Day et al. 2005).

Many evidences indicate that the route of entry of pollutants in sea turtles occurs mainly through food intake. Thus, blood samples could provide an approach on

Fig. 9.1 Location of the studies presented in this review. Different symbols were used to represent studies that sampled blood, tissues, and eggs. Different colors were used to represent each species of sea turtles (*Caretta caretta, Cc; Chelonia mydas, Cm; Dermochelys coriacea, Dc; Eretmochelys imbricata, Ei; Lepidochelys olivacea, Lo*)

contamination from the site where turtles feed. During the nesting season, sea turtles have rarely been observed foraging; nevertheless, females seem to ingest at least a significant volume of water to decrease their body temperature in warm waters of nesting tropical beaches (Southwood et al. 2005) and to ensure egg production (albumen is mainly composed of water) (Wallace et al. 2006), which could be related to their contamination during this period. Besides allowing the determination of different chemicals, blood can also be used to assess clinical parameters that could be adversely modulated by the pollutants (Camacho et al. 2013).

In Latin America 20 studies used blood as a biomarker (Table 9.1). The most investigated species is *Lepidochelys olivacea*, with nine publications from Mexico. There are four publications on *Chelonia mydas* (three from Brazil and one from Chile), one publication on *Chelonia mydas agassizii* from Mexico, three publications on *Eretmochelys imbricata* (two from El Salvador and one from Brazil), and two publications on *Caretta caretta* from Mexico and one publication on *Dermochelys olivacea* from French Guiana.

Only ten publications correlated the levels of contaminants with a toxicological endpoint. The majority of these publications investigated health parameters (Ley-Quiñónez et al. 2017; Álvarez-Varas et al. 2017; Tauer et al. 2017; Cortés-Gómez et al. 2017, 2018a), three publications correlated with oxidative stress (Labrada-Martagón et al. 2011; Silva et al. 2016; Cortés-Gómez et al. 2018b), two publications with fibropapillomatosis (Silva et al. 2016; Prioste 2016), and one with carapace asymmetry (Cortés-Gómez et al. 2018c).

In Mexico, Ley-Quiñónez et al. (2017) investigated associations among metals and biochemical parameters from 22 *Caretta caretta* captured in Bahía Magdalena, finding significant positive correlations between Cd, As, and Mn and ALP. The authors argument that this increase in ALP activity may be caused by Cd and As accumulation suggesting a possible liver damage. Other possibility may occur due to an incorrect excretion process of these elements, resulting in the development of pathologies.

However, they highlight that the explanation of such correlations remains speculative, due to probable involvement of immune responses and the lack of baseline information regarding the toxicological effects of metal accumulation in sea turtle and their immune response to it. Cortés-Gómez et al. (2018a) also found significant relationships between metals and biochemical substances in *Lepidochelys olivacea* from Oaxaca. In this study, Sr and As had positive correlations with AST and urea, while As and Cd had negative correlations with glucose. Esterase activity (EA) was the parameter with most negative correlations (with Pb, Ti, As, Cr, and Se), which reinforce the results of other researchers regarding the possible inhibition of EA by metals. The authors concluded that the several correlations detected between the elements analyzed and biochemical parameters indicate that these contaminants may have a negative effect on the health of these turtles.

Cortés-Gómez et al. (2017) also examined the concentrations of EA and cortisol in *Lepidochelys olivacea* from a Mexican population and evaluated the possible correlations with metals. The authors found significant correlations between EA and important metals, such as Cd, Pb, Ti, and Al, and between cortisol and Sr, Se, and

Table 9.1 Metal levels (mean ± SD, μg.g^{-1} wet weight) in blood of sea turtles at different areas in Latin America

Study site[a]	Sp	n	CCL	Capture	Method	Essential metals						Nonessential metals					Source
						Cr	Cu	Mn	Ni	Se	Zn	As	Cd	Hg	Pb	Sr	
Mexico – BS	*Cc*	22	69.0	DV	ICP-AES	–	2.8 ± 0.3	0.6 ± 0.7	1.5 ± 1.9	6.14 ± 1.4	44.8 ± 2.7	4.0 ± 1.2	1.8 ± 0.4	–	–	–	Ley-Quiñónez et al. (2011)
Brazil – CE	*Cm*	24	48.4	IC	ICP-MS	–	–	–	–	29.99 ± 126	7.58 ± 4.17	0.93 ± 0.90	0.016 ± 0.016	0.009 ± 0.008	0.029 ± 0.027	–	Prioste (2016)[b]
Brazil – ES	*Cm*	68	42.8	CN	ICP-MS	–	–	–	–	1.19 ± 1.85	21.53 ± 46.08	2.16 ± 5.15	0.009 ± 0.011	0.010 ± 0.017	1.12 ± 2.04	–	(Prioste (2016)[b]
Brazil – PE	*Cm*	31	72.0	I	ICP-MS	–	0.757	0.061	–	0.424	14.05	0.366	0.014	–	0.027	–	Prioste et al. (2015)[b]
Brazil – PE	*Cm*	71	68.7	DV	ICP-MS	–	–	–	–	0.331 ± 0.37	8.31 ± 4.26	5.04 ± 39.7	0.010 ± 0.009	0.0002 ± 0.0002	0.027 ± 0.020	–	Prioste (2016)[b]
Brazil – SP	*Cm*	70	39.3	IC	ICP-MS	–	–	–	–	1.23 ± 1.96	6.94 ± 5.79	2.17 ± 3.97	0.012 ± 0.013	0.022 ± 0.037	0.034 ± 0.029	–	Prioste (2016)[b]
Brazil – SP	*Cm*	13	37.0	DV/IC	AAS	–	0.92	–	8.94	–	0.68	–	0.078	–	0.954	–	Silva et al. (2016)[c]
Chile – AT	*Cm*	7	66.5	EN	AAS	–	2.26 ± 0.10	–	–	–	–	–	–	–	1.11 ± 0.06	–	Álvarez-Varas et al. (2017)
Mexico – BS	*Cm*	42	67.2[d]	FN	FAAS	–	–	–	76.47	1.59	13.92	–	0.06	–	–	0.28	Labrada-Martagón et al. (2011)
Mexico – BS	*Cm*	14	61.2[d]	FN	FAAS	–	–	–	73.30	1.81	13.58	–	0.03	–	–	0.18	Labrada-Martagón et al. (2011)
Mexico – SO	*Cma*	12	67.2[d]	DV/FN	ICP-OES	–	1.71 ± 0.73	1.22 ± 0.99	1.03 ± 1.01	7.66 ± 3.19	63.58 ± 17.0	–	0.99 ± 0.35	–	–	–	Ley-Quiñónez et al. (2013)
French Guiana – SLM	*Dc*	78	160	DN	ICP-MS/ DMA	–	1.34 ± 0.28	–	–	9.98 ± 0.05	11.10 ± 0.28	–	0.08 ± 0.03	0.011 ± 0.003	0.18 ± 0.05	–	Guirlet et al. (2008)
Brazil – PE	*Ei*	18	–	DN	XRF	0.085 ± 0.02	0.951 ± 2.005	–	1.711 ± 0.35	–	–	–	–	0.027 ± 0.012	0.729 ± 0.488	–	Simões (2016)[b]
ElSalvador–US	*Ei*	28	84.0	DN	GFAAS/ AAS	–	–	–	–	–	–	0.24 ± 0.38	–	0.019 ± 0.010	0.06 ± 0.009	–	Bardales and Benavides (2016)
ElSalvador–US	*Ei*	66	84.9	DN	GFAAS	–	–	–	–	–	–	0.245	–	0.008	0.045	–	Tauer et al. (2017)

Mexico – OA	*Lo*	17	27.98	DB	ICP-OES	0.5 ± 0.91	1.0 ± 0.9	–	0.06 ± 0.06	7.1 ± 4.5	–	0.9 ± 0.6	7.0 ± 2.0	–	0.08 ± 0.1	1.3 ± 1.8	Cortés-Gómez et al. (2018c)
Mexico – OA	*Lo*	25	66.4	DA	GFAAS/ CV-AAS	–	0.47 ± 0.08	–	0.56 ± 0.26	–	11.68 ± 0.94	–	0.09 ± 0.04	0.001 ± 0.000	0.019 ± 0.03	–	Páez-Osuna et al. (2010a, b, 2011)[c]
Mexico – OA	*Lo*	41	65.50	DA	ICP-OES	–	0.61 ± 0.11	0.59 ± 0.10	0.04 ± 0.02	5.75 ± 2.48	10.55 ± 3.68	1.16 ± 0.70	0.17 ± 0.08	–	0.02 ± 0.01	–	Cortés-Gómez et al. (2014)
Mexico – OA	*Lo*	44	65.89	DN	ICP-OES	–	–	–	–	6.54 ± 3.10	10.88 ± 2.33	1.05 ± 0.53	0.15 ± 0.06	–	0.07 ± 0.04	1.03 ± 0.29	Cortés-Gómez et al. (2017)
Mexico – OA	*Lo*	20	–	DA	ICP-OES	0.73 ± 0.10	0.56 ± 0.35	–	0.06 ± 0.03	7.26 ± 5.52	8.06 ± 4.70	1.41 ± 1.62	0.13 ± 0.08	–	0.01 ± 0.01	1.02 ± 0.62	Cortés-Gómez et al. (2018b)
Mexico – OA	*Lo*	100	–	DA	ICP-OES	0.17 ± 0.07	0.52 ± 0.2	0.41 ± 0.12	0.07 ± 0.07	6.72 ± 3.0	7.7 ± 2.4	1.27 ± 0.9	0.12 ± 0.05	–	0.02 ± 0.01	1.02 ± 0.45	Cortés-Gómez et al. (2018a)
Mexico – SI	*Lo*	19	63.15	–	ICP-AES	–	1.02	2.77	1.35	11.15	37.12	2.44	1.33	–	BDL	–	Zavala-Norzagaray et al. (2014)

As arsenic, *Cd* cadmium, *Cr* chromium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Ni* nickel, *Pb* lead, *Se* selenium, *Sr* strontium, *Zn* zinc, *Cc Caretta caretta*, *Cm Chelonia mydas*, *Cma Chelonia mydas agassizii*, *Dc Dermochelys coriacea*, *Ei Eretmochelys imbricata*, *Lo Lepidochelys olivacea*, *CN* casting net, *DA* during "arribada," *DB* dead in the beach, *DN* during nesting, *DV* diving, *I* intentionally, *IC* incidental capture, *EN* entanglement nets, *FN* fishing net, *AAS* atomic absorption spectrophotometry, *CV-AAS* cold vapor atomic absorption spectrometry, *CVAFS* cold vapor atomic fluorescence spectrometry, *DMA* direct mercury analysis, *FAAS* flame atomic absorption spectrophotometry, *GFAAS* graphite furnace atomic absorption spectroscopy, *ICP-AES* inductively coupled plasma-atomic emission spectrometry, *ICP-OES* inductively coupled plasma-optical emission spectrophotometry, *ICP-MS* inductively coupled plasma-mass spectrometry, *XRF* X-ray fluorescence spectrometer, *BDL* below detection limits

[a]Country-state/province/department abbreviation

[b]$\mu g.mL^{-1}$

[c]Results originally published in dry weight transformed into wet weight using the humidity percentage reported by Guirlet et al. (2008) (blood, 80%)

[d]Original publication in straight carapace long (SCL), transform into CCL using the following formula: $Cm = -0.028 + 1.051$ (SCL) (Bjorndal and Bolten 1989)

As, which has already been reported. According to them, it could be expected that during an acute stress episode, cortisol and EA levels increase and present positive correlation. However, a strong negative correlation between EA and cortisol was observed in this study. The authors correlated this information with previous studies in the same population (Cortés-Gómez et al. 2014) and assumed that these animals were chronically exposed to different inorganic elements, such as Pb and Cd. They also suggested that a prolonged period under a stressful condition generated by pollution drives to a higher consumption of esterase and to a prolonged cortisol elevation, which could explain the results they found. Therefore, they emphasize the need of further research to clarify this topic.

Indicators of oxidative stress (antioxidant enzyme activities and lipid peroxidation levels) and levels of metals and organochlorine pesticides (OC) were evaluated by Labrada-Martagón et al. (2011) along with body condition of *Chelonia mydas* caught alive by monofilament fishing nets. Turtles were captured in Punta Abreojos (PAO) and Bahía Magdalena-Almejas (BMA), Mexico.

The results showed higher concentrations of Si and Cd in turtles captured in PAO, while turtles from BMA had higher levels of OCs. Additionally, sea turtles captured in PAO had enzymatic antioxidants mostly correlated to the concentration of pesticides, while in individuals from BMA, the antioxidant enzyme activities were correlated with the trace element concentrations. The authors attributed these regional differences to the influence of habitat conditions. The location of PAO and its direct connection to the Pacific Ocean could explain the concentration of trace elements and higher frequency of OC residuals in sea turtles, in contrast to the inland channels of BMA. However, the highest concentration of OCs found in the sea turtles from BMA, compared to PAO, could be the result of the agriculture activity developed in the last 50 years in the region.

Cortés-Gómez et al. (2018b) also related the transcription rate and/or enzymatic activities of some antioxidant enzymes (SOD, CAT, and GR) and metallothionein (MT) to metals in blood samples and tissues (liver and kidney) of 40 *Lepidochelys olivacea* from Mexico. Gene expression of *sod*, *cat*, and *gr* was higher in blood than the liver and kidney, which could be influenced by the fact that tissues were collected from dying turtles. However, most of the significant correlations of gene expression and enzyme activities were found in the liver. This must be related to the role of this structure as the first filter organ, with all metals passing through it before going to their target organs and accumulate.

Additionally, the authors found very high Cd levels and several positive relationships of *sod*, *cat*, and *gr* gene expression in different tissues. This could mean that the turtles were responding to the metals inducing production of ROS and damage through high transcription levels of these antioxidant enzymes. They argued that multiple positive relationships with GR seem to be part of the compensatory effect of GR due to the decrease of SOD production against the high and chronic exposure to certain xenobiotics, such as Cd. On the other hand, CAT seems to be not used much, and glutathione detoxification of H_2O_2 may be more important in this species.

Despite the high Cd concentrations found in this population, the authors didn't find significant relationships between any tissue with metallothionein gene expression. These results, along with very high Cd concentrations and a negative relationship with Cu, lead the authors to consider some kind of disruption in *mt* gene expression in these turtles.

Environmental contaminants have been proposed as one of the possible factors contributing to the development of fibropapillomatosis (FP) in sea turtles by reducing immune function (Balazs 1991). Some studies have already found correlations with FP prevalence and pollution, which justifies the belief that there may be a relationship, but more studies are still need to elucidate this possible correlation.

In Brazil, Silva et al. (2016) determined the concentrations of some metals (Ag, Cd, Cu, Fe, Ni, Pb, and Zn) in the blood of 27 *Chelonia mydas*, 14 with FP and 13 without FP, which were capture alive by diving and pound nets at Ubatuba, São Paulo State, Brazil. Green sea turtles were grouped and analyzed according to the severity of tumors, and the levels of metals were compared with parameters of oxidative stress, cholesterol concentration, and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) activity.

The results indicate that the reduced concentration of serum cholesterol observed in green sea turtles afflicted with FP is associated with an inhibition of HMGR activity induced by increased concentrations of Cu and Pb. They also suggest that oxidative stress induced by elevated concentrations of Fe and Pb (higher LPO levels) may be involved in the etiology and development of the disease.

Prioste (2016) also evaluated the concentrations of metals in 233 blood samples and 488 tissues samples of *Chelonia mydas*, along the Brazilian coast and correlated with FP. The results obtained from tissues samples showed that green turtles with signs of fibropapillomatosis present lower As and Se levels in all analyzed organs and higher Pb in the liver, kidneys, and bone tissues. The higher Pb levels are in agreement with the results found by Silva et al. (2016). The studies emphasize that samples obtained from the same population with reduced biological variability (gender and age) provide the most reliable results in terms of biochemical parameters of healthy individuals and disease response.

9.3.2 Tissues

Tissues are widely used as biomarkers. The contaminants found in most tissues of sea turtles (except blood) tend to reflect their foraging sites, and the distribution of metals among organs is influenced by both duration and concentration of exposure.

Since sea turtles are listed from vulnerable to critically endangered (IUCN 2019), it is difficult to obtain proper licenses to collect specimens, leading to studies that rely on carcasses. Sometimes samples are from animals that died in rehabilitation centers or stranded on the beach, which can influence the results when comparing with healthy animals. The advantage of using tissues is that a large mass

can be collected and stored for a long time, allowing many analyses to be made (Keller et al. 2014).

In Latin America, majority of the studies were carried out in Brazil and Mexico (eight and seven studies, respectively), and the most analyzed tissues were the liver and kidney, followed by the muscle (Table 9.2). The highest concentrations of nonessential metals in the liver, kidney, and muscle were found in Brazilian *Chelonia mydas* for Hg (Bezerra et al. 2015) and in Mexican *Lepidochelys olivacea* for Pb (Frías-Espericueta et al. 2006) and Cd (Cortés-Gómez et al. 2018b, c).

In Brazil, Barbieri (2009) evaluated the concentrations of metals in the liver and kidney of 30 *Chelonia mydas* (15 adults and 15 juveniles). The animals were found stranded along the Cananéia estuary, in the state of São Paulo. The most striking feature of the study was the organotropism found, with Cd levels being higher in the kidney and Cu in the liver, while Mn, Ni, and Pb concentrations were not significantly different between organs. The same pattern was found by Macêdo et al. (2015) in *C. mydas* from Bahia state. The authors likewise noticed significant differences in the liver between adults and juveniles regarding Cd, Pb, Cu, and Ni levels. Adult livers had higher concentrations of these metals than juveniles. Since Cu and Ni can be classified as essential, it is possible that there is a metabolic regulation of them, whereas Cd and Pb are nonessential metals and its accumulation must be related to aging.

Silva et al. (2014) collected tissue samples from 29 juvenile *Chelonia mydas* found stranded along the southern coast of Brazil (Rio Grande do Sul State). Gonads were histologically analyzed for sex identification, resulting in nonsignificant differences between males and females in relation to metal contamination. A positive correlation was observed between nonessential (Ag, Cd, and Pb) and essential (Cu or Zn) metals in the liver and kidney. Silva et al. suggested that this correlation is likely due to an induced metallothionein synthesis induced by Zn and/or Cu to protect the tissue against the toxic effect of nonessential metals. The authors also found organotropism between metals evaluated with highest levels of Cu and Ag detected in the liver and Pb, Cd, and Zn in the kidney. Silva (2011) sampled tissues of *Chelonia mydas* in the same region and had similar results. The presence of high levels of Cd in the kidney and Hg in the liver is highlighted. Considering the results from both studies, it is clear that the populations of *C. mydas* are under heavy anthropogenic pressures in the coastal regions of Rio Grande do Sul State.

Andreani et al. (2008), like the aforementioned studies, found clear organotropism in tissues of *Chelonia mydas* from Tortuguero National Park, Costa Rica. The concentrations of Fe, Cu, and Mn were greater in the liver, whereas Cd was accumulated preferentially in the kidney. Pb did not show any clear tissue distribution pattern. The authors also evaluated hepatic and renal metallothionein (MT) as a biomarker of environmental metal exposure and compared the results obtained for *Chelonia mydas* with samples from *Caretta caretta* collected in the Mediterranean Sea. Metallothionein concentrations were higher in green than in loggerhead turtles. In addition, positive correlations were found between Cu and Cd concentrations and Cu–MT and Cd–MT in liver and kidney in both species, suggesting a pivotal role of MT in metal storage and detoxification.

They found significant differences between the two species. Green turtles had higher Cu and Cd levels in the liver and kidney and Fe concentrations in the liver. On the other hand, loggerhead turtle had higher Zn and Fe levels in the kidney. Andreani et al. attribute the variability in metal concentrations to differences in food habit. Food is probably the main source of exposure to trace elements. Certainly, feeding mainly on cephalopods results in higher Cd concentrations in loggerhead turtles. By contrast, green sea turtles are herbivorous and feed on macroalgae. Algae have the capacity to accumulate trace metals several thousand times higher than the concentration in sea water, so the foraging habits of this species could influence the high Cu concentration found in the liver. The authors emphasized that besides diet composition, age and gender could be important factors affecting metal accumulation in tissues.

Gardner et al. (2006) also found higher levels of some metals in *Chelonia mydas* compared to *C. caretta*, *L. olivacea*, and *E. imbricata* that died by incidental fisheries in the northwestern coast of Mexico. Metal concentrations in the liver of sea turtles from Baja California did not vary among the species. In the kidney, *C. mydas* had greater Zn concentrations as compared to the other species and greater Ni concentrations than *L. olivacea*. In adipose, the concentration of Zn in *C. mydas* was also greater than *L. olivacea*. The authors also attributed the higher exposure of these elements in *Chelonia mydas* to their food habits.

To verify the influence of anthropogenic activities in coastal areas, Bezerra et al. (2015) compared Hg concentrations in tissues of *Chelonia mydas* from two foraging grounds in the northeast coast of Brazil (Ceará and Bahia states). In Ceará, the study area is a nearly pristine region, while in Bahia it is located on an industrial site with chemical and petrochemical activities. They also evaluated the food items from both areas. The results showed significant differences among the liver and muscle. Hg concentration was higher in the liver and lower in the muscle; the kidney and scutes had median values. Comparing the two areas, they found similar food preferences in specimens from both areas, but liver Hg concentrations were significantly higher in green turtles from Bahia compared to Ceará. These variations in the amount of Hg reflect the influence of local Hg backgrounds in food items, since in Bahia the foraging ground is in a highly industrialized area suggesting that the turtles are exposed to Hg burdens from locally anthropogenic activities.

In Mexico, Talavera-Saenz et al. (2007) compared metal concentrations in the *C. mydas* kidney and liver with plant and algae species found in their stomach contents and with the same species of food items collected inside a sea turtle refuge area. The results showed concentrations of Cd and Zn in flora from the sea turtle stomach contents were greater than the same species of marine plants collected in the bay. For both metals, the concentrations in sea turtle liver were not significantly different from the stomach contents. The authors concluded that sea turtles residing in Estero Banderitas are feeding in areas outside of the bay, most likely in coastal regions with high upwelling. Additionally, the similar levels of metals between liver and stomach contents could be explained by the fact that the liver reflects the concentration of metal in the food and the analyses in this organ may provide a better indication of recent environmental exposure. Metal levels that were similar or higher in

Table 9.2 Metal levels (mean ± SD, μg.g^{-1} dry weight) in tissues of sea turtles at different areas in Latin America

Tissue	Study site[a]	Sp	*n*	CCL	Method	Essential metals						Nonessential metals				Source
						Fe	Cu	Mn	Ni	Se	Zn	As	Cd	Hg	Pb	
Liver	Mexico – BC	*Cc*	5	61.4[b]	AAS	301	33.94	1.29	0.35	–	69.14	–	1.75	–	BDL	Gardner et al. (2006)
	Mexico – BS	*Cc*	16	59.8	CVAFS	–	–	–	–	–	–	–	–	0.15 ± 0.02	–	Kampalath et al. (2006)
	Brazil – BA	*Cm*	10	35.6	ICP-OES/ ICP-MS	4542 ± 2783	36.7 ± 9.3	8.73 ± 2.45	0.79 ± 0.34	16.8 ± 7.8	132 ± 22	29.8 ± 26.5	18.8 ± 10.6	1.34 ± 0.61	0.53 ± 0.45	Macêdo et al. (2015)
	Brazil – BA	*Cm*	25	36.4	CV-AAS	–	–	–	–	–	–	–	–	9.82 ± 7.11	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	12	40.7	ICP-MS	–	–	–	–	4.14 ± 3.2	29.5 ± 14.0	2.97 ± 3.03	6.40 ± 6.01	0.42 ± 0.32	0.22 ± 0.21	Prioste (2016)
	Brazil – CE	*Cm*	15	40.42	CV-AAS	–	–	–	–	–	–	–	–	0.70 ± 1.02	–	Bezerra et al. (2013)
	Brazil – CE	*Cm*	16	35.2	CV-AAS	–	–	–	–	–	–	–	–	4.75 ± 1.98	–	Bezerra et al. (2015)
	Brazil – ES	*Cm*	30	40.7	ICP-MS	–	–	–	–	2.47 ± 1.65	28.7 ± 10.2	3.05 ± 5.0	3.99 ± 2.26	0.104 ± 0.07	0.15 ± 0.11	Prioste (2016)
	Brazil – RS	*Cm*	15	36.65	FAAS/ CV-AAS	–	–	–	–	–	–	–	10.49	0.74	0.18	Silva (2011)
	Brazil – RS	*Cm*	29	39	FAAS	–	100.9	–	–	–	45	–	5.9	–	4.5	Silva et al. (2014)
	Brazil – SP	*Cm*	30	J	FAAS	–	20.7 ± 2.46	4.81 ± 0.9	0.13 ± 0.04	–	–	–	0.279 ± 0.14	–	–	Barbieri (2009)
	Brazil – SP	*Cm*	30	A	FAAS	–	39.9 ± 1.94	4.32 ± 0.71	0.28 ± 0.08	–	–	–	0.957 ± 0.31	–	–	Barbieri (2009)
	Brazil – SP	*Cm*	41	40.7	ICP-MS	–	–	–	–	3.54 ± 2.54	33.2 ± 12.2	2.71 ± 2.90	5.96 ± 3.57	0.144 ± 0.14	0.19 ± 0.14	Prioste (2016)
	Costa Rica – LI	*Cm*	34	A	FAAS/ GFAAS	2482	100	8.92	–	–	82.5	–	10.6	–	0.07	Andreani et al. (2008)
	Mexico – BC	*Cm*	11	62.13	AAS	14.35	60.04	0.06	0.01	–	62.91	–	3.30	–	BDL	Gardner et al. (2006)
	Mexico – BS	*Cm*	8	62 b	FAAS	350[c]	76.52[c]	0.24[c]	0.00[c]	–	90.95[c]	–	16.92[c]	–	0.00[c]	Talavera-Saenz et al. (2007)
	Mexico – BS	*Cma*	42	57.3	CVAFS	–	–	–	–	–	–	–	–	0.091 ± 0.05	–	Kampalath et al. (2006)
	Brazil – BA	*Ei*	16	33.6	ICP-OES/ ICP-MS	5566 ± 1441	21.8 ± 9.2	7.97 ± 1.69	0.75 ± 0.39	29.5 ± 4.80	144 ± 21.0	30.3 ± 11.8	20.1 ± 5.43	1.36 ± 0.61	0.27 ± 0.19	Macêdo et al. (2015)
	Mexico – BC	*Ei*	1	51.2[b]	AAS	71.88	2.47	0.74	2.48	NA	25.89	NA	0.49	–	BDL	Gardner et al. (2006)
	Mexico – BC	*Lo*	6	62.1[b]	AAS	731	36.73	0.1	0.58	–	47.14	–	17.89	–	BDL	Gardner et al. (2006)
	Mexico – BS	*Lo*	23	59.2	CVAFS	–	–	–	–	–	–	–	–	0.21 ± 0.28	–	Kampalath et al. (2006)

	Mexico – OA	*Lo*	7	–	ICP-OES	9914 ± 8669	46.08 ± 19.9	–	0.24 ± 0.20	40.08 ± 27.1	230 ± 160	14.8 ± 10.8	301 ± 221	–	0.81 ± 0.53	Cortés-Gómez et al. (2018b)[d]
	Mexico – OA	*Lo*	13	–	ICP-OES	8681 ± 5604	45.34 ± 30	–	0.24 ± 0.16	44.93 ± 15.7	188 ± 42	24.2 ± 14.3	390 ± 200	–	0.28 ± 0.20	Cortés-Gómez et al. (2018b)[d]
	Mexico – OA	*Lo*	13	65.50	ICP-OES	–	66.81 ± 42.2	13.7 ± 5.46	0.032 ± 0.24	33.67 ± 9.8	190 ± 4.01	13.6 ± 6.16	338 ± 149	–	0.44 ± 0.32	Cortés-Gómez et al. (2014)[d]
	Mexico – OA	*Lo*	17	27.98	ICP-OES	–	53.06 ± 33.8	–	0.20 ± 0.16	37.5 ± 19.1	–	48.9 ± 40.8	302 ± 187	–	0.81 ± 0.40	Cortés-Gómez et al. 2018c)[d]
	Mexico – SI	*Lo*	7	80.0	FAAS	–	33.4	–	–	–	–	–	13.12 ± 1.5	–	13.3 ± 2.1	Frías-Espericueta et al. (2006)
Kidney	Mexico – BC	*Cc*	5	61.4[b]	AAS	237	4.35	6.0	0.04	–	32.47	–	73.11	–	0.03	Gardner et al. (2006)
	Mexico – BS	*Cc*	16	59.8	CVAFS	–	–	–	–	–	–	–	–	0.09 ± 0.05	–	Kampalath et al. (2006)
	Brazil – BA	*Cm*	10	35.6	ICP-OES/ ICP-MS	435 ± 232	13.6 ± 6.53	6.05 ± 2.81	1.92 ± 1.41	13.4 ± 6.3	151 ± 21	1205 ± 1054	54.5 ± 21.2	0.36 ± 0.14	0.15 ± 0.14	Macêdo et al. (2015)
	Brazil – BA	*Cm*	25	36.4	CV-AAS	–	–	–	–	–	–	–	–	4.29 ± 2.82	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	12	40.8	ICP-MS	–	–	–	–	2.03 ± 1.41	30.1 ± 19.3	2.5 ± 2.27	17.28 ± 20.8	0.23 ± 0.21	0.071 ± 0.07	Prioste (2016)
	Brazil – CE	*Cm*	16	35.2	CV-AAS	–	–	–	–	–	–	–	–	3.86 ± 3.02	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	17	39.42	CV-AAS	–	–	–	–	–	–	–	–	0.42 ± 0.36	–	Bezerra et al. (2013)
	Brazil – ES	*Cm*	30	40.8	ICP-MS	–	–	–	–	1.47 ± 1.17	28.6 ± 16.5	2.38 ± 3.63	13.4 ± 6.93	0.043 ± 0.03	0.11 ± 0.08	Prioste (2016)
	Brazil – RS	*Cm*	15	36.65	FAAS/ CV-AAS	–	–	–	–	–	–	–	33.45	0.46	0.17	Silva (2011)
	Brazil – RS	*Cm*	29	39	FAAS	–	12.2	–	–	–	54.3	–	28.3	–	5.4	Silva et al. (2014)
	Brazil – SP	*Cm*	30	J	FAAS	–	12.55 ± 1.04	3.82 ± 0.73	0.089 ± 0.01	–	–	–	1.0 ± 0.32	–	–	Barbieri (2009)
	Brazil – SP	*Cm*	30	A	FAAS	–	13.72 ± 1.15	4.17 ± 0.86	0.19 ± 0.02	–	–	–	2.18 ± 0.27	–	–	Barbieri (2009)
	Brazil – SP	*Cm*	41	40.8	ICP-MS	–	–	–	–	1.49 ± 0.85	30.8 ± 21.7	2.2 ± 2.67	19.5 ± 17	0.054 ± 0.06	0.10 ± 0.09	Prioste (2016)
	Costa Rica – LI	*Cm*	34	A	FAAS/ GFAAS	300	8.34	5.75	–	–	77.4	–	39.2	–	0.044	Andreani et al. (2008)
	Mexico – BC	*Cm*	11	65.2[b]	AAS	44.09	5.67	0.31	1.15	–	128	–	121	–	0.01	Gardner et al. (2006)
	Mexico – BS	*Cm*	8	62[c]	FAAS	93.16[c]	5.83[c]	1.51[c]	3.19[c]	–	189[c]	–	110[c]	–	0.05[c]	Talavera-Saenz et al. (2007)
	Mexico – BS	*Cma*	42	57.3	CVAFS	–	–	–	–	–	–	–	–	0.08 ± 0.08	–	Kampalath et al. (2006)

(continued)

Table 9.2 (continued)

Tissue	Study site[a]	Sp	*n*	CCL	Method	Essential metals						Nonessential metals				Source
						Fe	Cu	Mn	Ni	Se	Zn	As	Cd	Hg	Pb	
	Brazil – BA	*Ei*	16	33.6	ICP-OES/ ICP-MS	309 ± 145	7.03 ± 2.95	5.28 ± 1.88	0.72 ± 0.39	11.0 ± 1.9	121 ± 30	1271 ± 480	76.2 ± 38.1	0.57 ± 0.42	0.07 ± 0.09	Macêdo et al. (2015)
	Mexico – BC	*Ei*	1	51.2[b]	AAS	362	3.89	7.62	1.61	–	82.45	–	4.20	–	BDL	Gardner et al. (2006)
	Mexico – BC	*Lo*	6	62.1 a	AAS	–	4.86	5.31	0.02	–	6.68	–	60.03	–	0.03	Gardner et al. (2006)
	Mexico – BS	*Lo*	23	59.2	CVAFS	–	–	–	–	–	–	–	–	0.14 ± 0.19	–	Kampalath et al. (2006)
	Mexico – OA	*Lo*	7	–	ICP-OES	66.48 ± 22.5	2.86 ± 0.67	–	0.05 ± 0.05	3.99 ± 1.31	88.2 ± 27.0	1.47 ± 0.69	209 ± 141	–	0.08 ± 0.02	Cortés-Gómez et al. (2018b)[d]
	Mexico – OA	*Lo*	13	65.50	ICP-OES	–	3.80 ± 1.84	10.1 ± 3.21	0.18 ± 0.10	4.47 ± 2.09	108 ± 60.6	3.69 ± 2.30	404 ± 29.2	–	0.16 ± 0.08	Cortés-Gómez et al. (2014)[d]
	Mexico – OA	*Lo*	13	–	ICP-OES	273 ± 230	4.39 ± 1.63	–	0.08 ± 0.02	6.24 ± 3.91	114 ± 39.9	4.02 ± 2.06	603 ± 276	–	0.05 ± 0.05	Cortés-Gómez et al. (2018b)[d]
	Mexico – OA	*Lo*	17	27.98	ICP-OES	–	3.75 ± 1.60	–	0.05 ± 0.02	4.28 ± 1.34	–	3.21 ± 3.48	316 ± 222	–	0.10 ± 0.08	Cortés-Gómez et al. (2018c)[d]
	Mexico – SI	*Lo*	7	80.0	FAAS	–	17.15	–	–	–	–	–	15.84 ± 1.2	–	13.4 ± 1.9	Frías-Espericueta et al. (2006)
Muscle	Mexico – BC	*Cc*	5	61.4 a	AAS	77.44	0.41	0.84	0.01	–	31.11	–	0.1	–	0.01	Gardner et al. (2006)
	Mexico – BS	*Cc*	16	59.8	CVAFS	–	–	–	–	–	–	–	–	0.02 ± 0.01	–	Kampalath et al. (2006)
	Brazil – BA	*Cm*	25	36.4	CV-AAS	–	–	–	–	–	–	–	–	1.84 ± 1.93	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	12	40.9	ICP-MS	–	–	–	–	3.96 ± 5.36	8.0 ± 2.06	5.49 ± 6.18	0.06 ± 0.05	0.027 ± 0.04	0.008 ± 0.011	Prioste (2016)
	Brazil – CE	*Cm*	16	35.2	CV-AAS	–	–	–	–	–	–	–	–	1.73 ± 0.71	–	Bezerra et al. (2015)
	Brazil – CE	*Cm*	18	37.68	CV-AAS	–	–	–	–	–	–	–	–	0.20 ± 0.18	–	Bezerra et al. (2013)
	Brazil – ES	*Cm*	30	40.9	ICP-MS	–	–	–	–	1.42 ± 1.34	8.99 ± 5.66	4.48 ± 7.30	0.07 ± 0.09	0.006 ± 0.009	0.005 ± 0.007	Prioste (2016)
	Brazil – RS	*Cm*	15	36.65	FAAS/ CV-AAS	–	–	–	–	–	–	–	0.21	0.23	0.11	Silva (2011)
	Brazil – RS	*Cm*	29	39	FAAS	–	1.2	–	–	–	16.6	–	0.4	–	4.2	Silva et al. (2014)
	Brazil – SP	*Cm*	42	40.9	ICP-MS	–	–	–	–	1.69 ± 1.23	9.75 ± 4.07	3.64 ± 5.73	0.07 ± 0.05	0.006 ± 0.008	0.004 ± 0.006	Prioste (2016)
	Mexico – BC	*Cm*	11	65.2 a	AAS	20.99	0.03	0.003	0.03	–	38.26	–	0.01	–	0.01	Gardner et al. (2006)
	Mexico – BS	*Cma*	42	57.3	CVAFS	–	–	–	–	–	–	–	–	0.02 ± 0.02	–	Kampalath et al. (2006)

	Mexico – BC	*Ei*	1	51.2 a	AAS	258	3.68	1.78	BDL	–	102	–	1.02	–	0.38	Gardner et al. (2006)
	Mexico – BC	*Lo*	6	62.1 a	AAS	93.09	1.28	0.77	0.01	–	85.78	–	0.48	–	BDL	Gardner et al. (2006)
	Mexico – BS	*Lo*	23	59.2	CVAFS	–	–	–	–	–	–	–	–	0.05 ± 0.04	–	Kampalath et al. (2006)
	Mexico – SI	*Lo*	7	80.0	FAAS	–	15.5	–	–	–	–	–	2.48 ± 0.4	–	8.9 ± 1.0	Frías-Espericueta et al. (2006)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	–	4.16 ± 1.04	–	0.31 ± 0.52	40.1 ± 16.14	–	19.7 ± 9.37	3.64 ± 1.56	–	0.20 ± 0.15	Cortés-Gómez et al. (2018c)[d]
Adipose	Mexico – BC	*Cc*	5	61.4 a	AAS	1.33	0.69	1.82	0.17	–	12.66	–	0.5	NA	BDL	Gardner et al. (2006)
	Mexico – BS	*Cc*	16	59.8	CVAFS	–	–	–	–	–	–	–	–	0.008 ± 0.01	–	Kampalath et al. (2006)
	Costa Rica – LI	*Cm*	34	A	FAAS/ GFAAS	45.2	0.446	0.826	–	–	62.1	–	0.113	–	0.063	Andreani et al. (2008)
	Mexico – BC	*Cm*	11	65.2[b]	AAS	2.63	0.01	0.003	0.02	–	49.82	–	0.002	NA	0.03	Gardner et al. (2006)
	Mexico – BS	*Cma*	42	57.3	CVAFS	–	–	–	–	–	–	–	–	0.004 ± 0.003	–	Kampalath et al. (2006)
	Mexico – BC	*Ei*	1	51.2[b]	AAS	11.14	0.72	2.53	BDL	–	42.39	–	0.43	NA	BDL	Gardner et al. (2006)
	Mexico – BC	*Lo*	6	62.1[b]	AAS	27.91	0.83	2.1	0.03	–	3.7	–	0.69	NA	BDL	Gardner et al. (2006)
	Mexico – BS	*Lo*	23	59.2	CVAFS	–	–	–	–	–	–	–	–	0.03 ± 0.06	–	Kampalath et al. (2006)
Bone	Brazil – BA	*Cm*	10	35.6	ICP-OES/ ICP-MS	44.7 ± 33.6	1.26 ± 1.37	4.68 ± 1.63	3.52 ± 1.43	2.97 ± 0.45	196 ± 34	7422 ± 667	< 0.060	< 0.128	0.98 ± 0.61	Macêdo et al. (2015)
	Brazil – CE	*Cm*	10	40.4	ICP-MS	–	–	–	–	1.0 ± 0.63	51.3 ± 11.1	6.58 ± 6.07	0.08 ± 0.07	0.019 ± 0.013	1.06 ± 1.13	Prioste (2016)
	Brazil – ES	*Cm*	23	40.4	ICP-MS	–	–	–	–	0.98 ± 0.84	56.3 ± 27.8	2.94 ± 4.15	0.11 ± 0.06	0.011 ± 0.007	0.6 ± 0.47	Prioste (2016)
	Brazil – SP	*Cm*	40	40.4	ICP-MS	–	–	–	–	0.69 ± 0.61	57.3 ± 28.0	11.9 ± 15.2	0.14 ± 0.15	0.007 ± 0.006	0.6 ± 0.49	Prioste (2016)
	Brazil – BA	*Ei*	16	33.6	ICP-OES/ ICP-MS	41.5 ± 17.8	0.73 ± 0.73	7.10 ± 2.12	1.71 ± 0.3	1.65 ± 0.41	215 ± 18	7006 ± 421	0.56 ± 0.63	< 0.128	0.64 ± 0.48	Macêdo et al. (2015)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	–	2.0 ± 5.0	–	0.25 ± 0.12	1.62 ± 05	–	0.75 ± 0.62	1.0 ± 0.37	–	1.25 ± 0.87	Cortés-Gómez et al. (2018c)[d]
Salt gland	Brazil – CE	*Cm*	8	39.6	ICP-MS	–	–	–	–	1.5 ± 1.07	11.8 ± 2.08	206.3 ± 578	1.04 ± 0.68	0.032 ± 0.05	0.11 ± 0.30	Prioste (2016)
	Brazil – ES	*Cm*	29	39.6	ICP-MS	–	–	–	–	1.2 ± 1.06	13.8 ± 7.48	1.71 ± 2.83	0.91 ± 0.43	0.008 ± 0.013	0.95 ± 2.39	Prioste (2016)
	Brazil – SP	*Cm*	39	39.6	ICP-MS	–	–	–	–	1.31 ± 0.72	15.3 ± 6.39	1.54 ± 1.96	0.98 ± 0.64	0.009 ± 0.010	0.008 ± 0.007	Prioste (2016)

(continued)

Table 9.2 (continued)

Tissue	Study site[a]	Sp	*n*	CCL	Method	Essential metals						Nonessential metals				Source
						Fe	Cu	Mn	Ni	Se	Zn	As	Cd	Hg	Pb	
Spleen	Brazil – CE	*Cm*	10	40.8	ICP-MS	–	–	–	–	1.27 ± 1.16	29.6 ± 16.8	0.88 ± 0.74	1.14 ± 1.16	0.041 ± 0.05	0.023 ± 0.014	Prioste (2016)
	Brazil – ES	*Cm*	30	40.8	ICP-MS	–	–	–	–	1.57 ± 1.93	22.5 ± 12.2	1.68 ± 2.44	0.91 ± 1.26	0.006 ± 0.01	0.019 ± 0.011	Prioste (2016)
	Brazil – SP	*Cm*	40	40.8	ICP-MS	–	–	–	–	1.46 ± 0.8	19.8 ± 3.64	1.57 ± 1.69	0.80 ± 0.48	0.009 ± 0.009	0.022 ± 0.02	Prioste (2016)
Heart	Mexico – SI	Lo	7	80.0	FAAS	–	44.9 ± 4	–	–	–	–	–	11.0	–	10.1 ± 1.1	Frías-Espericueta et al. (2006)
Brain	Mexico – OA	Lo	17	27.98	ICP-OES	–	13.6 ± 38.8	–	0.8 ± 0.8	29.2 ± 68	–	6.0 ± 17.6	3.2 ± 6.4	–	0.4 ± 0.4	Cortés-Gómez et al. (2018c)[d]

As arsenic, *Cd* cadmium, *Cu* copper, *Fe* iron, *Hg* mercury, *Mn* manganese, *Ni* nickel, *Pb* lead, *Se* selenium, *Zn* zinc, *Cc Caretta caretta*, *Cm Chelonia mydas*, *Cma Chelonia mydas agassizii*, *Dc Dermochelys coriacea*, *Ei Eretmochelys imbricata*, *Lo Lepidochelys olivacea*, *AAS* atomic absorption spectrophotometry, *CV-AAS* cold vapor atomic absorption spectrometry, *CVAFS* cold vapor atomic fluorescence spectrometry, *FAAS* flame atomic absorption spectrophotometry, *GFAAS* graphite furnace atomic absorption spectroscopy, *ICP-OES* inductively coupled plasma- optical emission spectrophotometry, *ICP-MS* inductively coupled plasma- mass spectrometry, *J* juvenile, *A* adult

[a]Country-state/province/department abbreviation

[b]Original publication in straight carapace long (SCL), transform into CCL using the following formulas: *Lo* = (SCL − 9.244)/0.818 (Whiting et al. 2007); *Cc* = 1.388 + 1.053 (SCL) (Bjorndal et al. 2000); *Cm* = −0.028 + 1.051 (SCL) (Bjorndal and Bolten 1989) and *Ei* = (SCL − 0.449)/0.935 (Wabnitz and Pauly 2008)

[c]Median, *BDL* below detection limits

[d]Results originally published in wet weight transformed into dry weight using the humidity percentage reported by Frías-Espericueta et al. (2006) (liver, 75.5%; kidney, 62.7%; muscle, 80.8%), Garcia-Fernandez et al. (2009) (bone, 20%; brain, 75%), and Adballah and Abd-Allah (2011) (adipose, 20%)

the liver and kidney but lower than in the stomach contents may indicate metabolic processing of these metals and/or accumulation over time.

Cortés-Gómez et al. (2018c) developed a method to use carapace morphologies from photographs to quantify developmental instability (DI) and examined relationships between inorganic elements and asymmetry of the carapace. They compared the concentrations of 16 elements in tissues (liver, kidney, muscle, brain, bone, blood, and egg components) of stranded dead *Lepidochelys olivacea* from Mexico. The results suggested that individuals with more asymmetric carapaces seem to be more susceptible to accumulate the organic elements. They also found negative significant relationships between the DI of adult females and the concentration of metals in their eggs. However, the authors highlight that more studies are needed to validate the use of carapace asymmetry as a biomarker.

9.3.3 Eggs and Hatchlings

Egg samples provide many advantages for monitoring pollutants. It can be collected in a nonlethal manner if unhatched eggs and/or dead hatchlings are sampled after the live hatchlings have emerged or if the eggshell can be found in remaining nest, instead of sacrificing a fresh egg that has the potential to develop. Eggs are usually abundant, easy to collect, and more accessible for sampling compared to capturing juveniles or adults at sea (Keller et al. 2014).

In reptiles, the ovulation and the supply of albumen and eggshell for all the eggs to be laid during the season happen progressively throughout the nesting season (Palmer et al. 1993). Thus, the contents of sea turtle eggs represent the diet, nutrients, and chemical compounds ingested by adult females in their foraging sites and during the breeding season (Miller 1997). Eggs also constitute a potential tool for monitoring the excretion route or maternal transfer and are good indicators of the metal load of nesting colonies.

During incubation, contaminants could also be transferred from the nest environment into the eggs. Indeed, during incubation, the number of permeable open pores on eggshell of turtles increases due to water or gas exchange between eggs and nest environment, facilitating the transfer of contaminants from nest material into eggs (Hewavisenthi and Parmenter 2001; Canas and Anderson 2002). Permeability of eggshells to soil contaminants should also be considered as a way of contamination that could affect hatching success.

Early life stages of oviparous organisms seem to exhibit higher sensitivity to chemical contaminants than adults (Russell et al. 1999). In reptiles, ovo exposure to toxic elements has been shown to impact the development of the embryo, resulting in hatchlings deformities, disorientation, and lower fitness, thus increasing the risk of predation and negatively affecting migration to feeding sites (Bishop et al. 1991, 1998).

In Latin America few studies were found regarding the contamination of eggs, unhatched eggs, and hatchlings (Table 9.3). In these studies, some authors analyzed

eggs contents individually (Cortés-Gómez et al. 2018c; Páez-Osuna et al. 2010a, b, 2011; Dyc et al. 2015) and others used the whole egg (Guirlet et al. 2008; Ross et al. 2016; Roe et al. 2011) or only the eggshells found in the nest after the hatching (Simões 2016; Vazquez et al. 1997). The eggs were collected at the time of oviposition (Guirlet et al. 2008; Páez-Osuna et al. 2010a, b, 2011; Dyc et al. 2015), within 12 hours of oviposition (Ross et al. 2016) or 2 days after hatchling ceased (unhatched eggs) (Roe et al. 2011) and from the oviduct of dead animals (Cortés-Gómez et al. 2018c).

The highest concentration of Cu, Cd, and Se in the yolk and eggshell were found in eggs collected directly from the oviduct of dead *L. olivacea* in Mexico (Cortés-Gómez et al. 2018c). The highest Pb levels in the yolk and albumen were also detected in *L. olivacea* from the same area, however, in a different study (Páez-Osuna et al. 2010a). In the eggshell the highest Pb concentrations were found in *D. coriacea* from Mexico (Vazquez et al. 1997).

The highest Hg levels in yolk, albumen, and eggshell were found in *E. imbricata* from Guadeloupe Islands (Dyc et al. 2015), *C. mydas* from Guadeloupe Islands (Dyc et al. 2015), and *E. imbricata* from Brazil (Simões 2016), respectively.

Considering the whole egg, the highest concentrations of Cu, Mn, and Cd were from *D. coriacea* collected in Mexico (Roe et al. 2011), while the highest concentrations of nonessential metals (Pb and Hg) were found in eggs of *D. coriacea* from French Guiana (Guirlet et al. 2008).

Vazquez et al. (1997) collected egg samples from *Dermochelys coriacea* in the field and held them under three different conditions prior to contaminant analyses. In "natural" condition eggs were kept at a preservation area (Playon de Mexiquillo); in "container" and "artificial" conditions, eggs were brought to the laboratory and kept in plastic containers or in an artificial environment of beach sand, respectively. In the last cases, eggs were kept at temperatures of 24–31 °C. The authors also analyzed metal concentrations in seawater and sand from the nesting area. They found significantly higher levels of metals in the sand than seawater and similar concentrations of contaminants in all three samples of eggshells. So, they concluded that the beach sand might be responsible for the eggshell contaminations.

In French Guiana, Guirlet et al. (2008) evaluated the levels of some metals in blood and eggs of *Dermochelys coriacea* and sampled multiple clutches from each female to assess the variations of trace element concentrations, according to the number of nesting events (time).

The results found in blood show that time had no effect on Hg, Cd, Se, and Zn concentrations indicating that concentrations remain constant throughout the nesting season, whereas Cu concentrations in blood decreased significantly with time and Pb concentrations increased. The decrease of Cu during nesting season could be a result of an important maternal transfer to albumen combined with a low dietary intake and insufficient reserves of this metal in the liver and kidney, resulting in Cu limitation at the end of the nesting period. In contrast, the increasing trend that occurred in Pb is likely to suggest Pb mobilization from bones associated with Ca requirement for egg formation and eggshell secretion.

On the other hand, in eggs, no fluctuation had been observed for trace elements concentrations between the different clutches laid suggesting a constant maternal transfer to egg along the nesting season. Moreover, the amount of elements transferred

Table 9.3 Metal levels (mean ± SD, μg.g^{-1} wet weight) in eggs and hatchlings of sea turtles at different areas in Latin America

Tissue	Study site[a]	Sp	*n*	CCL	Method	Essential metals				Nonessential metals				Source
						Cu	Ni	Se	Zn	As	Cd	Hg	Pb	
Yolk	Guadeloupe Islands	*Cm*	12	–	ICP-MS/ DMA	0.59	–	0.17	–	–	<0.003	0.0020	0.010	Dyc et al. (2015)
	Guadeloupe Islands	*Ei*	4	–	ICP-MS/ DMA	0.49	–	1.37	–	–	<0.003	0.017	0.017	Dyc et al. (2015)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	1.2 ± 0.4	BDL	2.8 ± 1.1	–	0.2 ± 0.07	0.2 ± 0.09	–	0.03 ± 0.03	Cortés-Gómez et al. (2018c)
	Mexico – OA	*Lo*	25	66.4	GFAAS/ CV-AAS	0.82 ± 0.55	1.23 ± 0.22	–	27.11 ± 1.51	–	0.09 ± 0.03	0.010 ± 0.003	0.30 ± 0.03	Páez-Osuna et al. (2010a, b, 2011)[b]
Albumen	Guadeloupe Islands	*Cm*	12	–	ICP-MS/ DMA	0.85	–	0.27	–	–	<0.014	0.018	0.019	Dyc et al. (2015)
	Guadeloupe Islands	*Ei*	3	–	ICP-MS/ DMA	0.86	–	4.6	–	–	<0.014	0.013	<0.019	Dyc et al. (2015)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	0.2 ± 0.2	BDL	0.8 ± 0.5	–	0.1 ± 0.1	0.02 ± 0.05	–	0.01 ± 0.0	Cortés-Gómez et al. (2018c)
	Mexico – OA	*Lo*	25	66.4	GFAAS/ CV-AAS	0.09 ± 0.07	0.10 ± 0.10	–	0.90 ± 0.16	–	0.005 ± 0.002	0.00003 ± 0.00002	0.029 ± 0.005	Páez-Osuna et al. (2010a, b, 2011)[b]
Eggshell	Guadeloupe Islands	*Cm*	12	–	ICP-MS/ DMA	1.63	–	0.15	–	–	0.083	0.0018	0.006	Dyc et al. (2015)
	Mexico – MI	*Dc*	5	–	GFAAS	–	3.11	–	4.87	–	–	–	5.69	Vazquez et al. (1997)[b]
	Mexico – MI	*Dc*	5	–	GFAAS	–	2.75	–	4.18	–	–	–	3.11	Vazquez et al. (1997)[b, c]
	Mexico – MI	*Dc*	5	–	GFAAS	–	4.3	–	5.61	–	–	–	2.53	Vazquez et al. (1997)[b, d]
	Brazil – PE	*Ei*	20	–	XRF	0.10 ± 0.07	1.92 ± 2.03	–	–	–	–	0.006 ± 0.006	0.016 ± 0.015	Simões (2016)[b]
	Guadeloupe Islands	*Ei*	3	–	ICP-MS/ DMA	2.64	–	1.39	–	–	0.07	0.0033	<0.0076	Dyc et al. (2015)
	Mexico – OA	*Lo*	17	27.98	ICP-OES	8.6 ± 2.3	0.02 ± 0.0	6.6 ± 3.3	–	BDL	0.4 ± 0.7	–	BDL	Cortés-Gómez et al. (2018c)
	Mexico – OA	*Lo*	25	66.4	GFAAS/ CV-AAS	3.06 ± 1.06	19.88 ± 5.28	–	5.08 ± 0.61	–	0.19 ± 0.03	0.0035 ± 0.0001	0.43 ± 0.08	Páez-Osuna et al. (2010a, b, 2011)[b]

(continued)

Table 9.3 (continued)

Tissue	Study site[a]	Sp	*n*	CCL	Method	Essential metals				Nonessential metals				Source
						Cu	Ni	Se	Zn	As	Cd	Hg	Pb	
Whole	Panama	*Cm*	31	–	ICP-MS/ ICP-OES	0.5 ± 0.1	–	–	14.0 ± 3.0	0.12 ± 0.04	0.09 ± 0.04	0.006 ± 0.002	0.003 ± 0.002	Ross et al. (2016)
Egg	Costa Rica – G	*Dc*	38	–	ICP-AES/ ICP-OES	25.9	1.9	–	14.2	–	1.6	–	–	Roe et al. (2011)
	French Guiana – SLM	*Dc*	76	160	ICP-MS/ DMA	0.63 ± 0.10	–	1.44 ± 0.38	14.16 ± 2.23	–	0.024 ± 0.001	0.012 ± 0.003	0.036 ± 0.001	Guirlet et al. (2008)
	Panama	*Lo*	30	–	ICP-MS/ ICP-OES	0.6 ± 0.2	–	–	16.0 ± 2.0	0.12 ± 0.06	0.07 ± 0.02	0.009 ± 0.005	0.004 ± 0.002	Ross et al. (2016)
Hatchlings	Costa Rica – G	*Dc*	38	–	ICP-AES/ ICP-OES	4.2	BDL	–	22.5	–	BDL	–	–	Roe et al. (2011)

As arsenic, *Cd* cadmium, *Cu* copper, *Hg* mercury, *Ni* nickel, *Pb* lead, *Se* selenium, *Zn* zinc, *Cm Chelonia mydas*, *Dc Dermochelys coriacea*, *Ei Eretmochelys imbricata*, *Lo Lepidochelys olivacea*, *CV-AAS* cold vapor atomic absorption spectrometry, *DMA* direct mercury analysis, *FAAS* flame atomic absorption spectrophotometry, *GFAAS* graphite furnace atomic absorption spectroscopy, *ICP-AES* inductively coupled plasma- atomic emission spectrometry, *ICP-OES* inductively coupled plasma- optical emission spectrophotometry, *ICP-MS* inductively coupled plasma- mass spectrometry, *XRF* X-ray fluorescence spectrometer, *BDL* below detection limits

[a]Country-state/province/department abbreviation

[b]Results originally published in dry weight transformed into wet weight using the humidity percentage reported by Páez-Osuma et al. (2010a, b, 2011) (albumen, 97.3%; yolk, 62.5%; eggshell, 59.0%)

[c]Eggs incubated in a container (plastic)

[d]Eggs incubated in an artificial environment of beach sand

by the females to eggs were in the order of Zn > Se > Cu > Pb > Cd > Hg. The authors concluded that females transfer a higher burden of essential elements than toxic elements, during the nesting season. This result is in concordance with data reported by Páez-Osuna et al. (2010a, b, 2011) for Mexican *Lepidochelys olivacea* from a nesting colony at Oaxaca State.

In this study, maternal transfer of trace metals via egg-laying, in terms of metal burden in the whole body, was 21.5%, 7.8%, 3.4%, 2.0%, 0.5%, and 0.2% for Ni, Cu, Zn, Hg, Pb, and Cd, respectively. The excretion rates of trace metals through egg-laying followed the same pattern (Ni > Cu > Zn > Hg > Pb > Cd). It indicates that egg-laying is not a major route for transferring nonessential metals (perhaps with the exception of Ni), but essential metals are transferred at a higher rate, possibly as a source mechanism for the hatchlings.

Considering the proportions of each egg fraction (albumen, 4.9%; yolk, 80.9%; eggshell, 14.2% in dry weight) and concentrations of each metal in each case, the authors observed that the highest percentage (or load) of metals was incorporated in the yolk, except of Ni. This result confirms the importance of yolk in the accumulation of heavy metals in sea turtles hatchlings. Additionally, the concentration of essential metals in yolk is important because they contribute to the physiological processes and good development of the embryo. For example, Cu and Zn are required for normal growth, metabolism, and structure and function of many proteins vital for cell function. Thus, a maternal transfer of metals to eggs is necessary for successful development of the embryos.

As mentioned earlier in this chapter, sea turtles are an important food source in many coastal communities in Latin America; for this reason Ross et al. (2016) evaluated the overall health risks from heavy metal intake through *Chelonia mydas* and *Lepidochelys olivacea* egg consumption along the Pacific coast of Panama. The authors used the average body weights of human consumers according to age, sex, and socioeconomic factors to calculate the consumption rates and correlated with metal levels found in eggs. The results suggest that, except in cases of consistent extreme consumption, heavy metal exposure through sea turtle egg consumption alone is unlikely to pose a threat to those who regularly eat green turtle and/or olive ridley eggs in Panama. However, average consumption rates may contribute substantially to lifetime Cd intake.

In Guadeloupe Islands, Dyc et al. (2015) investigated the effects of contaminants on sea turtles performing a screening-level risk assessment, using hazard quotients (HQ) as a measure of the ratio between measured concentrations, and predicted no effect concentrations. For this, the authors collected eggs from *C. mydas* and *E. imbricata* during the oviposition.

The HQ results indicated that Se, Cd, and Hg exposure may represent a threat for developing marine turtle embryos ($HQ_{worst} > 1$ for Se and Hg and HQ_{best} and $HQ_{worst} > 1$ for Cd, for both species). Se may reduce the embryo viability and Hg could induce embryo deformities and/or reduce the survival of green and hawksbill turtle embryo. However, the authors argument that if Guadeloupian sea turtles are tolerant, the levels found may be harmless, which may be most likely considering the higher hatchling rate of both species. Due to species-specific difference in toxi-

cological responses, screening-level risk assessments may inaccurately estimate the effects of chemical pollutants in sea turtles, and improved risk assessments would rely on more detailed toxicological data being generated.

Investigations of the impact of metal contaminants on hatchlings and unhatched eggs have been conducted in only one publication, in Costa Rica. In this study, Roe et al. (2011) sampled two clutches for each female *D. coriacea*, the first and the fourth nest of the season. Two days after hatching, unhatched eggs and hatchlings were collected to determine metal levels. They also measured 20 hatchlings from each nest during emergence to determine the body condition index. The results of this study indicate that leatherback embryos accumulate a suite of essential metals including Cu, Fe, Mn, and Zn, as well as nonessential metals Cd and Ni. In part, such concentrations reflect the maternal transfer of metals to eggs but also may be influenced by exposure in the nest environment, a contamination route that the authors did not measure.

Regarding differences between clutches, egg trace element concentrations did not vary between nests laid earlier and later in the season. Roe et al. found little evidence that metal levels in leatherback eggs had any significant influence on clutch success, hatchling size, or hatchling body condition. Therefore, the authors concluded that maternal genotype, maternal health, or nest environment may have a more profound influence on clutch viability in leatherbacks than environmental contaminants at current exposure levels in the eastern Pacific Ocean.

Further investigations into the effects of contaminants in sea turtle hatchlings and eggs may be a useful tool in ecotoxicology as a reduction in hatchling success may have big effects on population dynamics.

9.3.4 Carapace

Scutes from the carapace have increasingly been used as reliable tissue to evaluate metal contents because they can be collected noninvasively and nonlethally, either in juvenile or adult animals. Heavy metals are known to bind with keratin and studies have revealed that elements such as mercury maintain a strong association with keratin following prolonged exposure to UV radiation and extreme temperatures. The keratinized carapace scutes could therefore provide a reliable and temporarily robust measure of determining heavy metal concentration in sea turtles.

A recent study on *C. caretta* found significant positive correlations between scute mercury levels and mercury concentrations in the liver, muscle, kidney, and spinal cord (Day et al. 2005). Another study on *C. caretta* accidentally caught in fishing nets off the coast of Japan found significant positive correlations between carapace concentrations and whole-body burdens for zinc, manganese, and mercury (Sakai et al. 2000).

In Latin America, all studies that used carapace as a biomarker were made in Brazil. The samples were collected from alive and dead animals that were trapped in fishing artifacts, found stranded or nesting on the beach, or died during rehabilita-

tion. The only metal analyzed was mercury and the species sample were *Chelonia mydas* and *Caretta caretta* from Bahia and Ceará states (Table 9.4).

Comparing the studies, juveniles of *C. mydas* from Bahia and adults of *Caretta caretta* from Ceará had the highest Hg levels found (Bezerra et al. 2015; Rodriguez 2017). On the other hand, the lowest levels were observed in adults of *C. mydas* from Ceará (Bezerra et al. 2012) and adults of *Caretta caretta* from Bahia (Rodriguez et al. 2018).

Bezerra et al. (2012, 2015) observed highest levels of mercury in juvenile *C. mydas*, with a significant negative correlation between size of the animal (CCL) and Hg levels. The authors related this correlation factor to the change in eating habits between juvenile and adults. When *C. mydas* are recruiting to coastal habitats, their diet changes from an omnivorous to herbivorous diet which results in feeding mostly on benthic algae and seagrass. However, juveniles that feed on a more omnivorous diet are exposed to higher levels of organic mercury than when feeding on benthic plants.

The differences in Hg levels found in adults of *C. caretta* from Ceará (Rodriguez 2017) and Bahia (Rodriguez et al. 2018) could be explained by the differences in the regions sampled. Ceará coast is characterized as nearly pristine regarding Hg contamination because of low industrial development. On the other hand, the northern coast of Bahia is under influence by a petrochemical industrial complex and an extensive industrial development. As a result, this area has been receiving a large input of heavy metal contaminants in the last several decades, which potentially enhances the exposure of marine species, threatening biodiversity and human safety.

Table 9.4 Mercury levels (mean ± SD, $\mu g.g^{-1}$ dry weight) in the carapace of sea turtles at different areas in Brazil

Study Site[a]	Sp	*n*	CCL	Capture	Method	Hg	Source
Brazil-BA	*Cc*	8	97.5	AL	CV-AAS	0.86 ± 0.95	Rodriguez et al. (2018)
Brazil- BA	*Cc*	76	99 ± 5	DN	CV-AAS	1.71 ± 2.63	Rodriguez (2017)
Brazil- CE	*Cc*	8	95.0	AL	CV-AAS	2.07 ± 1.69	Rodriguez et al. (2018)
Brazil- CE	*Cc*	6	76 ± 17	DN	CV-AAS	3.46 ± 2.38	Rodriguez (2017)
Brazil- BA	*Cm*	8	45.1	AL	CV-AAS	0.19 ± 4.71	Rodriguez et al. (2018)
Brazil- BA	*Cm*	25	36.4	FS/DR	CV-AAS	3.91 ± 3.39	Bezerra et al. (2015)
Brazil- CE	*Cm*	8	44.3	AL	CV-AAS	1.59 ± 1.83	Rodriguez et al. (2018)
Brazil- CE	*Cm*	25	50.5	AFW/DB	CV-AAS	0.154	Bezerra et al. (2012)
Brazil- CE	*Cm*	10	43.4	FS/DR	CV-AAS	0.42 ± 0.37	Bezerra et al. (2013)
Brazil- CE	*Cm*	16	35.2	FS/DR	CV-AAS	3.54 ± 2.75	Bezerra et al. (2015)

Hg mercury, *Cc Caretta caretta*, *Cm Chelonia mydas*, *AFW* alive animals trapped in fish weirs, *AL* alive (unknown capture method), *DB* dead in the beach, *DN* during nesting, *DR* died during rehabilitation, *FR* found stranded on the beach (dead), *CV-AAS* cold vapor atomic absorption spectrometry

[a]Country and state abbreviation

9.4 Conclusion

This chapter presented metal contamination data from Latin American sea turtles, and in spite of all the studies and the importance of nesting and foraging sites in Latin America to the development and reproduction of these animals, there is still a lack of information for several countries. The differences among methods to quantify metals difficult a precise comparison among studies, once some methods are more sensitive. Additionally, the results in the publications do not follow a pattern regarding metal values units. The non-reported moisture percentage could difficult the conversion of values and the results may be over- or underestimated.

The studies described in this chapter show that the differences in the accumulation rate vary depending on several factors, including geographical location, species, sizes, and type of tissue, along with environmental differences between species including pelagic or sedentary life strategy, trophic levels, food items, and growth rates (Guirlet et al. 2008; Andreani et al. 2008; Gardner et al. 2006; Talavera-Saenz et al. 2007). It is important to consider the mobility that some elements have in sick, moribund, or stranded turtles, which could result in a concentration variance between debilitated and healthy turtles (Camacho et al. 2014). Further, it is known that global factors are more relevant than local factors in the distribution of some metals (Fraga et al. 2018), so it is important to know the migration routes of sea turtles to investigate all the areas they inhabit, improving our understanding about the toxicological effects of contaminants and how they might impact sea turtles during key periods of life.

Although there are some studies that evaluated the interactions of heavy metals with biochemical and physiological processes, it is necessary to generate baseline information of health parameters, biochemical reference intervals, and pollution levels for each species and regional populations. The levels in environments with low anthropogenic impact could enable comparison with populations in polluted areas and will help to determine the impact of these contaminants on sea turtle's health, reproduction, and survival.

Additionally, few studies evaluated the variability of heavy metal levels in sea turtles' eggs and hatchlings and their relationship with maternal transfer. Future studies should focus on the influence of pollution in embryo development, reproduction success, and hatchling fitness.

The understanding of the action mechanisms allows a more precise risk assessment, helping to predict and prevent wildlife damage, and being essential in guiding regulatory decisions for the development of national conservation plans.

References

Abdallah MAM, Abd-Allah MAM (2011) Bioaccumulation of toxic metals in loggerhead turtles from Mediterranean Sea coast, Egypt. In: Özhan E (ed). Proc 10th Int Conf on the Mediterannean Coastal Environment, MEDCOAST, Rhodes, Greece, pp 569–580

Aguirre AA, Lutz PL (2004) Marine turtles as sentinels of ecosystem health: is fibropapillomatosis an indicator? EcoHealth 1:275–283

Aguirre AA, Balazs GH, Zimmerman B et al (1994) Organic contaminants and trace metals in the tissues of green turtles (*Chelonia mydas*) afflicted with fibropapillomas in the Hawaiian Islands. Mar Pollut Bull 28(2):109–114

Álvarez-Varas R, Contardo J, Heidemeyer M et al (2017) Ecology, health and genetic characterization of the southernmost green turtle (*Chelonia mydas*) aggregation in the eastern Pacific: implications for local conservation strategies. Lat Am J Aquat Res 45(3):540–554

Anan Y, Kunito T, Watanabe I et al (2001) Trace element accumulation in hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*) from Yaeyama Islands, Japan. Environ Toxicol Chem 20(12):2802–2814

Anan Y, Kunito T, Sakai H et al (2002) Subcellular distribution of trace elements in the liver of sea turtles. Mar Pollut Bull 45:224–229

Andreani G, Santoro M, Cottignoli S et al (2008) Metal distribution and metallothionein in loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles. Sci Total Environ 390:287–294

Athar M, Vohora S (1995) Heavy metals and environment. New Age International Publishers Ltd, Wiley Eastern Ltd, New Delhi, 216p

Balazs GH (1991) Current status of fibropapillomas in the Hawaiian green turtle, *Chelonia mydas*. In: Balazs GH, Pooley SG (eds) Research plan for marine turtle fibropapilloma. US Department of Commerce NOAA Technical Memorandum, Honolulu, pp 47–57

Barbieri E (2009) Concentration of heavy metals in tissues of green turtles (*Chelonia mydas*) sampled in the Cananéia Estuary, Brazil. Braz J Oceanogr 57(3):243–248

Bardales FAL, Benavides MGQ (2016) Determinación de concentraciones de metales pesados en sangre de hembras anidantes de tortugas carey (*Eretmocheys imbricata*) en la Bahía de Jiquilisco, Departamento de Usulután, El Salvador. Bachelor Thesis, Universidad de El Salvador, 101p

Bezerra MF, Lacerda LD, Costa BGB, Lima EHSM (2012) Mercury in the sea turtle *Chelonia mydas* (Linnaeus, 1958) from Ceará coast, NE Brazil. An Acad Bras Cienc 84(1):123–128

Bezerra MF, Lacerda LD, Lima EHSM et al (2013) Monitoring mercury in green sea turtles using keratinized carapace fragments (scutes). Mar Pollut Bull 77:424–427

Bezerra MF, Lacerda LD, Rezende CE et al (2015) Food preferences and Hg distribution in *Chelonia mydas* assessed by stable isotopes. Environ Pollut 206:236–246

Bishop C, Brooks RJ, Carey JH et al (1991) The case for a cause-effect between environmental contamination and development in eggs of the common snapping turtle (*Chelydras. serpentine*) from Ontario, Canada. J Toxicol Environ Health 33:5121–5547

Bishop CA, Ng P, Pettit KE et al (1998) Environmental contamination and developmental abnormalities in eggs and hatchlings of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes-St Lawrence River basin (1989-1991). Environ Poll 101:143–156

Bjorndal KA, Bolten AB (1989) Comparison of straight-line and over-the-curve measurements for growth rates of green turtles, *Chelonia mydas*. Bull Mar Sci 45:189–192

Bjorndal KA, Bolten AB, Martins HR (2000) Somatic growth model of juvenile loggerhead sea turtles *Caretta caretta*: duration of pelagic stage. Mar Ecol Prog Ser 202:265–272

Camacho M, Orós J, Boada LD (2013) Potential adverse effects of inorganic pollutants on clinical parameters of loggerhead sea turtles (*Caretta caretta*): results from a nesting colony from Cape Verde, West Africa. Mar Environ Res 92:15–22

Camacho M, Orós J, Henríquez-Hernández LA et al (2014) Influence of the rehabilitation of injured loggerhead turtles (*Caretta caretta*) on their blood levels of environmental organic pollutants and elements. Sci Total Environ 487:436–442

Canas JE, Anderson TA (2002) Organochlorine contaminants in eggs: the influence of contaminated nest material. Chemosphere 47:585–589

Cortés-Gomes AA, Romero D, Girondot M (2017) The current situation of inorganic elements in marine turtles: a general review and meta-analysis. Environ Pollut 229:567–585

Cortés-Gómez AA, Fuentes-Mascorro G, Romero D (2014) Metals and metalloids in whole blood and tissues of Olive Ridley turtles (*Lepidochelys olivacea*) from La Escobilla Beach (Oaxaca, Mexico). Mar Pollut Bull 89(1–2):367–375

Cortés-Gómez AA, Tvarijonaviciute A, Teles M et al (2017) p-Nitrophenyl acetate esterase activity and cortisol as biomarkers of metal pollution in blood of olive ridley turtles (*Lepidochelys olivacea*). Arch Environ Contam Toxicol 75(1):25–36

Cortés-Gómez AA, Tvarijonaviciute A, Girondot M et al (2018a) Relationship between plasma biochemistry values and metal concentrations in nesting olive ridley sea turtles. Environ Sci Pollut Res 36:36671–36679

Cortés-Gómez AA, Morcillo P, Guardiola FA et al (2018b) Molecular oxidative stress markers in olive ridley turtles (*Lepidochelys olivacea*) and their relation to metal concentrations in wild populations. Environ Pollut 233:156–167

Cortés-Gómez AA, Romero D, Girondot M (2018c) Carapace asymmetry: a possible biomarker for metal accumulation in adult olive ridleys marine turtles? Mar Pollut Bull 129:92–101

Day RD, Christopher SJ, Becker PR et al (2005) Monitoring mercury in the loggerhead sea turtle, *Caretta caretta*. Environ Sci Technol 39:437–446

Dyc C, Covaci A, Debier C et al (2015) Pollutant exposure in green and hawksbill marine turtles from the Caribbean region. Reg Stud Mar Sci 2:158–170

Fraga NS, Martins AS, Faust DR et al (2018) Cadmium in tissues of green turtles (*Chelonia mydas*): a global perspective for marine biota. Sci Total Environ 637–638:389–397

Frías-Espericueta MG, Osuna-López JI, Ruiz-Telles A et al (2006) Heavy metals in the tissues of the sea turtle *Lepidochelys olivacea* from a nesting site of the Northwest Coast of Mexico. Bull Environ Contam Toxicol 77:179–185

Garcia-Fernandez AJ, Gomez-Ramirez P, Martinez-Lopez E et al (2009) Heavy metals in tissues from loggerhead turtles (*Caretta caretta*) from the southwestern Mediterranean (Spain). Ecotox Environ Safe 72(2):557–563

Gardner SC, Fitzgerald SL, Vargas BA, Rodríguez LM (2006) Heavy metal accumulation in four species of sea turtles from the Baja California Peninsula, Mexico. Biometals 19:91–99

Gilman E, Zollett E, Beverly S et al (2007) Reducing sea turtle bycatch in pelagic longline fisheries. Fish Fish 7:1–22

Guirlet E, Dasb K, Girondot M (2008) Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana. Aquat Toxicol 88:267–276

Hewavisenthi S, Parmenter CJ (2001) Influence of incubation environment on the development of the flatback turtle (*Natator depressus*). Copeia 2001:668–682

IUCN (2019) Red list of threatened species. https://www.iucnredlist.org. Accessed 27 Jan 2019

Kampalath R, Gardner SC, Méndez-Rodríguez L et al (2006) Total and methylmercury in three species of sea turtles of Baja California Sur. Mar Pollut Bull 52:1784–1832

Keller JM, Peden-Adams MM, Aguirre AA (2006) Immunotoxicology and implications for reptilian health. In: Gardner SC, Oberdörster E (eds) Toxicology of reptiles. Taylor & Francis, Boca Raton, pp 199–240

Keller JM, Pugh RS, Becker PR (2014) Biological and environmental monitoring and archival of sea turtle tissues (BEMAST): rationale, protocols, and initial collections of banked sea turtle tissues. National Institute of Standards and Technology, Gaithersburg, 76p

Labrada-Martagón V, Rodríguez PAT, Méndez-Rodríguez LC et al (2011) Oxidative stress indicators and chemical contaminants in East Pacific green turtles (*Chelonia mydas*) inhabiting two foraging coastal lagoons in the Baja California Peninsula. Comp Biochem Physiol C Toxicol Pharmacol 154:65–75

Lam JC, Tanabe S, Chan SK et al (2004) Trace element residues in tissues of green turtles (*Chelonia mydas*) from South China waters. Mar Pollut Bull 48:174–182

Ley-Quiñónez C, Zavala-Norzagaray AA, Espinosa-Carreón TL et al (2011) Baseline heavy metals and metalloid values in blood of loggerhead turtles (*Caretta caretta*) from Baja California Sur, Mexico. Mar Pollut Bull 62:1979–1983

Ley-Quiñónez CP, Zavala-Norzagaray AA, Réndon-Maldonado JG et al (2013) Selected heavy metals and selenium in the blood of black sea turtle (*Chelonia mydas agassizii*) from Sonora, Mexico. Bull Environ Contam Toxicol 91:645–651

Ley-Quiñónez CP, Rossi-Lafferriere NA, Espinoza-Carreon TL et al (2017) Associations between trace elements and clinical health parameters in the North Pacific loggerhead sea turtle (*Caretta caretta*) from Baja California Sur, Mexico. Environ Sci Pollut Res 24(10):9530–9537

Livingstone DR (1993) Biotechnology and pollution monitoring: use of molecular biomarker in the aquatic environment. J Chem Technol Biotechnol 57:195–211

Macêdo GR, Tarantino TB, Barbosa IS et al (2015) Trace elements distribution in hawksbill turtle (*Eretmochelys imbricata*) and green turtle (*Chelonia mydas*) tissues on the northern coast of Bahia, Brazil. Mar Pollut Bull 94(1–2):284–289

Marcovaldi MA, Sales G, Thome JC et al (2006) Sea turtle interactions in Brazil: identifying and mitigating potential conflicts. Mar Turt Newsl 112:4–8

Miller JD (1997) Reproduction in sea turtles. In: Lutz PL, Musick JA (eds) The biology of sea turtles. CRC Press, Boca Raton, pp 51–82

Páez-Osuna F, Calderón-Campuzano MF, Soto-Jiménez MF et al (2010a) Lead in blood and eggs of the sea turtle, *Lepidochelys olivacea*, from the Eastern Pacific: concentration, isotopic composition and maternal transfer. Mar Pollut Bull 60:433–439

Páez-Osuna F, Calderón-Campuzano MF, Soto-Jiménez M et al (2010b) Trace metals (Cd, Cu, Ni, and Zn) in blood and eggs of the sea turtle *Lepidochelys olivacea* from a nesting colony of Oaxaca, Mexico. Arch Environ Contam Toxicol 59:632–641

Páez-Osuna F, Calderón-Campuzano MF, Soto-Jiménez MF et al (2011) Mercury in blood and eggs of the sea turtle *Lepidochelys olivacea* from a nesting colony in Oaxaca, Mexico. Mar Pollut Bull 62:1320–1323

Palmer BD, Demarco VG, Guillette LJ (1993) Oviductal morphology and eggshell formation in the lizard *Sceloporus woodi*. J Morphol 217:205–217

Prioste FES (2016) Detection and quantification of selected inorganic chemical elements in blood and tissue samples from green turtles – *Chelonia mydas* (Linnaeus, 1758) – from the Brazilian coast: possible correlation with fibropapillomatosis. Thesis, Universidade de São Paulo, 115p

Prioste FES, Souza VCO, Queiroz MR et al (2015) Chemical element concentrations in the blood of green turtles (*Chelonia mydas*) captured at Fernando De Noronha Marine National Park, Brazil. J Environ Anal Toxicol 5:325

Rodriguez CAB (2017) Mercúrio e metil mercúrio em carapaça da tartaruga marinha *Caretta caretta* (Linnaeus, 1758), no nordeste do Brasil, e seu uso potencial em monitoramento ambiental. Dissertation, Universidade Federal do Ceará, 57p

Rodriguez CAB, Bezerra MF, Lacerda LD (2018) Use of carapace for monitoring mercury in two species of sea turtles in northeast of Brazil. Arq Ciên Mar, Fortaleza 51(1):93–100

Roe JH, Sill NS, Columbia MR et al (2011) Trace metals in eggs and hatchlings of pacific leatherback turtles (*Dermochelys coriacea*) nesting at Playa Grande, Costa Rica. Chelonian Conserv Biol 10(1):3–9

Ross DA, Guzmán HM, Hinsberg VJV, Potvin C (2016) Metal contents of marine turtle eggs (*Chelonia mydas*; *Lepidochelys olivacea*) from the tropical eastern pacific and the implications for human health. J Environ Sci Health B 10:675–687

Ross DA, Guzman HM, Potvin C et al (2017) A review of toxic metal contamination in marine turtle tissues and its implications for human health. Reg Stud Mar Sci 15:1–9

Russell RW, Gobas F, Haffner GD (1999) Maternal transfer and in ovo exposure of organochlorines in oviparous organisms: a model and field verification. Environ Sci Technol 33:416–420

Sakai H, Saeki K, Ichihashi H et al (2000) Species-specific distribution of heavy metals in tissues and organs of Loggerhead turtle (*Caretta caretta*) and Green turtle (*Chelonia mydas*) from Japanese Coastal Waters. Mar Pollut Bull 40:701–709

Silva LM (2011) Metais pesados em tecidos de *Chelonia mydas* encalhadas no litoral do Rio Grande do Sul, Brasil. Bachelor Thesis, Universidade Federal do Rio Grande do Sul, 40p

Silva CC, Varela AS, Barcarolli IB, Bianchini A (2014) Concentrations and distributions of metals in tissues of stranded green sea turtles (*Chelonia mydas*) from the southern Atlantic coast of Brazil. Sci Total Environ 466–467:109–118

Silva CC, Klein RD, Barcarolli IF et al (2016) Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. Aquat Toxicol 170:42–51

Simões TN (2016) Metais pesados em sedimento, cascas de ovos e sangue de tartarugas marinhas da espécie *Eretmochelys imbricata* (Linnaeus, 1766). Dissertation, Universidade Federal de Pernambuco, 79p

Southwood AL, Andrews RD, Paladino FV et al (2005) Effects of diving and swimming behavior on body temperatures of Pacific leatherback turtles in tropical seas. Physiol Biochem Zool 78:285–297

Storelli MM, Storelli A, D'Addabbo R et al (2005) Trace elements in loggerhead turtles (*Caretta caretta*) from the eastern Mediterranean Sea: overview and evaluation. Environ Pollut 135(1):163–170

Talavera-Saenz A, Gardner SC, Rodriquez RR et al (2007) Metal profiles used as environmental markers of Green Turtle (*Chelonia mydas*) foraging resources. Sci Total Environ 373:94–102

Tauer AM, Liles MJ, Chavarría S et al. (2017) Hematology, biochemistry, and toxicology of wild hawksbill turtles (*Eretmochelys imbricata*) nesting in mangrove estuaries in the eastern Pacific Ocean. BioRxiv 238956

Vazquez GF, Reyes MC, Fernandez G et al (1997) Contamination in marine turtle (*Dermochelys coriacea*) egg shells of Playon de Mexiquillo, Michoacan, Mexico. Bull Environ Contam Toxicol 58:326–333

Wabnitz C, Pauly D (2008) Length-weight relationships and additional growth parameters for sea turtles. Fish Cent Res Rep 16:92–101

Wallace BP, Sotherland PR, Tomillo PS et al (2006) Egg components, egg size, and hatchling size in leatherback turtles. Comp Biochem Physiol Mol Integr Physiol 145:524–532

Whiting SD, Long JL, Hadden KM et al (2007) Insights into size, seasonality and biology of a nesting population of the Olive Ridley turtle in northern Australia. Wildl Res 34:200–210

Zavala-Norzagaray AA, Ley-Quiñónez CP, Espinosa-Carreón TL et al (2014) Trace elements in blood of sea turtles *Lepidochelys olivacea* in the Gulf of California, Mexico. Bull Environ Contam Toxicol 93:536–541

Chapter 10
Evaluation of the Toxicity of an Industrial Effluent Before and After a Treatment with Sn-Modified TiO_2 Under UV Irradiation Through Oxidative Stress Biomarkers

Leobardo Manuel Gómez-Oliván, Dora Alicia Solís-Casados, Hariz Islas-Flores, and Nely San Juan-Reyes

10.1 Introduction

Due to the growth of populations and the human activities, water quality is increasingly threatened; the main pollutants that affect its quality are metals, persistent organic compounds, pesticides, and emerging contaminants (Richardson 2009). Some of them are brominated flame retardants, chloroalkanes, polar pesticides, perfluorinated compounds, pharmaceuticals, food additives, and drugs of abuse, as well as metabolites and/or degradation products of the classes of the previous categories (Petrović et al. 2003).

The appearance of drugs in water represents a high risk to the environment for many reasons; one of them is they contain active ingredients that were designed to induce specific pharmacological effects in humans but, when they dissolve in water, can reach nontarget populations such as amphipods, amphibians, crustacean, and fish, among others, which produces toxicological effects. In addition, most drugs are designed to be persistent. Some do not degrade in the environment and others are degradable but at a very slow pace, and although some others

L. M. Gómez-Oliván (✉) · H. Islas-Flores · N. San Juan-Reyes
Laboratorio de Toxicología Ambiental, Facultad de Química,
Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico
e-mail: lmgomezo@uaemex.mx

D. A. Solís-Casados
Centro Conjunto de Investigación en Química Sustentable UAEM-UNAM,
Toluca, State of Mexico, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_10

are not persistent in the environment, they can be transformed through natural processes. The continuous introduction of this type of products from various sources (municipal, hospital, and industrial effluents) prolongs and maintains its presence in the waters, increasing any possible impact on aquatic life (Geissen et al. 2015).

Drugs in aquatic environments cause many deleterious effects in organisms. The toxicity of these compounds has been demonstrated in several studies, for example, antibiotics, such as tetracycline, oxytetracycline, and chlortetracycline, affect the growth, reproduction, and mobility of different organisms (Pépin 2006). Nonsteroidal anti-inflammatories (NSAIDs), such as ibuprofen, diclofenac, naproxen, ketorolac, and acetylsalicylic acid, produce growth inhibition and mobility on certain functions, oxidative stress, genotoxicity, citotoxicity, embryotoxicity, and teratogenesis in vertebrates and invertebrates (Pérez-Alvarez et al. 2018; Cardoso-Vera et al. 2017; Islas-Flores et al. 2017; Galar-Martínez et al. 2016; Lucero et al. 2015; Saucedo-Vence et al. 2015; Cleuvers 2004). 17-α-Ethinylestradiol and 17-β-estradiol, components of the contraceptive tablets, inhibit growth and reproduction and produce morphological changes, feminization of fish, oxidative stress, and mortality (Pépin 2006; Orozco-Hernández et al. 2018). Antineoplastic drugs (cytostatic) have a low biodegradability in the environment and are capable of producing cytotoxic, genotoxic, mutagenic, carcinogenic, or teratogenic effects in organisms (Zounkova et al. 2010; Jolibois and Guerbet 2005).

The first studies on the quantification of drug concentrations in the environment and the induction of toxic effects in aquatic organisms date back to the 1990s. Since then, concern about the presence of small concentrations of these pollutants and the health risk of the agencies they represent has increased in various sectors of society in different parts on the world. Although this field of study has not yet been fully developed, joint efforts have been made to mitigate the possible ecotoxicological effects of these pollutants and regulate their presence in surface water, groundwater, and even drinking water.

In this context, it is imperative to establish reaction and /or removal systems that guarantee a safe discharge of treated effluents for the organisms present in the water bodies. Several solutions have been proposed to reduce or eliminate the concentration of drugs in wastewater from hospital and industrial effluents, focused on the use of advanced oxidation processes (AOPs). Among the most studied are the photocatalytic processes. The most widely used photocatalyst has been TiO_2, because of its capability to oxidize drugs dissolved in water, nontoxicity, low cost, and long-term photostability, and also this photocatalyst can carry out the degradation of up to 75% of some organic compounds. In particular, incorporation of Sn into the TiO_2 has been reported as the most facile doping of TiO_2. Although these processes are efficient to remove drugs, it is necessary to verify that these treatments will not generate toxic effects in aquatic organisms in order to evaluate their biological efficiency.

The aim of this study was to evaluate the toxicity of an industrial effluent (wastewater from a pharmaceutical industry dedicated to the manufacture of

NSAIDs) before and after a treatment using Sn-modified TiO_2 under UV irradiation on *Hyalella azteca*. The toxicity assessment was performed using acute toxicity test (LC_{50}) and biomarkers of oxidative stress.

10.2 Methods

Unless otherwise stated, reagents in this section were obtained from Sigma-Aldrich (St. Louis MO).

10.2.1 Sampling of Industrial Effluent

A pharmaceutical industry was selected in the State of Mexico, Toluca City, which specializes in the production of NSAID. The samples were collected from the outlet draining the production area which is directly connected to the drainpipe exiting the plant. Dark containers of 20 l were used. Before sampling the containers were washed properly using 30% nitric acid and then rinsed with deionized water perfectly. The samplings were made taking into account the official Mexican norm on wastewater sampling (NMX-AA-003-1980). Samples were labeled and transported to the laboratory where they were stored at 4 degrees Celsius, a part of the water was immediately analyzed to assess the physicochemical parameters, another part was selected to perform the determination of NSAID concentrations, another part to perform the tests oxidative stress, and another to perform the photocatalytic treatment. It is very important to indicate that the wastewater generated by this pharmaceutical industry does not receive any chemical or biological treatment and that it goes directly to the municipal discharges of Toluca City and that they are capable of causing harmful effects on the organisms that live in the bodies of water receivers.

10.2.2 Physicochemical Characterization

For the physicochemical characterization of the industrial effluents under study, the methodologies proposed in the standard methods referred to in APHA, AWWA, and WPCF, 1995, were considered and in accordance with the one stipulated in the official Mexican norms NOM-001-SEMARNAT-1996 and NOM-073-ECOL-1994. The characterization of the effluents was carried out in triplicate and was carried out before and after the photocatalytic treatment.

10.2.3 Quantification of NSAIDs by Liquid Chromatography-Tandem Mass Spectrometry (LC–MS/MS) Before and After Treatment of Industrial Effluent

The determination of NSAIDs was carried out using the methodology described by (SanJuan-Reyes et al. 2015). Chemical analyses were performed using an Agilent 1290 Infinity HPLC unit (Santa Clara, CA). The RRHD Eclipse Plus C18 chromatography column (2.1 × 50 mm, 1.8 μm) was used, and this was maintained at 40 °C. The mobile phase was a 60:40 v/v mixture of acetonitrile and 10 mM ammonium formate. Flow rate was 0.3 mL min^{-1}, run time 1.8 min, and injection volume 2 μL. NSAIDs were quantified on an Agilent 6430 triple-quadrupole MS equipped with electrospray ionization (ESI). The ESI positive mode was used throughout. Electrospray voltage operated at 4000 V as the MS collected data in the negative ion mode.

Aliquots of 5 mL were taken and refrigerated at 4 °C for the determination of NSAIDs before and after treatment. The samples were vacuum-filtered through 1–0 μm GF/C glass microfiber filters, followed by 0.45 μm nylon membrane filters (Whatman, Cambridge, UK). A liquid–liquid extraction with 5 mL (1:1, v/v) hexane/ethyl acetate was performed to extract NSAIDs from 1 mL water samples. These samples were centrifuged at 1800 × g for 10 min and the upper organic layer was extracted again. This extraction was repeated and organic layers were combined and evaporated to dryness. The procedure was carried out in triplicate. When the water samples analyzed were outside the range of the standard curve, dilutions were performed. Finally, the result was multiplied by the dilution. Results were expressed as time-weighted average concentrations of DCF, IBP, NPX, and PCT.

10.2.4 Photocatalytic Performance

The obtained materials of Sn-modified TiO_2 were evaluated in the degradation of some drugs as the NSAIDs singles under UV light of 250 Watts at 250 nm to assess their photocatalytic response to radiation. As the first step for the photocatalytic response measurements, it was determined the relation of the absorbance as a function of the concentration of the drug (paracetamol, naproxen, diclofenac, ibuprofen). The photocatalytic process was carried out using a photochemistry reactor Q200 from PRENDO, where 100 mL of the industrial effluent is placed, and the photocatalyst is introduced into the system. Then the reaction system was kept in dark condition to reach adsorption equilibrium between the solution and the photocatalyst. Afterward the reaction system was irradiated using a UV lamp (250 nm) for different times taking aliquots to measure the absorbance at each time. The degradation was calculated from the intensity of the drug absorption band, assuming that the absorbance values are related to concentrations.

10.2.5 Procurement, Culturing, and Maintenance of Specimens

The test organism selected for this study was *Hyalella azteca*, because this is a species that is widely used in toxicology tests. In addition, it has been shown to be a sensitive organism, is easy to grow in the laboratory, and can generate reliable results in reference to the toxicity of contaminants present in aquatic environments. The test organism was collected from its natural habitat in the municipality of Capulhuac (State of Mexico) in San Miguel de Amaya lake and transported to the laboratory in plastic bags with constant aeration. Breeding stock was transported to the laboratory using the source of water in which the organisms were reared. Water used for transporting animals was well oxygenated (90–100% saturated). Once in the laboratory, the organisms were acclimated, gradually, to the laboratory conditions, such that the organisms were not stressed. Test organisms were in good health, and the mortality rate for juvenile *Hyalella* did not exceed 10% (Harmon et al. 2009).

The collected organisms were morphologically characterized according to (Pennak 1978). In this study we used organisms from the same clade that had been in culture under the same feeding conditions, temperature, and photoperiod for approximately 4 months (third-generation neonates obtained by sexual reproduction).

The culture of *Hyalella*, during the period of acclimatization, was maintained in reconstituted water with different concentrations ($NaHCO_3$, 174 mg/L; $MgSO_4$, 120 mg/L; KCl, 8 mg/L; and $CaSO_4.2H_2O$, 120 m/L). The culture conditions were pH 7.5–8.5 at room temperature with constant oxygen (6.4–6.6 mg/L, O_2) and a 12 h/12 h light/dark photoperiod and were fed ground lettuce ad libitum.

10.2.6 Toxicity Assays

In order to assess the efficiency of the photocatalytic process, biological tests were carried out to ensure that the water treated by the process was no more toxic than the original industrial waters. For this purpose, acute toxicity tests (LC_{50}) were performed, and also biomarkers of cellular oxidation and antioxidant activity (oxidative stress) were evaluated.

10.2.7 Determination of the Median Lethal Concentration (LC50)

The LC_{50} was determined before and after treatment. To determine the LC_{50}, test systems were used consisting of 50 mL dark precipitated beakers. Artificial sediment and previously aerated and reconstituted with salts water were placed in them.

The water/sediment ratio was 3:1. The test was conducted at a daily mean temperature (overlying water) of 23 ± 1 ° C. The system used in the study was a semi-static system with renewal of medium; in the period of exposure, the food was not provided to the study specimens.

Five systems were used with different proportions of the industrial effluent in the study: 0.71, 0.73, 0.75, 076, and 0.78% (before the photocatalytic treatment). A sixth system was also run; it was free of industrial effluent and served as control. All systems were added with ten 2-day-old neonatal organisms, and these were assigned to each system in a random way. The test vessels were covered, and the water in each system was aerated with a low air flow to avoid stress of the test organisms. The assay was performed in triplicate. After the photocatalytic treatment, the test was repeated using the same conditions; in this case the proportions used were 2.5, 3, 3.5, 4, and 4.5%.

The LC_{50} of 96 h of industrial effluent before and after treatment and its 95% confidence limits ($P < 0.05$) were estimated by probit analysis (EPA, v1.5). The data obtained were used to estimate the concentration used in the oxidative stress tests.

10.2.8 Oxidative Stress Determination

The phenomenon of oxidative stress was evaluated before and after the photocatalytic treatment. The proportions of industrial effluents used for (1) before treatment was 0.074% and (2) after treatment was 0.4%. These proportions corresponded to the LOAEL (lowest-observed-adverse-effect level) value obtained from the LC_{50} experiment.

For the evaluation of biomarkers of oxidative stress, 20 test systems were established, of which 5 were for the determination of the oxidative stress biomarkers before treatment and for each exposure time (12, 24, 48, 72, and 96 h), another 5 for the industrial effluent after treatment at each exposure time, and the remaining 10 corresponded to control systems of each time before and after treatment (free of the effluent). Each test system was added with 150 mg wet tissue of *H. azteca.*

After the exposure times, the specimens were extracted from each test system and homogenized using 1 mL of Tris buffer solution pH 7. The supernatant was centrifuged at 13, 000 × g for 15 min at −4 °C. The biomarkers of oxidative stress were evaluated to determine the cellular oxidation; the following tests were carried out: hydroperoxide content [HPC] (Jiang et al. 1992), lipid peroxidation [LPX] (Buege and Aust 1978), and protein carbonyl content [PCC] (Levine et al. 1994; Parvez and Raisuddin 2005; Burcham 2007) in order to assess oxidized protein levels. Antioxidant activity was also determined by superoxide dismutase activity [SOD] (Misra and Fridovich 1972) and catalase activity [CAT] (Radi et al. 1991). The aforementioned tests were carried out in triplicate before and after the photocatalytic treatment.

10.2.9 Statistical Analysis

In the acute toxicity assay (96 h LC50 of industrial effluent) before and after treatment, probit analysis was performed and significance assessed by the degree of 95% LC_{50} overlap, and also the value of LOAEL was calculated (EPA Analysis Program v1.5). The χ^2 linear adjustment test was not significant at $P < 0.05$. In the sublethal toxicity assays before and after treatment, statistical evaluation of results was done with one-way analysis of variance (ANOVA), and differences between means were compared using the Bonferroni's test, with P set at <0.05. Statistical determinations were made with the SPSS v10 software package (SPSS, Chicago IL).

10.3 Results

10.3.1 Physicochemical Characterization of Industrial Effluent Before and After Treatment

Table 10.1 shows the results of the physicochemical parameters determined in the industrial effluent under study before and after the photocatalytic treatment. These results were contrasted with the Mexican regulatory framework, especially with the Mexican official norms NOM-001-SEMARNAT, 1996, and NOM-073-ECOL, 1994. Some of the physicochemical parameters do not exceed the values of the mentioned norms as temperature, pH, chlorides, fluorides, hardness, total suspended solids, total phosphorus, total nitrogen, COD, and BOD. It is important to note that most of these parameters decreased drastically after the photocatalytic treatment of the industrial effluent. However, many of the parameters evaluated in this study are not considered in Mexican regulations. For example, among these parameters we can mention dissolved oxygen, conductivity, ammonia, NaClO, and TOC that had values of 13.1 mg/L, 147.2 µS/cm, 0.77 m/L, 1 mg /L, and 384 m/L, respectively, before treatment. However, the values of these parameters decreased substantially in 2.3, 16.3, 85.7, 100, and 94.3%, respectively, after treatment.

10.3.2 NSAID Concentrations Before and After Treatment of Industrial Effluent

The concentrations of NSAIDs, DCF, IBP, NPX, and PCT are shown in Table 10.2. As can be highlighted, the NSAIDs that were found in the highest concentrations in the industrial effluent were NPX and PCT. As can be seen, the concentration of DCF, IBP, NPX, and PCT decreased drastically by 78.8, 82.3, 82.7, and 86.9%, respectively, after the photocatalytic treatment.

Table 10.1 Physicochemical characteristics of the industrial effluent evaluated before and after treatment

Physicochemical characteristics	NOM-001-SEMARNAT-1996	NOM-073-ECOL-1994	Industrial effluent before treatment	Industrial effluent after treatment
Temperature (°C)	40	40	16.3	18
Dissolved oxygen (mg/L)	NI	NI	13.1	12.8
Conductivity (μS/cm)	NI	NI	147.2	123.1
pH	6.5–8.5	6–9	6.8	6.5
Chlorides (mg/L)	Maximum 250	NI	132	12
Fluorides (mg/L)	0–15	NI	4.3	0.2
Hardness (mg/L)	Maximum 500	NI	238.4	102.7
Ammonia (mg/L)	NI	NI	0.77	0.11
Total suspended solids (mg/L)	60	150	38	0
Total phosphorous (mg/L)	10	10	7.6	2.8
Total nitrogen (mg/L)	25	NI	19	8
Chemical oxygen demand (COD) (mg/L)	NI	300	193	51
Biochemical oxygen demand (BOD) (mg/L)	60	100	37	16
NaClO (mg/L)	NI	NI	1.0	0
Total organic carbon (TOC) (mg/L)	NI	NI	384	22

Table 10.2 NSAID concentrations of the industrial effluent before and after treatment. Values are the mean of three replicates ± SEM

NSAIDs	Industrial effluent before treatment	Industrial effluent after treatment
DCF μg/L	104.63 ± 0.05	22.1 ± 0.03
IBP μg/L	100.40 ± 0.03	17.8 ± 0.02
NPX μg/L	1717.31 ± 0.03	296.8 ± 3.1
PCT μg/L	3034.41 ± 0.02	425.3 ± 4.9

10.3.3 Median Lethal Concentration (LC50) 96 h of Industrial Effluent Before and After Treatment

Before treatment, the LC_{50} value at 96 h of the industrial effluent on the amphipod *H. azteca* was 0.732% with 95% confidence intervals between 0.725 and 0.741. The χ^2 linear adjustment test was not significant at $P \leq 0.05$. After the photocatalytic treatment, the LC_{50} value was of 3.889% with 95% confidence intervals of 3.542–4.276. As can be seen, after the treatment, there was a reduction in acute toxicity by approximately 430% with respect to the untreated industrial effluent.

With the results of LC_{50}, the values of the LOAEL were determined, which in the case of the untreated effluent was 0.074% and the treated effluent of 0.4%. These proportions of the effluent were those that were used to expose the organisms at different times to evaluate the oxidative stress biomarkers.

10.3.4 Oxidative Stress Evaluation

10.3.4.1 Hydroperoxide Content

Figure 10.1 shows the concentrations of hydroperoxides induced on the amphipod *H. azteca* exposed to the industrial effluent, before and after the photocatalytic treatment. As can be seen in the untreated effluent, there was an increase in the biomarker evaluated at all exposure times, 12, 24, 48, 72, and 96 h, compared to the control group of 33.3, 55.2, 84.6, 81.8, and 146.2%, respectively. After treatment, a significant decrease in HPC was observed compared to the amphipods exposed before treatment ($P \leq 0.05$) in all exposure times. These decreases were 20, 24.4, 42.5, 43, and 56.3%, respectively.

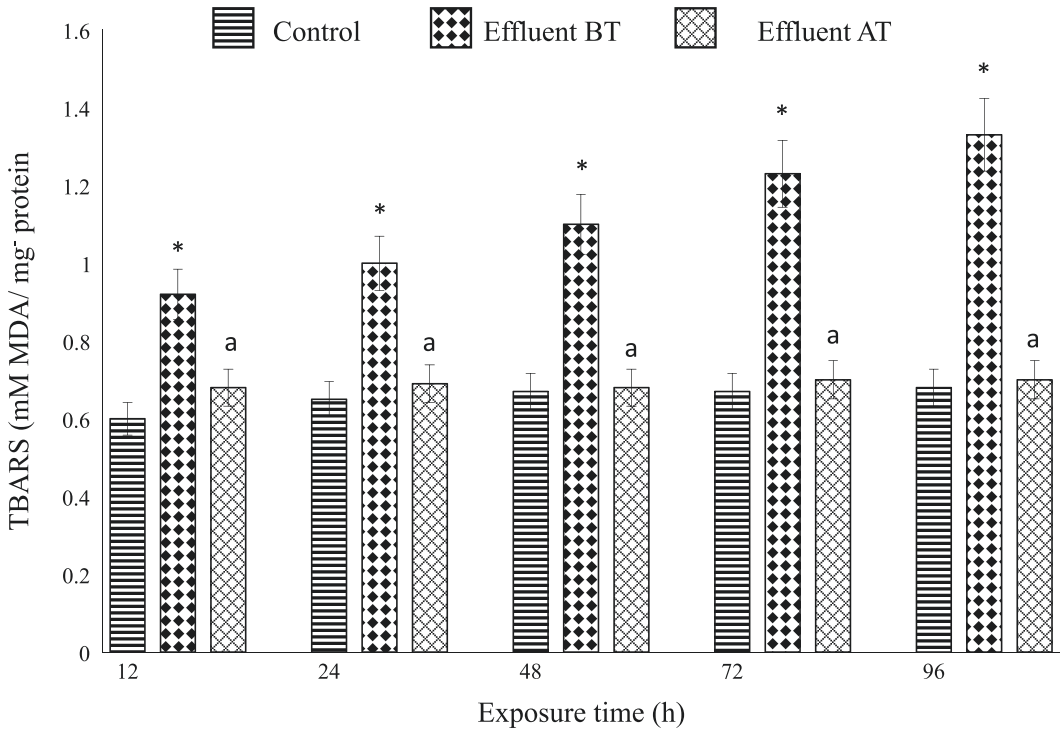

Fig. 10.1 Hydroperoxide content (HPC) in *H. azteca* exposed to industrial effluent before and after treatment at 12, 24, 48, 72, and 96 h. Values are the mean of three replicates ± SE. CHP cumene hydroperoxide, BT before treatment, AT after treatment, *Significantly different from control values. [a]Significantly different from before treatment, ANOVA and Bonferroni ($P \leq 0.05$)

10.3.4.2 Lipid Peroxidation (LPX)

The amount of malondialdehyde induced by the exposure of the amphipod to the industrial effluent before and after treatment is shown in Fig. 10.2. As can be seen in the industrial effluent before treatment, there was an increase in the MDA at all exposure times, 12, 24, 48, 72, and 96 h, compared to the control group of 33, 41, 50.6, 50.6, and 54.2%, respectively. After treatment, a significant decrease in MDA was observed compared to *H. azteca* exposed before treatment ($P \leq 0.05$) in all exposure times. These decreases were 23, 36.1, 41.3, 46.8, and 47%, respectively.

10.3.4.3 Protein Carbonyl Content (PCC)

Protein carbonyl content in specimens exposed to industrial effluent before and after photocatalytic treatment is shown in Fig. 10.3 (Buege and Aust 1978). Significant increases in this oxidation cellular marker with respect to control were observed ($P \leq 0.05$) in all exposure times before treatment. These increases were 55.3, 54, 64.2, 83.6, and 95.6% at 12, 24, 48, 72, and 96 h respectively. After photocatalytic treatment, a significant decrease was observed in PCC compared to the amphipods

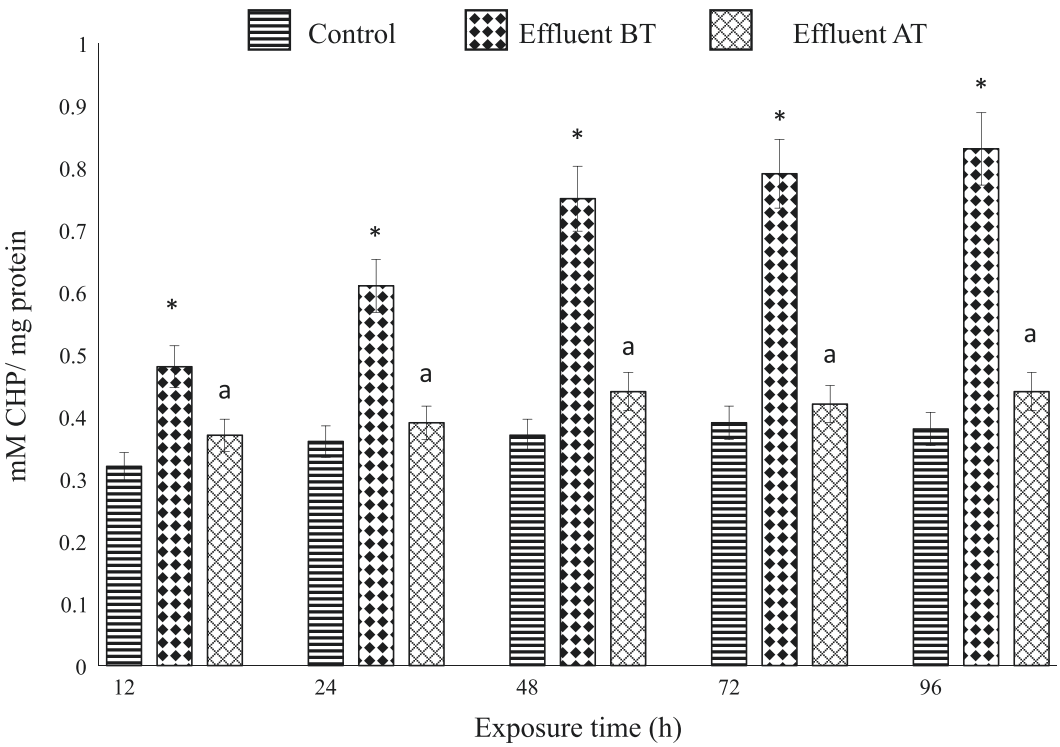

Fig. 10.2 Lipid peroxidation (LPX) in *H. azteca* exposed to industrial effluent before and after treatment at 12, 24, 48, 72, and 96 h. Values are the mean of three replicates ± SE. MDA, malondialdehyde. BT before treatment, AT after treatment. *Significantly different from control values. [a]Significantly different from before treatment, ANOVA and Bonferroni ($P \leq 0.05$)

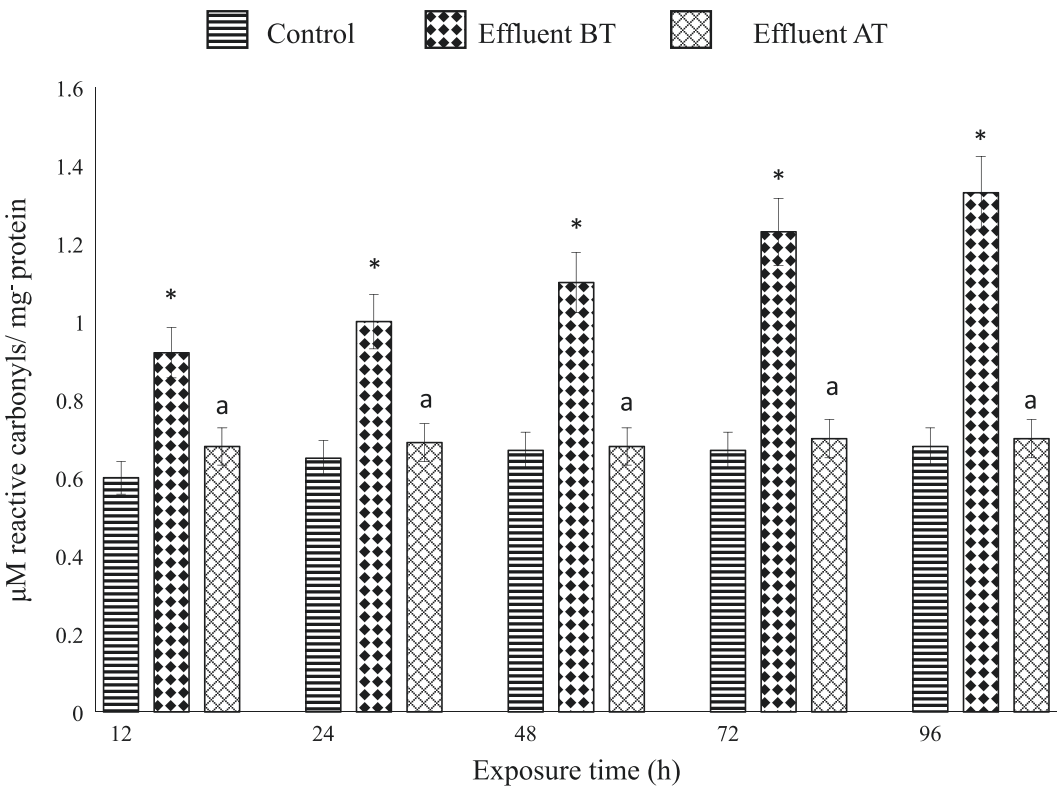

Fig. 10.3 Protein carbonyl content (PCC) in *H. azteca* exposed to industrial effluent before and after treatment at 12, 24, 48, 72, and 96 h. Values are the mean of three replicates ± SE. BT before treatment. AT after treatment. *Significantly different from control values. [a]Significantly different from before treatment, ANOVA and Bonferroni ($P \leq 0.05$)

exposed before treatment ($P \leq 0.05$) in all exposure times. These decrements were 26.1, 31, 38.2, 43.1, and 47.4, respectively.

10.3.4.4 Superoxide Dismutase Activity

Industrial effluent before photocatalytic treatment induced significant increases with respect to the control group ($P \leq 0.05$) which is shown in Fig. 10.4**.** The observed increases were 83.7, 81.8, 108.3, 100, and 121.4% at 12, 24, 48, 72, and 96 h, respectively. After photocatalytic treatment, a significant decrease was observed in SOD activity compared to the amphipods exposed before treatment ($P \leq 0.05$) in all exposure times. These decrements were 44.4, 35, 36, 38.6, and 38.7, respectively.

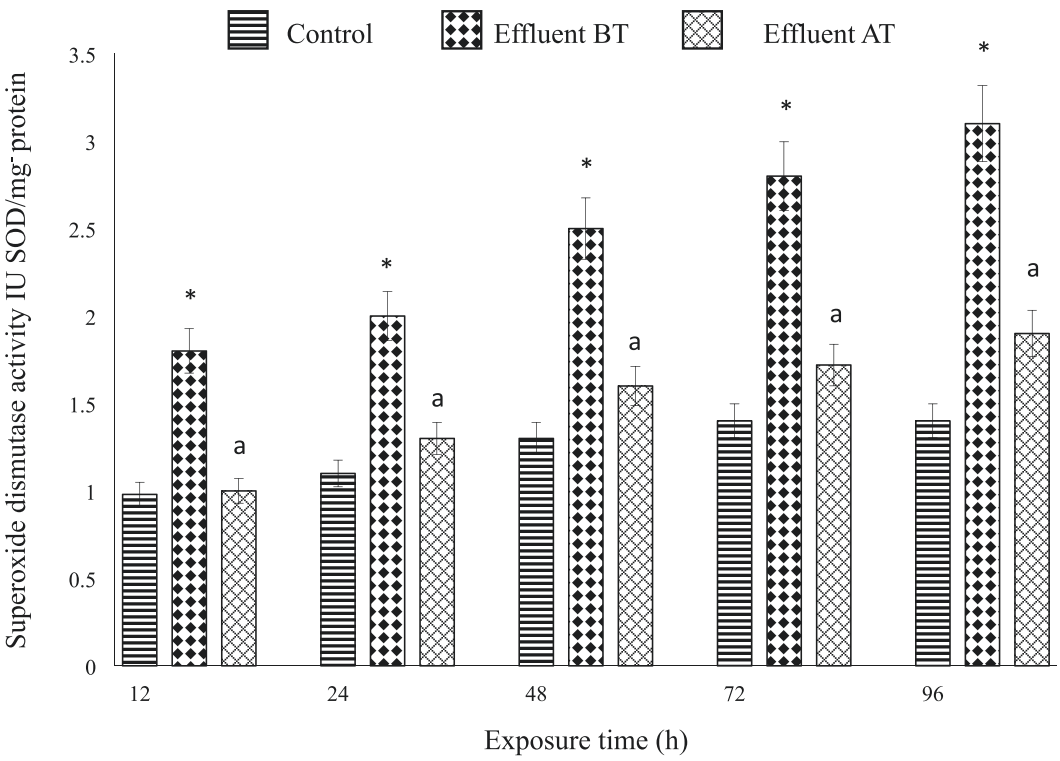

Fig. 10.4 Superoxide dismutase (SOD) activity in *H. azteca* exposed to industrial effluent before and after treatment at 12, 24, 48, 72, and 96 h. Values are the mean of three replicates ± SE. BT before treatment, AT after treatment. *Significantly different from control values. [a]Significantly different from before treatment, ANOVA and Bonferroni ($P \leq 0.05$)

10.3.4.5 Catalase Activity

The industrial effluent before photocatalytic treatment induced significant increases with respect to the control group in the biomarker evaluated ($P \leq 0.05$). The observed increases in CAT activity were 41.6, 46.2, 50, 71.4, and 86.6% at 12, 24, 48, 72, and 96 h, respectively (Fig. 10.5). After photocatalytic treatment, a significant decrease was observed in CAT activity compared to the amphipods exposed before treatment ($P \leq 0.05$) in all exposure times. These decrements were 17.6, 26.3, 23.8, 29.2, and 32.1%, respectively.

10.4 Discussion

The indiscriminate use of drugs and their improper elimination have generated the entry of these compounds into aquatic ecosystems by municipal, hospital, and industrial discharges, which has caused various deleterious effects on the environment and on species of ecological interest (Pérez-Alvarez et al. 2018; Perez-Coyotl et al. 2017; Olvera-Néstor et al. 2016; Morachis-Valdez et al. 2015). The relevance

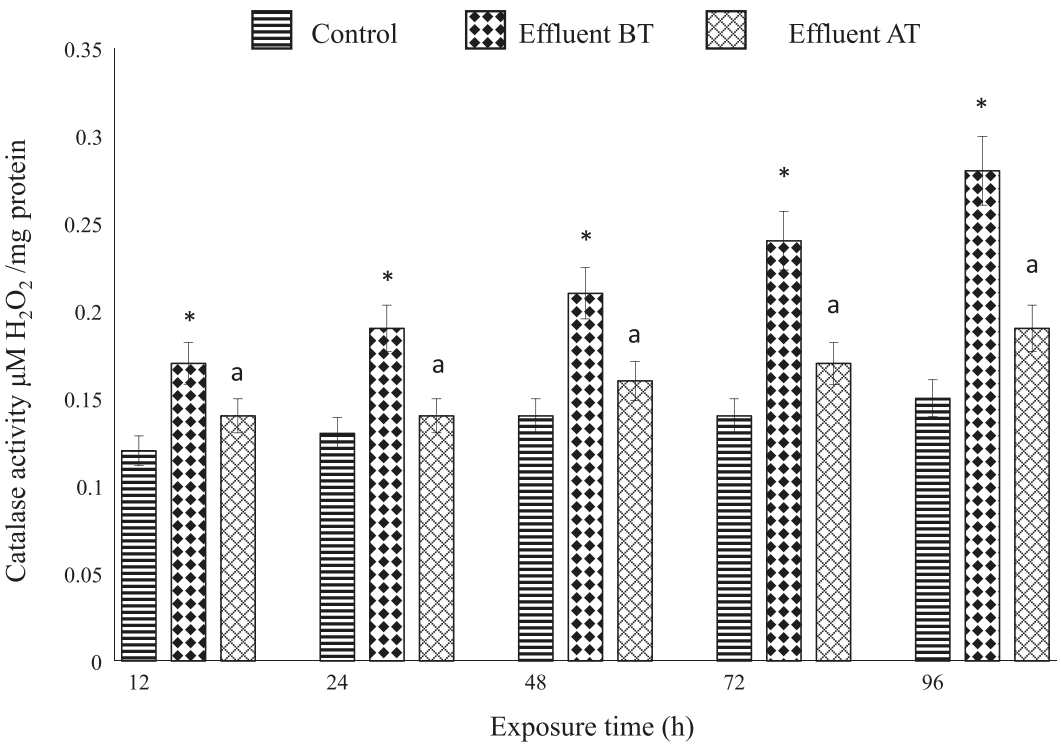

Fig. 10.5 Catalase (CAT) activity in *H. azteca* exposed to industrial effluent before and after treatment at 12, 24, 48, 72, and 96 h. Values are the mean of three replicates ± SE. BT before treatment, AT after treatment. *Significantly different from control values. [a]Significantly different from before treatment, ANOVA and Bonferroni ($P \leq 0.05$)

of this work lies in the fact that the proposed methodologies will establish the biosecurity of a photocatalytic process, which shows to be efficient for the removal of emerging contaminants present in an effluent from a pharmaceutical industry.

One of the indicators that is usually used to verify water quality are the physicochemical characteristics; however these are not useful to verify the toxicity of the water. One of the physicochemical parameter of water, which are useful for the organisms that inhabit it to survive adequately is the pH. In the case of the bioindicator *H. azteca*, the pH is fundamental since it cannot resist abrupt changes of this parameter and that can put its integrity at risk. It is also well known that pH is a determinant in the ionization of some pollutants and that in some cases it may favor the absorption of compounds by organisms (Olvera-Néstor et al. 2016). In this study, the pH value was kept within Mexican official norms, before and after the photocatalytic treatment, and in addition, the organisms under study were well adapted to these pH values.

Ammonia and conductivity are other deterministic physicochemical parameters to assess the quality of water, since they are considered as confounding factors in the bioeffects of the micropollutants found in the industrial effluent such as DCF, IBP, NPX, and PCT. A study conducted by (Postma et al. 2002) refers that the highest values at which they observed no significant effects in microcontaminants were ammonia 13–60 mg L^{-1} and conductivity <650 µS cm^{-1}. The results obtained in our

study showed that before the photocatalytic treatment, the values of these parameters cannot be considered as confounders. In addition, after the photocatalytic treatment, these parameters decreased substantially for the case of conductivity in 16.4% and for ammonia in 91.53%.

One of the compounds identified in the industrial effluent prior to the treatment was NaClO, which is generally used as a disinfectant and sanitizer in the pharmaceutical industry. This compound is important because when combined with humic and fulvic acids normally present in surface water, it can form highly toxic compounds such as haloalkanes, haloacetic acids, haloacetonitriles, and haloaldehydes (Boorman et al. 1999; Galal-Gorchev et al. 1993). However, the NaClO after the photocatalytic treatment was completely eliminated, favoring the decrease in toxicity, as can be seen later with the evaluated biomarkers.

In general, the parameters FQ characterized in the industrial effluent under study were drastically reduced after the photocatalytic treatment. Howover, although some of the physicochemical parameters of the effluent studied are within the Mexican regulations, this does not imply that the effluents evaluated have a good quality, since within the regulatory framework of the country, the presence of emerging contaminants is not mentioned, and as already mentioned, it can generate deleterious effects in aquatic organisms. In addition, it must be considered that Mexican norms have not been updated for more than 20 years.

The chemical characterization of the effluent showed that it contained NSAIDs such as DCF, IBP, NPX, and PCT in concentrations between 100.4 and 3034 μg L^{-1}. But after the photocatalytic treatment, these pollutants were reduced between 78.8 and 86.9%. These data reinforce the chemical efficiency of the photocatalytic treatment. But it is important to consider that these compounds can be transformed by the abiotic characteristics of the system, such as photodegradation, or by biotic factors, such as biotransformation by organisms (*H. azteca*).

For the reasons mentioned above, the physicochemical characterization and the determination of micropollutants are not the only factors that are important for evaluating water quality. Therefore, water quality was evaluated in this study through the use of biological markers such as oxidative stress.

The results found in this study in the assessment of the biomarkers of oxidative stress, as well as those of the physicochemical characterization and NSAID concentrations, show that the photocatalytic treatment is very efficient and that it is also friendly with the organisms that may be present in the environment, once the industrial waters are discharged to the municipal discharges of the city.

As expected, due to the presence of various contaminants in the industrial effluent (NSAID and NaClO), the cell oxidation biomarkers (HPC, LPX, PCC) prior to treatment were found to be increased with respect to the control group. In the case of HPC, the increases were between 33.3 and 146.2%, in the LPX between 33 and 54.2%, and in the PCC between 55.3 and 95.6%. The increase in biomarkers can be attributed to all the components of industrial effluent; and mainly by the presence of NSAIDs, DCF, IBP, NPX, and PCT.

Several studies that have been done in our research group have shown that NSAIDs such as DCF, IBP, NPX, and PCT are able to increase the biomarkers of cellular oxidation in the amphipod *Hyalella azteca* (Gómez-Oliván et al. 2012, 2014; Oviedo-Gómez et al. 2010). The mechanisms through which these molecules can induce these responses are through their photodegradation, since generally the degradation products that are generated are hydroxylated compounds such as 5,4-dihydroxy-DCF, 3-hydroxy-DCF, 4-hydroxymethyl-DCF, 3-hydroxy 4-hydroxymethyl-DCF, 4-hydroxy-DCF, and 5-hydroxy-DCF in the case of DCF; 4-isobutyl acetophenone 1-(6-methoxy-2-naphthyl) ethanol and 2-acetyl-6-methoxy naphthalene for the IBP and NPX; and p-hydroxyacetanilide, p-hydroxyacetanilide glucuronide, and N-acetyl benzoquinoneimine for the PCT. These compounds have proved to be very reactive and have the possibility of forming adducts with macromolecules such as lipids, nucleic acids, and nucleophilic amino acid residues (Boelsterli 2003).

But in addition to abiotic transformations, especially by light activity, NSAIDs have also shown that they can undergo biotransformation by aquatic organisms as *Hyalella azteca*. This amphipod is capable of transforming these compounds, by the system of monooxygenase (cytochrome P450) through phase 1 producing an oxygenated intermediate called oxycytochrome P450 complex [P450 (Fe^{3+}) 0^{2-}], which is very unstable and which is decoupled during the biotransformation of NSAIDs, with the subsequent formation of radical anion superoxide and hydrogen peroxide, which are highly oxidant (Gómez-Oliván et al. 2014). These reactive oxygen species are highly reactive and can interact with the lipids of the membranes and with the proteins, generating the oxidation of these macromolecules and making them nonfunctional.

In addition to the fact that by PCT photodegradation N-acetyl benzoquinoneimine can be formed, the formation of this compound is also catalyzed by cytochrome P450 mixed-function oxidase and more specifically by the prostaglandin hydroperoxidase (Kemper 2008). Studies conducted by (Yen et al. 2007) have shown that N-acetyl benzoquinoneimine is an important inducer of the production of ROS and reactive nitrogen species and especially the peroxynitrite. The latter has a high affinity for the sulfhydryl group of the proteins promoting their oxidation (Chilo 1999).

The biotic transformations, mainly by photodegradation and the metabolic activity of *H. azteca* are variables that could have contributed directly to the increase of cell oxidation biomarkers in industrial effluents without previous treatment.

On the other hand, the results of the evaluation of antioxidant activity, in the untreated effluent, showed significant increases between 83.7 and 121.4% for SOD and between 41.6 and 86.6% for CAT, with respect to the control group ($P \leq 0.05$). Increase in the activity of these enzymes can be explained by the repair mechanisms that the amphipod has, since by O_2^* and H_2O_2 increase in *Hyalella azteca*, the elevation in SOD and CAT activity is promoted, since the SOD enzyme is able to dismute

the superoxide anion radical to form H_2O_2 that is metabolized to O_2 and water by CAT (van der Oost et al. 2003).

Also, since NSAIDs including DCF, IBP, NPX, and PCT affect the mitochondrion and consequently also oxidative phosphorylation, increased ROS production particularly of $O^{\bullet}{}_2^-$ may occur, resulting in increased SOD activity and higher levels of hydrogen peroxide (Asensio et al. 2007), as evident in the present study with exposure of *H. azteca* to these compounds, present in the industrial effluent before the photocatalytic treatment.

It is important to note that the results of the biomarkers of cellular oxidation and cellular antioxidation were drastically reduced after the industrial effluent was treated photocatalytically. In the case of HPC from 20 to 56.3%, LPX from 23 to 47%, PCC from 26.1 to 47.4%, SOD activity from 35 to 44.4%, and CAT from 17.6 to 32.1%, these results are very relevant, since they can indicate that the degradation of the compounds present in the untreated industrial effluent is very efficient, which is also verified with the concentrations of the NSAIDs in the effluent after the treatment; also they suggest that the process is reaching the mineralization without the formation of intermediaries that are more toxic than the original compounds.

As can be seen, the results of the physicochemical characterization; the concentrations of DCF, IBP, NPX, and PCT; the LC_{50} values; and the biomarkers of cellular oxidation and antioxidation before and after the photocatalytic treatment show that treatment with Sn-modified TiO_2 powders under UV irradiation is an efficient process that guarantees the biosecurity of treated effluents.

10.5 Conclusions

The toxicity of a pharmaceutical effluent before and after of photocatalytic treatment was evaluated. The physicochemical parameters of the industrial effluent decreased drastically after the treatment with TiO_2 doped with Sn. Prior to photocatalytic treatment, the studied industrial effluent was determined to be toxic to amphipod *Hyalella azteca*. After photocatalytic treatment there was a reduction in LC50 by approximately 430% with respect to the untreated industrial effluent. Biomarkers of cell oxidation and antioxidation (oxidative stress phenomenon) were drastically reduced after treatment. The treatment used is effective both chemically and biologically. The treatment with Sn-modified TiO_2 powders under UV irradiation can be proposed as an effective process for the elimination of emerging contaminants in effluents, being environmentally friendly.

References

Asensio C, Levoin N, Guillaume C, Guerquin MJ, Rouguieg K, Chrétien F, Chapleur Y, Netter P, Minn A, Lapicque F (2007) Irreversible inhibition of glucose-6-phosphate dehydrogenase by the coenzyme A conjugate of ketoprofen: a key to oxidative stress induced by non-steroidal anti-inflammatory drugs? Biochem Pharmacol 73:405–416

Boelsterli UA (2003) Mechanistic toxicology: the molecular basis of how chemicals disrupt biological targets. Taylor & Francis/CRC Press. [cited 3 September 2018]. Available from http://books.google.com/books?hl=en&lr=&id=lyFpIWr1H1MC&oi=fnd&pg=PA1&dq=Mechanistic+toxicology+:+the+molecular+basis+of+how+chemicals+disrupt+biological+targets&ots=2yBeUKp6u5&sig=AsHnQBSg7IoeqZb2yuf4qPwSVdw%5Cn http://www.worldcat.org/oclc/53003932

Boorman GA, Dellarco V, Dunnick JK, Chapin RE, Hunter S, Hauchman F, Gardner H, Mike C, Sills RC (1999) Drinking water disinfection byproducts: review and approach to toxicity evaluation. Environ Health Perspect 107:207–217

Buege JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310

Burcham PC (2007) Modified protein carbonyl assay detects oxidised membrane proteins: a new tool for assessing drug- and chemically-induced oxidative cell injury. J Pharmacol Toxicol Methods 56:18–22

Cardoso-Vera JD, Islas-Flores H, SanJuan-Reyes N, Montero-Castro EI, Galar-Martínez M, García-Medina S, Elizalde-Velázquez A, Dublán-García O, Gómez-Oliván LM (2017) Comparative study of diclofenac-induced embryotoxicity and teratogenesis in Xenopus laevis and Lithobates catesbeianus, using the frog embryo teratogenesis assay: Xenopus (FETAX). Sci Total Environ 574:467–475

Chilo NH (1999) El citocromo P450 y su rol en la hepatotoxicidad inducida por drogas. Enfermedades del Aparato digestivo 2:34–37

Cleuvers M (2004) Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxicol Environ Saf 59:309–315

Galal-Gorchev H, Ozolins G, Bonnefoy X (1993) Revision of the WHO guidelines for drinking water quality. Annali dell'Istituto Superiore di Sanita 29:335–345

Galar-Martínez M, García-Medina S, Gómez-Olivan LM, Pérez-Coyotl I, Mendoza-Monroy DJ, Arrazola-Morgain RE (2016) Oxidative stress and genotoxicity induced by ketorolac on the common carp Cyprinus carpio. Environ Toxicol 31:1035–1043

Geissen V, Mol H, Klumpp E, Umlauf G, Nadal M, van der Ploeg M, van de Zee SEATM, Ritsema CJ (2015) Emerging pollutants in the environment: a challenge for water resource management. Int Soil Water Conserv Res 3:57–65

Gómez-Oliván LM, Neri-Cruz N, Galar-Martínez M, Vieyra-Reyes P, García-Medina S, Razo-Estrada C, Dublán-García O, Corral-Avitia AY (2012) Assessing the oxidative stress induced by paracetamol spiked in artificial sediment on hyalella azteca. Water Air Soil Pollut 223:5097–5104

Gómez-Oliván LM, Neri-Cruz N, Galar-Martínez M, Islas-Flores H, García-Medina S (2014) Binary mixtures of diclofenac with paracetamol, ibuprofen, naproxen, and acetylsalicylic acid and these pharmaceuticals in isolated form induce oxidative stress on Hyalella azteca. Environ Monit Assess 186:7259–7271

Harmon RS, Remus J, McMillan NJ, McManus C, Collins L, Gottfried JL, DeLucia FC, Miziolek AW (2009) LIBS analysis of geomaterials: geochemical fingerprinting for the rapid analysis and discrimination of minerals. Appl Geochem 24:1125

Islas-Flores H, Manuel Gómez-Oliván L, Galar-Martínez M, Michelle Sánchez-Ocampo E, SanJuan-Reyes N, Ortíz-Reynoso M, Dublán-García O (2017) Cyto-genotoxicity and oxidative stress in common carp (Cyprinus carpio) exposed to a mixture of ibuprofen and diclofenac. Environ Toxicol 32:1637–1650

Jiang Z-Y, Hunt JV, Wolff SP (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 202:384–389

Jolibois B, Guerbet M (2005) Evaluation of industrial, hospital and domestic wastewater genotoxicity with the Salmonella fluctuation test and the SOS chromotest. Mutat Res – Genet Toxicol Environ Mutagen 565:151–162

Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. Ecol Ind 8:1–13

Levine RL, Williams JA, Stadtman EP, Shacter E (1994) [37] Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–357

Lucero GMA, Marcela GM, Sandra GM, Manuel GOL, Celene RE (2015) Naproxen-enriched artificial sediment induces oxidative stress and genotoxicity in Hyalella azteca. Water Air Soil Pollut 226:195

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Morachis-Valdez G, Dublán-García O, López-Martínez LX, Galar-Martínez M, Saucedo-Vence K, Gómez-Oliván LM (2015) Chronic exposure to pollutants in Madín Reservoir (Mexico) alters oxidative stress status and flesh quality in the common carp Cyprinus carpio. Environ Sci Pollut Res 22:9159–9172

Olvera-Néstor CG, Morales-Avila E, Gómez-Olivan LM, Galár-Martínez M, García-Medina S, Neri-Cruz N (2016) Biomarkers of cytotoxic, genotoxic and apoptotic effects in Cyprinus carpio exposed to complex mixture of contaminants from hospital effluents. Bull Environ Contam Toxicol 96:326–332

Orozco-Hernández L, Gutiérrez-Gómez AA, SanJuan-Reyes N, Islas-Flores H, García-Medina S, Galar-Martínez M, Dublán-García O, Natividad R, Gómez-Oliván LM (2018) 17B-Estradiol induces cyto-genotoxicity on blood cells of common carp (Cyprinus carpio). Chemosphere 191:118–127

Oviedo-Gómez DGC, Galar-Martínez M, García-Medina S, Razo-Estrada C, Gómez-Oliván LM (2010) Diclofenac-enriched artificial sediment induces oxidative stress in Hyalella azteca. Environ Toxicol Pharmacol 29:39–43

Parvez S, Raisuddin S (2005) Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish Channa punctata (Bloch). Environ Toxicol Pharmacol 20:112–117

Pennak RW (1978) Freshwater invertebrates of the United Sates. 2nd edn. Wiley, New York

Pépin J-M (2006) Impacts Écotoxicologiques De Certains Médicaments Dans L'Environnement:77. doi:http://hdl.handle.net/11143/7379

Pérez-Alvarez I, Islas-Flores H, Gómez-Oliván LM, Barceló D, López De Alda M, Pérez Solsona S, Sánchez-Aceves L, SanJuan-Reyes N, Galar-Martínez M (2018) Determination of metals and pharmaceutical compounds released in hospital wastewater from Toluca, Mexico, and evaluation of their toxic impact. Environ Pollut 240:330–341

Perez-Coyotl I, Martinez-Vieyra C, Galar-Martinez M, Gomez-Olivan LM, Garcia-Medina S, Islas-Flores H, Perez-Pasten Borja R, Gasca-Perez E, Novoa-Luna KA, Dublan-Garcia O (2017) DNA damage and cytotoxicity induced on common carp by pollutants in water from an urban reservoir. Madin reservoir, a case study. Chemosphere 185:789–797

Petrović M, Gonzalez S, Barceló D (2003) Analysis and removal of emerging contaminants in wastewater and drinking water. TrAC – Trends Anal Chem 22:685–696

Postma JF, De Valk S, Dubbeldam M, Maas JL, Tonkes M, Schipper CA, Kater BJ (2002) Confounding factors in bioassays with freshwater and marine organisms. Ecotoxicol Environ Saf 53:226–237

Radi R, Turrens JF, Chang LY, Bush KM, Crapo JD, Freeman BA (1991) Detection of catalase in rat heart mitochondria. J Biol Chem 266:22028–22034

Richardson SD (2009) Water analysis: emerging contaminants and current issues. Anal Chem 81:4645–4677

SanJuan-Reyes N, Gómez-Oliván LM, Galar-Martínez M, García-Medina S, Islas-Flores H, González-González ED, Cardoso-Vera JD, Jiménez-Vargas JM (2015) NSAID-manufacturing

plant effluent induces geno- and cytotoxicity in common carp (Cyprinus carpio). Sci Total Environ 530–531:1–10

Saucedo-Vence K, Dublán-García O, López-Martínez LX, Morachis-Valdes G, Galar-Martínez M, Islas-Flores H, Gómez-Oliván LM (2015) Short and long-term exposure to diclofenac alter oxidative stress status in common carp Cyprinus carpio. Ecotoxicology 24:527–539

van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149

Yen FL, Wu TH, Lin LT, Lin CC (2007) Hepatoprotective and antioxidant effects of Cuscuta chinensis against acetaminophen-induced hepatotoxicity in rats. J Ethnopharmacol 111:123–128

Zounkova R, Kovalova L, Blaha L, Dott W (2010) Ecotoxicity and genotoxicity assessment of cytotoxic antineoplastic drugs and their metabolites. Chemosphere 81:253–260

Chapter 11
Effects of River Pollution on Its Biota: Results from a 20-Year Study in the Suquía River Basin (Córdoba, Argentina)

María Valeria Amé, María Laura Ballesteros, María de los Ángeles Bistoni, Andrea Cecilia Hued, Magdalena Victoria Monferrán, and Daniel Alberto Wunderlin

11.1 Introduction

The Suquía River receives a complex mixture of pollutants from anthropogenic activities, sewages, agricultural and industrial effluents from point and nonpoint pollution sources. Furthermore, it is particularly vulnerable to pollution due to its scarce and seasonal flow, short length, and endorheic basin (Merlo et al. 2011). The presence of pollutants in a compartment of the aquatic ecosystem is not, by itself, a direct indication of harmful effects on the inhabiting biota. Associations must be established between external levels of exposure, internal levels of tissue contamination, and early adverse effects. Therefore, the exposure to and effects of chemical contaminants or pollutants on the aquatic ecosystem have been extensively studied by environmental toxicologists (van der Oost et al. 2003).

The identity and concentration of pollutants in the Suquía River Basin has been extensively reported (Monferrán 2018; Santiago et al. 2018). Here, we will examine the accumulation of several chemicals in the exposed native biota (Biomarkers of

M. V. Amé · M. V. Monferrán
Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Córdoba, Argentina

CONICET, Centro de Investigaciones en Bioquímica Clínica e Inmunología – CIBICI, Córdoba, Argentina

M. L. Ballesteros · M. Bistoni · A. C. Hued
Universidad Nacional de Córdoba, Facultad de Ciencias Exactas, Físicas y Naturales, Córdoba, Argentina

CONICET, Instituto de Diversidad y Ecología Animal – IDEA, Córdoba, Argentina

D. A. Wunderlin (✉)
Universidad Nacional de Córdoba, Facultad de Ciencias Químicas, Córdoba, Argentina

CONICET, Instituto de Ciencia y Tecnología de Alimentos Córdoba – ICYTAC, Córdoba, Argentina
e-mail: dwunder@fcq.unc.edu.ar

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_11

exposure) and the biological responses that could be associated with a gradient of pollution in the Suquía River (Biomarkers of effect). These biological responses will cover different biological organization levels: from molecular to community level.

11.2 Biomarkers of Exposure in Native Biota

11.2.1 Cyanotoxins in Fish and Shrimp

Serious eutrophication accompanied by the presence of massive cyanobacterial blooms and cyanotoxins has been documented in many rivers, lakes, and wetlands worldwide. The San Roque Reservoir is not an exception and, according to measured concentrations of nutrients and associated biomass production, it could be classified as eutrophic or hypereutrophic (Amé et al. 2003).

Cyanobacterial blooms have occurred in the San Roque Reservoir for about 40 years (Scarafia et al. 1995; Pizzolón et al. 1997). *Dolichospermum*, *Microcystis*, *Chroococcus*, *Oscillatoria*, *Pseudoanabaena*, *Phormidium*, *Lyngbya*, and *Nodularia* are among the cyanobacteria genus more frequently described in this water body (Brandalise et al. 2007). The presence of cyanotoxins in the San Roque Reservoir has been informed since 1997. Particularly, the occurrence of microcystins (MC) in different monitoring campaigns reached 97% of the analyzed water samples, with concentrations ranging from nondetectable levels to 450 μg L^{-1}. Maximum values were found in summer and autumn (Amé et al. 2003; Ruibal Conti et al. 2005). Moreover, the hepatotoxin nodularin and the neurotoxin anatoxin-a have also been detected in the San Roque Reservoir (Galanti et al. 2013; Ruiz et al. 2013). Noduralin concentrations in the reservoir water reached 0.25 μg L^{-1}, while the maximum value for anatoxin-a was 13 ng L^{-1}. Both levels are low when compared with other water bodies, showing that the main health hazard to humans, aquatic animals, livestock, and wildlife in the San Roque Reservoir is associated with MC occurrence.

In the aquatic environment, cyanotoxins are mainly released into the surrounding water during cyanobacterial cell senescence, death, and lysis. Therefore, the biota may take up and accumulate cyanotoxins from two main routes: dissolved toxins via a transdermal route, or ingestion via food intake (Ibelings and Chorus 2007).

In order to evaluate the accumulation of MC in wild fish exposed to toxic cyanobacterial blooms in the San Roque Reservoir (SRR, 31°22′21.84″ South; 64°28′8.03″ West; Fig. 11.1), Cazenave et al. (2005a) captured *Odontesthes bonariensis* (Pisces, Atherinidae) during the wet season (November to March) and during the dry season (May to October) of 2004. Analyses of gastrointestinal tract of *O. bonariensis* showed presence of cyanobacterial cells, evidencing the ingestion of bloom material by this fish species. MC-RR was found in both external (gills) and internal tissues (liver, muscle); hence, the ingestion can be proposed as the most probable route of toxin uptake in this wild fish. Nevertheless, since dissolved MC-RR was recorded in the water where fish were captured, uptake via gills would not be discarded.

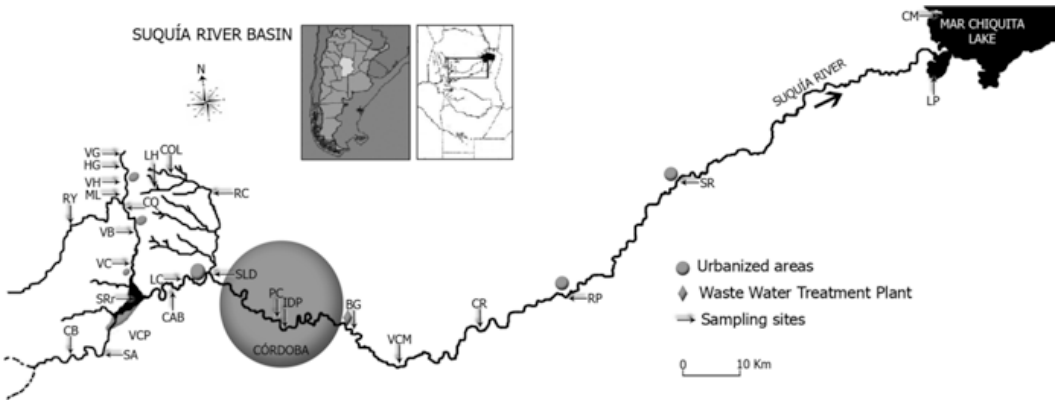

Fig. 11.1 Suquía River Basin with sampling sites: COL: Colanchanga Brook; LH: Los Hornillos Brook; RC: Río Ceballos Brook; VG: Villa Giardino, San Francisco River; HG: Huerta Grande, San Francisco River; VH: Valle Hermoso, San Francisco River; ML: Molinari, San Francisco River; CQ:Cosquín, Cosquín River; VB: Villa Bustos, Cosquín River; VC: Villa Caeiro, Cosquín River; RY: Río Yuspe, Yuspe River; CB: Cuesta Blanca, San Antonio River; SA: San Antonio de Arredondo, San Antonio River; VCP: Villa Carlos Paz, San Antonio River; SRr: San Roque Reservoir; CAB: Casabamba, Suquía River; LC: La Calera, Suquía River; SDN: Saldán, Suquía River; PC: Puente Cantón, Suquía River; IDP: Isla de los Patos, Suquía River; BG: Bajo Grande, Suquía River; VCM: Villa Corazón de María, Suquía River; CR: Capilla de los Remedios, Suquía River; RP: Río Primero, Suquía River; SR: Santa Rosa de Río Primero, Suquía River; CM: Campo Mare, Mar Chiquita Lake; LP: Laguna del Plata, Mar Chiquita Lake

Once absorbed by either gills or intestinal epithelia, a rapid spread of the toxin via the bloodstream with further distribution throughout the fish body may take place. The relative distribution of MC-RR in *O. bonariensis* revealed highest amounts of toxin in liver, followed by muscle and gills. Other field studies have shown that MC concentrations in fish tend to be highest in liver and intestine, rather lower in kidneys and gonads, and much lower in muscle tissue (Ibelings and Chorus, 2007 and other authors referenced therein). Therefore, the highest concentration of MC-RR in liver of *O. bonariensis* is in good agreement with this report. Nevertheless, compared to muscle tissues of the other studied fish species, *O. bonariensis* muscle accumulated the highest amounts of cyanotoxins. An analysis of seasonal differences showed that the concentration of MC-RR recovered from muscle ranged from traces during the dry season to a maximum of 0.339 μg g^{-1} during the wet season. Considering the average value for MC-RR in muscle of *O. bonariensis* (0.05 ± 0.11 μg g^{-1}), and assuming a 70-kg person eating 100 g fish muscle per day, the calculation reveals an average consumption of 5 μg MC-RR per day, which exceeds the tolerable daily intake (TDI) of 0.04 μg kg^{-1} body weight per day, recommended by the World Health Organization (WHO 1998). The risk of consumption of contaminated fish increases during the wet season, where the highest concentration of MC-RR in muscle was observed.

A cage study was also conducted in the San Roque Reservoir in order to evaluate cyanotoxin accumulation in the freshwater shrimp *Palaemonetes argentinus* (Galanti et al. 2013). During the 4-week exposure after a cyanobacteria bloom, MC levels in water were below quantification limits (0.1 μg L^{-1}). On the contrary,

nodularin was detected in all water samples analyzed during the study. Consistently, nodularin content in *P. argentinus* in the San Roque Reservoir ranged from 0.06 to 0.11 μg g^{-1}. However, the shrimp needed a 3-week exposure to accumulate nodularin at detectable levels, which is reasonable considering the low level of this toxin found in the water. *P. argentinus* is not habitually used for human consumption. However, this shrimp is preyed by fish present in the reservoir, thus constituting an important link for translating this toxin to humans through the food chain.

11.2.2 Persistent Organic Pollutants in Fish

Persistent organic pollutants (POPs), such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs), represent persistent, bioaccumulative, and toxic compounds of global concern (Wurl and Obbard 2005). During decades, the production and intensive agricultural or industrial use of these substances have led to widespread contamination of the environments. The high molecular weight and the low water solubility of most of these compounds lead to their bioaccumulation in the biota, mainly in fatty tissues as well as their biomagnification in the food chain (Newman and Unger 2003).

In Argentina, most of the OCPs and PCBs uses have been banned since 1998 and 2005, respectively, although their residues are still found in aquatic environments. Particularly, technical grade and active ingredients of endosulfan were included in the POPs list on April 2011 (UNEP 2011). There is not yet any legislation regarding PBDEs, and they have been used as flame retardants since 1970 worldwide (de Wit 2002).

The Mar Chiquita Lake (30°–30°55′ South; 62°–63°West) is located in the central region of Argentina, and is the largest continental saline shallow lake of South America. Because of its high salinity (>35 g L^{-1}), wildlife diversity, and great extension, since 1993, it is considered an important wetland of the Western Hemisphere Shorebird Reserve Network, and it was declared a RAMSAR site in 2002. Also, it is the final destination of three endorheic rivers, Dulce, Xanaes, and Suquía that run along urban and agricultural areas (Fig. 11.1). On the other hand, this lake is a stopover and final destination of around 60 migrant bird species (42 aquatic birds) from North America and other countries, including shorebirds of the Charadriidae and Scolopacidae families, and up to 500,000 individuals of the migrant Wilson's Phalarope (*Phalaropus tricolor,* Osinaga-Acosta et al. 2006) were registered in the lake. Most of the bird species inhabiting the Mar Chiquita Lake feed on fish and invertebrates, and therefore, they could be exposed to risk levels of POPs and also transport these compounds far away from this area during migration cycles. Among fish, silverside (*Odontesthes bonariensis*) is one of the two fish species capable of living in this high salinity waters; it is consumed by local populations and used for sport fishing activities, adding economic importance to the region. Ballesteros et al. (2014) conducted a study evaluating the levels of POPs in silverside of the Mar

Chiquita Lake. Three monitoring samplings were carried out (dry, rainy, and post-rainy seasons) at two locations in the southeastern coast of the Lake: (1) Laguna del Plata (LP, 30° 50′09.6″ South; 62° 53′21.6″ West; Fig. 11.1), located close to the Suquía River mouth where this freshwater system contributes to both urban and industrial wastes and (2) Campo Mare (CM, 30°48′49.4″ South/62°52′02.8″ West; Fig. 11.1), surrounded mainly by crops. The POP levels (OCPs: DDTs, HCH, endosulfans, PCBs, PBDEs, Heptachlors, Chlordanes, and Drins) were registered in the gills, liver, muscle, and digestive tracts. These compounds were determined and quantified according to Metcalfe and Metcalfe (1997) through gas chromatography with electron capture detection.

The levels of contaminants present in *O. bonariensis* organs showed a predominance of endosulfans and PCBs (15–85%), followed by PBDEs, DDTs, and HCHs, and, finally, Heptachlors, Chlordanes, and Drins, as a consequence of the recent use of endosulfan and the influence of industrial activities primarily contributed by the Suquía River. The accumulation varied according to the organ considered. Higher levels were found in liver and digestive tract content, followed by gills, and, finally, muscle. As it is well known, the liver is located in a strategic position within the body, receiving a large quantity of blood, which contributes to the distribution of toxics and its metabolites to other organs, and also accumulating organic contaminants (Ballesteros et al. 2011). On the other hand, the muscle is a good indicator of chronic exposure to pesticides. Therefore, the accumulation in both organs could represent good biomarkers of exposure to POPs in fish.

The highest levels of HCHs, endosulfans, and PCBs in silverside organs were registered on the postrainy season when compared to the other sampling times. In the study area, rainy periods are mainly concentrated on spring and summer seasons (from November to March). Contaminants enter to the aquatic ecosystems through runoff during rain events along the river basins, and finally end up in the Mar Chiquita Lake. Particularly, silverside of LP had the highest levels of POPs, because this station is located near the Suquía River mouth; this river is the main input of this pesticide into the lake during the rainy period. With respect to DDTs and PBDEs, the residue levels were low and similar between sampling stations and among periods.

Because *O. bonariensis* has an economic importance for the local population, it becomes necessary to highlight the levels of POPs in muscle of silverside and its relationship with the Oral Reference Dose (RfD) of this contaminants. Most POPs (HCHs, endosulfans, DDTs, and PBDEs) did not represent a risk for human health (Table 11.1). However, even though PCBs have been forbidden since 2005, the residue levels in fish muscle exceeded the RfD at both sampling stations (Table 11.1). Moreover, the PCB congener #118 was found in almost all fish tissues. This is a mono-ortho substituted molecule, and due to this chemical structure, it has the ability to pass through biological membranes easily, and therefore, it is considered highly toxic to organisms. This clearly shows that even though most of these contaminants have been phased out decades ago, they are still in the environment and could exert deleterious effects on the biota and, consequently, on human health.

Table 11.1 Persistent organic pollutants in a 300-g filet of *Odontesthes bonariensis*, oral reference dose (RfD), and µg in a 70-kg individual

SITE	POPs	Burden (µg)			RfD (µg kg^{-1} d^{-1})	µg in a 70 kg individual
		Post-rainy	Dry	Rainy		
Laguna del Plata	γ-HCH#	0.4	0.1	N/A	0.3	21.0
	∑ENDO#	7.2	1.0	N/A	6.0	420.0
	∑DDTs#	0.3	0.2	N/A	0.5[(d)]	35.0
	∑PCBs#	0.7	3.2	N/A	0.02[(c)]	1.4
	∑PBDEs∗	1.0	2.2	N/A	0.1[(a)], 2[(b)]	7.0
Campo Mare	γ-HCH#	0.3	0.1	0.2	0.3	21.0
	∑ENDO#	0.8	0.4	3.2	6.0	420.0
	∑DDTs#	0.5	0.1	0.3	0.5[(d)]	35.0
	∑PCBs#	3.3	0.6	1.7	0.02[(c)]	1.4
	∑PBDEs∗	0.4	0.4	1.5	0.1[(a)], 2[(b)]	7.0

∗IRIS EPA database (http://www.epa.gov/IRIS/ accessed may 2012); #ATSDR (http://www.atsdr.cdc.gov/accessed may 2012);); [(a)]BDE-47, [(b)]Penta BDE, [(c)]Arochlor 1254/1248, [(d)]*p p´*-DDT.∑HCHs: α−, β− and γ− isomers; ∑ENDO: α− + β− isomers + endosulfan sulfate; ∑DDTs: *p p´*-DDT+ *p p´*-DDE; ∑PCBS: #18, #44, #66, #101, #110, #118, #138, #153, #156, 167, #180 and #187; ∑PBDEs: #47, #100, #99 and #154; N/A: Not analyzed. Fish were not captured at this station during the rainy season

11.2.3 Metals in Plankton, Shrimp, and Fish

The concentrations of Ag, Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, Hg, Li, Mn, Mo, Ni, P, Pb, Rb, Se, Sr, Tl, U, V, and Zn were analyzed in plankton, shrimp (*Palaemonetes argentinus*), and fish (*Odontesthes bonariensis*) collected in the San Roque Reservoir (SRr, Fig. 11.1) during the wet and dry seasons (Griboff 2017).

In general, the concentrations of Ag, Al, As, Cu, Ga, Hg, Li, Ni, Pb, Rb, Se, and V in the biota (plankton, shrimp and fish) were either higher or presented no statistical differences during the dry season with respect to the values observed during the wet season ($p < 0.05$).

Accumulation of inorganic elements in plankton depends on several factors, such as the productivity of the water body, the physicochemical properties of the water, the quantitative and qualitative composition of metals and metalloids present in the environment, as well as the composition of the plankton (Mazej et al. 2010), which can lead to changes in the capacity of organisms to accumulate metals from the environment, increasing in dry season and decreasing in wet season; on the other hand, there was no clear seasonal pattern in the concentrations of elements in shrimp, which could indicate that the highest concentrations accumulated in dry season are due to changes in metabolic activity or to the growth rate or reproductive cycle of these organisms (Çoğun et al. 2005). The accumulation of metals and metalloids in fish results mainly from surface contact with water, respiration, and food. The capture of these three routes depends on the environmental levels of the elements in the organism's habitat. One possible explanation for the results obtained in the SRr is that *O. bonariensis* is a planktophagous fish, which feeds in free waters;

in addition to that, Griboff (2017) reported the highest concentrations of the studied elements in water from SRR during the dry season.

The highest levels of Ag, Al, As, Co, Cr, Fe, Li, Mn, Ni, U, and V were found in plankton, while Cu, P, Sr, and Zn showed the highest concentration in shrimp, and Se and Rb had the highest values in fish in wet season and Hg in dry season. Boron, Be, Bi, Mo, and Tl were not detected in any of the analyzed samples. Cadmium concentration in plankton was higher to those observed in shrimps during both seasons. However, Cd was not detected in fish. Zinc, Cu, Fe, Mn, Ni, Mo, and Al are essential elements with important metabolic functions. Thus, most organisms have biochemical mechanisms to regulate the amount of these elements within their cells. On the other hand, Cd is a nonessential element that competes with calcium for enzymatic sites. Even though Ca and Cd have long half-lives in the biota, they are poorly absorbed from food (Regoli et al. 2012). Although the above information could help interpret the intraspecific differences in metal concentrations between both studied seasons, a great number of studies demonstrate that the bioaccumulation of metals in organisms depends on the particular element and species involved. The handling strategy of each species, determined by the metal assimilation efficiency, metal efflux rates, and ingestion activity, as well as physiological requirements of the organisms (Wang et al. 2011), determine, among other factors, the final concentration of the element. For instance, invertebrates like shrimp are known to be strong net metal accumulators, especially Cu (Cui et al. 2011). This is because shrimps have a hemocyanin molecule in their hemolymph, whose function is to transport oxygen through the body, replacing hemoglobin, for which they have two Cu atoms instead of Fe.

Concentrations of Ni, Mn, P, Fe, Cr, and Al were higher in plankton than in shrimp or fish. The concentration of Fe in plankton was the highest among all measured elements, being over 10,000 μg g^{-1} in both seasons. Phosphorus was the second abundant element in plankton, ranging from 5600 to 63,008 μg g^{-1}. The concentration of Al reposted a mean of 4900 μg g^{-1}. The mean concentration of Ni in plankton was 9 μg g^{-1}, without significant differences between both sampling seasons. Plankton consists of zooplankton and phytoplankton; the latter may consist of different varieties of unicellular algae, which are rich in oligoelements and minerals such as Fe, Al, Mn, and Ni, as they have a great capacity to store elements from the surrounding environment and, therefore, these algae are usually selected as bioindicators of environmental pollution (Jun and Min 2012). The concentration of metals in the plankton of the SRr was similar to, or lower than, that reported by Tao et al. (2012) in lakes with low wastewater contamination.Summarizing our current results, we found higher concentrations of Al, Ag, As, Co, Cr, Fe, Li, Mn, Ni, U, and V in plankton, with the highest levels of Cu, P, Sr, and Zn in invertebrates (shrimp), while Hg, Se, and Rb were elements found in the highest concentration in fish. Farag et al. (2007) also reported higher levels of Cu and Zn bioaccumulated in invertebrates in comparison with fish from the Boulder River watershed from Montana. This behavior can be caused by differences in mechanisms involved in the accumulation and detoxification of metals; for instance, metallothioneins (MTs) can bind metals, increasing their concentration in certain tissues/organs, such as the liver (Laura, 2009).

On a previous work, the distribution of metals in different organs of *O. bonariensis* was studied (Monferrán et al. 2016). The comparison of the extent of heavy metal accumulation in analyzed tissues showed that differences in the distribution among different tissues were statistically significant for all studied elements ($p < 0.05$). As, Cu, Mo, and Fe showed the highest concentrations in fish liver during the studied period, while Ag and Cr were present at high concentration only during the wet season. On the other hand, no statistically significant differences were observed in any studied organ during the dry season. Concentrations of Al, Ni, Zn, Mn, Nd, and Ce were the highest in gills during both studied seasons, also in agreement with a previous report on the Pontic shad (*Alosa immaculata*) from the Danube River (Visnjic-Jeftic et al. 2010).

Overall, the highest concentrations of most of the analyzed elements were recorded in the liver and gills, while the lowest ones were present in the brain, with some exceptions such as Ni, Cu, and Cr. Such pattern has been observed in a number of other studies, covering a wide range of fish species (Dural et al. 2006; Storelli et al. 2006, Ploetz et al. 2007). Muscle is generally considered to have a weak accumulating potential (Jarić et al. 2011). Conversely, the high cumulative ability of the liver is the result of the activity of metallothioneins, proteins that can be bound to some metals, such as Cu and Zn, thus reducing their toxicity and allowing the liver to accumulate them at high concentrations (Ploetz et al. 2007). Due to the reasons discussed above, liver has been recommended by many authors as the best environmental indicator of both water pollution and chronic exposure to heavy metals (Jarić et al. 2011). Gills could be important as they present a site for direct contact with metals and other elements dissolved or suspended in water. High metal concentrations in gills can point out to water as the main source of pollution (Jarić et al. 2011). According to Dural et al. (2006), and Erdoğrul and Erbilir (2007), the levels of total metals in gills can be influenced by the absorption of metals onto the gill surface, but also by the element complexation with the mucous, that is very difficult to remove from lamellae prior to the analysis.As, Cr, Fe, Mn, and Zn concentrations in muscle of *O. bonariensis* from the SRr were higher than those reported in other Argentinean lakes and lagoons for the same species (Avigliano et al. 2015); however, Hg, Ni, and Cd concentrations were in agreement with those results.

11.3 Effects of Pollutants on Biomarkers from Biota Inhabiting the Suquía River Basin

11.3.1 Effects on Macrophytes, Mussels, and Fish at Molecular Level

11.3.1.1 Biotransformation and Antioxidant Response

It is practically impossible to control all pollutants from anthropogenic and natural origin that may be a potential threat to the environment. In order to assess the overall quality of the aquatic environment, a more promising approach is to examine

biochemical responses reflecting the potential of contaminants to impair physiological processes in exposed biota. For this purpose, various biochemical parameters have been extensively studied, and the enzymes involved in the detoxification of xenobiotics and their metabolites (biotransformation enzymes, antioxidant enzymes) are among the most studied ones (van der Oost et al. 2003).

In the Suquía River, the activity of biotransformation and antioxidant enzymes has been measured in macrophytes, invertebrates, and fish through active and passive biomonitoring.

Macrophytes from the *Myriophyllum quitense* species were collected during the spring season, from an almost unpolluted site (Colanchanga Brook, COL; 31° 8′32.08″ South; 64°21′23.55″ West), and then transplanted to five stations: Los Hornillos (LH Brook, 31° 9′43.66″ South; 64°22′58.67″ West) located in a second unpolluted area; Río Ceballos (RC Brook, 31°10′33.23″ South; 64°18′51.19″ West); La Calera (LC, Suquía River, 31°21′24.7″ South; 64°23′18.7″ West, 18 km upstream from Córdoba city); Isla de los Patos (IDP, Suquía river, 31°23′59.05″ South; 64°12′15.63″ West, at Córdoba downtown); and Bajo Grande (BG, Suquía River, 31°24′32.98″ South; 64° 4′49.26″ West, downstream from Córdoba city and 3 km downstream from the sewage of the Waste Water Treatment Plant (WWTP) of the city) (Fig. 11.1). After a 3-week exposure, *M. quitense* responded to the pollution, inducing its antioxidant and biotransformation enzyme system, expressed through an increased activity of glutathione S-transferase (GST), guaiacol peroxidase (POD), and glutathione reductase (GR) in highly polluted sites (RC and BG). Increased POD and GR activities of *M. quitense* exposed to BG may be attributed to an elevated nutrient concentration, combined with high lead contents in the sediment, and low oxygen levels in water. At RC station, the only significant detectable pollution source was the Al content in the sediment, elevating significantly the activities of GR and GST, reflecting possible consequences of oxidative damage originated by the aluminum ions in the organism (Nimptsch et al. 2005).

A similar study was conducted with the invasive and nonnative golden mussel *Limnoperna fortunei* (Contardo-Jara et al. 2009). For this cage study, organisms were collected from the San Roque Reservoir and, after a 2-week acclimatization period under laboratory conditions, exposed for 1, 4, and 7 days at Río Yuspe (RY, 31°14′18.55″ South; 64°31′13.31″ West, another unpolluted area), LC, IDP, and Villa Corazón de María (VCM, 31°26′50.19″ South; 63°59′26.58″ West, located 20 km downstream from Córdoba City and 16 km downstream from the WWTP). The antioxidant enzymes GPx, GR, and CAT were sensitive to respond to the different pollution scenarios and correlated in their response intensity to the Water Quality Index (WQI), which, in turn, integrates the chemical characterizations for the sampling sites. GPx, GR, and CAT, together with GST measured in the microsomal fraction, responded by either induction or inhibition at the most polluted sampling site (VCM) within 1 day. Subsequently, the same enzymes showed an induction after a 4-day exposure, but further sampling was restricted at this site due to the nonsurvival of the animals after 1 week. Certain response patterns might only be explained by a closer look on contaminants or unexpected charges, e.g., the pronounced antioxidant response in mussels exposed at Río Yuspe, a quasipristine, but iron- and nickel-charged sampling site.

In another study, adult specimens of the native species *Jenynsia multidentata* were sampled in the Suquía River twice seasonally during two years. Fish were captured at five selected sites: LC, VCM, Capilla de los Remedios (CR, 31°26′4.83″ South; 63°49′53.61″ West), Río Primero (RP, 31°20′16.53″ South; 63°36′31.01″ West), and Santa Rosa de Río Primero (SR, 31° 9′26.99″ South; 63°23′38.06″ West). The last three sites were located at 35, 50, and 80 km, respectively, downstream from the WWTP, and primarily were affected by the input of pollutants from the city sewage and agricultural runoff. GST, GR, GPX, and CAT tended to increase their activity with worse water quality (VCM), and decreased their activity downstream, where the consequences of sewage discharge were reduced. Comparing the enzymes activity in different tissues, it is observed that gills and brain present a more clear response to the pollution gradient in comparison to liver. Moreover, in GST and GR activities, the effects are more pronounced during the dry season in the gills and brain. On the other hand, GPx and CAT responded well to pollution only in the wet season. Thus, increased GST, GR, CAT, and GPX activities observed in *J. multidentata* from VCM may be attributed once more to low WQI values, combined with high heavy metal contents (Pb, Cu, Cr and Zn) in sediments, and low oxygen concentrations at this highly polluted site (Monferrán et al. 2011). Nevertheless, unexpected responses were also observed in the studied fish. Maggioni et al. (2012) reported that according to GPx activity, the worst conditions for *J. multidentata* in the Suquía River occur at LC during the wet season. This response could be associated with a punctual reaction to the toxics introduced at this site by runoff after a rainfall event.

All things considered, these results could mean that biotransformation and oxidative stress response are activated during both the wet and dry seasons along the Suquía River and change with the pollution gradient.

11.3.1.2 Endocrine Disruption

Endocrine disruption in wildlife is being extensively and increasingly reported worldwide, mainly on aquatic environments, potentially affecting many physiological processes, including reproduction. The presence of pesticides, hormones, pharmaceuticals, and heavy metals in the Suquía River has been reported (Merlo et al. 2011; Monferrán et al. 2011; Maggioni et al. 2012; Bonansea et al. 2013; Valdés et al. 2014); thus, an impact on the endocrine system of the inhabiting biota should not be surprising. In this context, the study of changes in gene aromatase expression is increasingly used as indicators of endocrine disruption. Therefore, *J. multidentata* was used as a model to study not only natural fluctuations in both brain and gonadal aromatase expression, but also the effects of water pollution of the Suquía River. Males of *J. multidentata* were monthly collected over a year in RY and in RP and showed that brain aromatase of fish coming from the reference site (RY) fluctuates along the reproductive cycle with maximum values during the breeding season of this species. A significant mismatch in the increase of brain gene expression was observed, which begins with a 1-month delay in the contaminated site (RP) in relation to Río Yuspe. This mismatch is also evidenced in the fluctuations of the gonadosomatic

index, where testes weight increased with a 1-month delay in the contaminated site. These effects could interfere with reproduction, altering the beginning and length of the reproductive cycle due to pollution (Guyón 2013).

11.3.2 Effects on Fish at Tissue Level

11.3.2.1 Histopathology Analysis

Histopathology studies are a useful method to evaluate the effects of pollutants on freshwater fish. Thus, histopathological changes are sensitive and reliable indicators of fish health, and have been reported in several studies (Schlenk and Benson 2001). Particularly in fish, the most studied organs are those in direct contact with the environment or those that perform functions closely related to it. In this sense, gills have a large respiratory surface of lamellae as well as an extensive epithelium outlining the filaments, which represent an important area of contact between animals and the surrounding environment. Also, this organ is one of the main routes of entry for toxic agents in teleost fish and, therefore, it is considered as a target for the toxic action of contaminants (Wood 2001). Damage in gill epithelia has been considered as a good indicator of xenobiotics effects (Ballesteros et al. 2007). Another organ highly affected by environmental pollution is liver because of its central position in the circulatory system and its function in the detoxification of xenobiotics. When toxic compounds exceed the detoxification level of this organ, they tend to accumulate at high concentrations in the liver and modify its structure. As many toxic compounds tend to accumulate in this organ, their cells could be exposed to higher levels of contaminants that those found in the environment, or other organs (Heath 1995). Histopathological changes in the livers of fish exposed to a wide range of organic compounds and heavy metals have been reported. Histological damage was also reported in the brain, kidney, and skin (Schlenk and Benson, 2001).

The Suquía River water quality has been analyzed through histological biomarkers in two hydrological seasons, dry and wet. Maggioni et al. (2012) registered histological injuries in two sampling sites along the middle-lower basin of the Suquía River based on the contamination gradient showed by previous investigations (Bistoni et al. 1999; Pesce and Wunderlin 2000). La Calera City (LC) was considered the reference site, because it is located 18 km upstream from Córdoba city, while Río Primero (RP) site is situated 50 km downstream from Córdoba city, in an agricultural area (Fig. 11.1). López (2012) added other sampling sites to those mentioned before, Villa Corazón de María (VCM), Capilla de los Remedios (CR), Río Primero (RP), and Santa Rosa de Río Primero (SR) (Fig. 11.1). This last sampling station was supposed to be a potential site of water-quality recuperation due to the self-purification processes of the river.

The native fish *Jenynsia multidentata* was used as bioindicator in both studies. These authors described the histological changes in the liver and gill, and they used semiquantitative indexes to evaluate the observed damage. They calculated an index by organ (IH_{Gill}, IH_{Liver}), which represents the degree of damage to each organ and a

total index (IH_{Total}) (the sum of organ indexes) to determine the extent of damage in the body, a measure of the general health, based on the histological lesions (Bernet et al. 1999). It is important to note that the higher the values each index takes, the more severely the organs are affected.

Histological alterations were more frequently observed in the dry season with respect to the wet season in the different sampling sites of the river. This difference between the hydrological stations may be due to a decrease in river flow in this period that generates higher concentrations of contaminants in water and sediment.

Regarding damage to gills, epithelial lifting, hypertrophy, and hyperplasia were injuries equally frequent at all sites of the Suquía Basin. However, alterations such as fusion and secondary lamellae shortening were frequently found in sampling sites located after Córdoba city, with the exception of the site further downstream from the sewage treatment plant (SR). So, the sampling stations located near this plant showed the greatest impact on gills structure.

Regarding liver alterations, both López (2012) and Maggioni et al. (2012) pointed out that water quality in the sites after Córdoba city, except SR, is the main responsible for the hepatic damage. In these stations, all individuals registered regressive changes (hydropic degeneration, lipid metamorphosis, necrosis, and fibrosis) as well as inflammatory changes (leukocyte infiltration). In addition, during the dry season at RP site, hypertrophy and pyknosis of hepatocytes were observed in all fish samples. CR, VCM, and RP showed higher values for all studied indexes (organ and total indexes), which highlights the deterioration in fish health due to poor water quality along the river (Table 11.2). For organ and total indexes, López (2012) reported lower values for the SR site in relation to other sites located after Córdoba city, and even lower than those values mentioned by Maggioni et al.

Table 11.2 Histopathological indices for *J. multidentata* from the middle-lower basin of the Suquía River, according to the wet and dry hydrological stations reported by López (2012) and Maggioni et al. (2012)

		IH_{Gill}		IH_{Liver}		IH_{Total}	
		López (2012)	Maggioni et al. (2012)	López (2012)	Maggioni et al. (2012)	López (2012)	Maggioni et al. (2012)
LC	Wet		22 ± 3		22.5 ± 3		36 ± 4
	Dry		23 ± 3		13 ± 5		45 ± 5.2
VCM	Wet	29.2 ± 4.4		32 ± 5.7		61.2 ± 5.9	
	Dry	32.8 ± 5.9		32.4 ± 9.7		65.2 ± 15.6	
CR	Wet	26.6 ± 1.6		30 ± 4.6		56.6 ± 6	
	Dry	26.4 ± 4.6		28 ± 1.4		54.4 ± 5.5	
RP	Wet	29.1 ± 2	22.6 ± 4.1	35.1 ± 4.3	19 ± 6.2	64.2 ± 4.5	42.7 ± 9
	Dry	30.8 ± 3	28 ± 1.6	36 ± 6.2	21.5 ± 6.4	66.8 ± 8.8	47 ± 6
SR	Wet	14.8 ± 3		11.2 ± 4.1		26 ± 6.8	
	Dry	16.8 ± 6.7		10.8 ± 3		27.6 ± 9.4	

Sampling sites: CL, La Calera; VCM, Villa Corazón de María; CR, Capilla de los Remedios; RP, Río Primero; SR, Santa Rosa de Río Primero

(2012) for the reference site (LC). Although in SR histological damages were also recorded, and others authors indicated for this site an increased activity of antioxidant enzymes in response to the presence of contaminants (Monferrán et al. 2011), the intensity and frequency of damage found in both liver and gill are much lower compared to other sites.

SR is located 80 km downstream from Córdoba city, and the self-purifying power of the river helps improve the water quality (Fig. 11.1). Ballesteros et al. (2017) studied through histological analysis in gill and liver the effect that different pollutants produce in the locally edible and commercial fish *O. bonariensis*. The study was carried out in the south coast of the RAMSAR site, Mar Chiquita Lake where the Suquía River mouth is located (Fig. 11.1). This river contributes, as it was previously mentioned, to various kinds of contaminants from agriculture, industries, and wastewater sources. In gills, the most frequently alterations registered were epithelial lifting and shortening of secondary lamellae in 93% and 60% of all the individuals analyzed, respectively. The alterations such as chloride and pavement cell hypertrophy and hyperplasia were the least frequently found in the samples (<27%). Aneurisms (circulatory disturbances) were found in 40% of the samples. In the liver, dilation of sinusoids and vascular congestion were found in 100% and 93% of the samples, respectively, meanwhile, the lipid degeneration was found only in the 27% of the individuals. The authors finally concluded that the histological changes detected in *O. bonariensis* could be considered as reversible if the environmental conditions improve (Ballesteros et al. 2007). When the alterations found in *O bonariensis* were analyzed through histopathological indexes, the score obtained by gills (IH_{Gill}) was close to 8–10% of the maximum value that the index can reach, assuming that the highest value is the poorest condition of the organ. According to this, gills of fish inhabiting Mar Chiquita Lake were slightly affected and the values observed for this index did not show relation between rainy, postrainy, and dry seasons. However, the liver index (IH_{Liver}) and the total index (IH_{Total}) differed significantly between sampling periods, showing the highest scores during the postrainy season.

The histological damage registered in the Suquía River Middle-Lower Basin has been registered in previous works as unspecific histopathological responses against residual waters (Bernet et al. 1999), pesticides (Ballesteros, et al. 2007; Pesce et al. 2008; Hued et al. 2012) and heavy metals (Figereido-Fernandes et al. 2007).

As expected, in sites located downstream from Córdoba city, water quality had a strong impact on the overall health of *J. multidentata* individuals. However, the power of self-purification of the river noticeably improved fish health far downstream from Córdoba city.

These results support the spatial deterioration in water quality along the Suquía River Basin. The greatest impact was produced by anthropogenic activities, human population growth, together with a lack of urban planning, with an important negative effect on the water quality due to the WWTP from Córdoba city. This strong negative effect is partially reduced downstream from WWTP, but the use of different pesticides in the low basin, between the main city and Mar Chiquita Lake (Fig. 11.1), adds new families of contaminants, also having deleterious effect on the inhabiting biota.

On the other hand, Rautenberg et al. (2014) analyzed the effect of contamination of the Suquía river in *Gambusia affinis* (a nonnative fish species) in two sites stations. One of them located previously to Cordoba City, La Calera (LC), and the other one after the city (RP). These authors registered in gills same damages mentioned for *J. multidentata* although they were more frequently found in LC instead of RP. In the first sampling station, the concentration in water of the mercury and the pesticide alpha-cypermethrin exceed the established limits for the protection of the aquatic biota proposed by Canadian Water Quality Guidelines (CCME, 2013) or Argentinean Environmental Water Quality Guidelines (Niveles Guía Nacionales de Calidad de Agua Ambiente) (SRHN, 2017). In liver, instead, the highest frequency of hydropic degeneration and necrosis was registered at RP station. These lesions are a general response associated with chronic exposure to different toxic compounds (pesticides, heavy metals, and wastewaters) (Pesce et al. 2008; Costa et al. 2011; Hued et al. 2012).

11.3.2.2 Hematological Parameters

The hematological parameters in fish are influenced by external factors like seasonal dynamics, water temperature, environmental quality, and stress, among other variables (Rios et al. 2002). The variation of hematological features could serve as a biomarker of sublethal environmental stress (Bridges et al. 1976). Cazenave et al. (2005b) conducted a study in sites located before and after Córdoba city. The city produces a serious impact on the Suquía River water quality, as was explained in this chapter. *Corydoras paleatus* was chosen by the mentioned authors as bioindicator because of its wide distribution and ornamental value. Individuals were collected from the Suquia River in LC, identified as a quasipristine site (Hued and Bistoni 2002) (Fig. 11.1). The other station, VCM, has been classified as a highly polluted site by Pesce and Wunderlin (2000). The hematological parameters tested were erythrocyte counts (Er), hematocrit (Ht), hemoglobin concentration (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC).

Fish from the polluted site presented higher values of Er, Ht, Hb, MCH, and MCHC than those individuals captured at the reference site. The Hb concentration was the key parameter to point out differences between fish from sites with different environmental conditions. This result is in agreement with Saint-Paul (1984) who suggested that an increase in Hb concentration could be considered as an especially reliable first indicator of an adaptation to improve the oxygen-transporting capacity of the blood. In addition to behavioral and morphological adjustments, fish could respond to low oxygen levels by adjusting several physiological and biochemical parameters (Val et al. 1998). Besides being an air-breathing fish (Gómez, 1993), *Corydoras paleatus* may increase its respiratory blood function, mainly through an increase in hemoglobin content.

Although hematological parameters are nonspecific in their responses toward chemical stressors, it is well known that toxic substances can significantly damage

the hematological system of fish (van der Oost et al. 2003). Therefore, hematology could provide important information on the general physiology and health status of the organisms living under environmental stress.

11.3.3 Effects on Fish at Individual Level

Several field studies have provided direct evidence that water quality degradation seriously impacts fish reproductive biology. Changes in external sexual characteristics, delay in gonadal development and maturation, reduction in the number and quality of gametes, alterations of courtship patterns, decrease in fertilization rates, and changes in vitellogenin levels are some of the effects registered in different investigations (Leusch et al. 2006, Jessica et al. 2007, Rey Vazquez et al. 2009).

Among the fish species used to evaluate the quality of freshwater systems, the neotropical fish species *J. multidentata* and *C. decemmaculatus* are widely distributed through South America, and have been found by Hued and Bistoni (2005) in pristine as well as severely degraded habitats of Argentina. These fish species were successfully used as a sentinel species in many ecotoxicological researches, prompting their inclusion as suitable native fish species for the determination of the lethal acute toxicity of xenobiotics by the Argentinean Institute of Standardization and Certification (IRAM, 2008). On the other hand, the nonnative species, the mosquito fish, *Gambusia affinis* (Poeciliidae, Cyprinodontiformes), has been used as an excellent bioindicator in field studies, and it is considered an important sentinel, due to its ability to establish itself in a wide range of habitats (Orlando et al. 2005). The three mentioned species are viviparous with external sexual dimorphism. Males have morphological traits that make them uniquely suited to study the effects of environmental endocrine disruptors, since the anal fin differentiates into a complex structure called gonopodium that is used for the transfer of sperm during copulation (Turner 1941). Development of the gonopodium is androgen-dependent and normally occurs in males at the time of sexual maturation. However, its normal development can be affected by xenobiotic compounds. Therefore, the gonopodial morphology could be used as an excellent biomarker of xenobiotic exposure (Angus et al. 2001).

Three studies by Hued et al. (2013), Rautenberg et al. (2014), and Zambrano et al. (2018) were carried out to determine whether in a freshwater system polluted by anthropogenic activities, sewage, agricultural, and industrial discharges into the Suquía River Basin could cause alterations in gonopodium morphology. To achieve the main goal, authors selected sampling sites upstream and downstream from Córdoba city (Fig. 11.1). Hued et al. (2013) collected males of *J. multidentata* upstream from Córdoba, in La Calera (LC) and downstream, in Villa Corazón de María (VCM), Rautenberg et al. (2014) captured males of *G. affinis* at LC and Río Primero (RP) and Zambrano et al. (2018) collected males of *C. decemmaculatus* at four sites on Suquía River basin: Río Yuspe (RY), Casabamba (CAB), and Villa Corazón de María (VCM; Fig. 11.1). The following parameters were measured: total body weight, standard length, gonopodium length (GL), and the gonopodium

somatic index (Gonop-SI = GL · 100/SL). In the tubular gonopodium of *J. multidentata*, the gonopodium angle (GA) and the area of the tip of the 6th anal-fin ray (GTA) were also measured. In adult males, this ray is an unbranched structure, which is displaced forward during copulation. For *G. affinis* and *C. decemmaculatus,* the presence or absence of hooks on the distal portion of the gonopodium (Doyle and Lim 2002) was also corroborated and counted. These hooks act as holdfast devices during copulation, and their presence on the gonopodial tip indicates that gonopodium has fully developed (Turner 1947).

Those gonopodia from fish collected at LC showed normal external morphology. The tubular intromittent organ of *J. multidentata*, supported by the anal fin rays, presents a normal inclination, which determines an angle on the distal part of the gonopodium. Males from VCM presented noticeable structural abnormalities of the anal fin, with and shorter standard length and lower body weight compared to those collected in LC. The GL, Gonop-SI, and GTA were significantly lower in VCM, whereas GA was higher, indicating an impairment of secondary sexual characteristics. On the other hand, Rautenberg et al. (2014) initially assumed that *G. affinis* from RP would be influenced by the contribution of the sewage discharge from the WWTP, located downstream from Córdoba city (Fig. 11.1). However, neither the Gonop-SI nor the percentage of fish with hooks and serrae differed significantly between sampling sites. Although Rautenberg et al. (2014) reported no differences in the gonopodium length between sampling sites, it is not possible to conclude that the studied individuals are not affected by water quality, since the males from the reference site (LC) and those from the polluted site (RP) expressed equal levels of vitellogenin (Vtg). It is important to note that the normal expression of Vtg occurs only in females, so the expression of this protein in males is indicative of the effects of endocrine disruptors on the environment (Denslow et al. 1999). The morphological responses registered in *J. multidentata* and *G. affinis* at the same site (LC) would indicate a different sensitivity at morphological levels in similar environmental conditions. According to Merlo et al. (2011) and Maggioni et al. (2012), LC site has a similar degree of degradation than RP site, which is characterized by low water quality. These results suggest that LC, considered as a reference site, presented clear water quality deterioration with respect to previous studies conducted at this site (Hued and Bistoni, 2005). Rautenberg et al. (2014) indicated that LC showed important levels of some metals (mercury, manganese, lead, zinc) and pesticides (β-endosulfan, acetochlor, and atrazine) that could act as endocrine disruptors and impact on the normal fish sexual development as was registered in *J. multidentata* and affect *G. affinis* at the molecular level of organization as was observed through the Vtg expression in males. On the other hand, males of *C. decemmaculatus* collected by Zambrano et al. (2018) presented significant differences in Gonop-SI between sampling sites, showing the lowest values at Puente Cantón (PC), located at the middle of Córdoba city (Fig. 11.1). This site receives the downtown runoff, which is composed largely of the harmful pollutants coming from vehicles. The presence of pesticides with known estrogenic effect, such as dieldrin, HCB, and β-hexachlorocyclohexane, was also registered. Taking into account this situation,

the decrease in Gonop-SI values could reflect the effects of degraded waters at this site on reproductive structures of fishes.

From the mentioned works, the three species evaluated at different sites evidenced the water quality degradation of on Suquía River basin. All the authors also demonstrated that reproductive structures such as gonopodium are excellent biomarkers of environmental degradation.

11.3.4 Effects on Fish at the Community Level

Although many experimental studies have explored the response of fish to environmental factors, fish behavior in relation to complex interactions among diverse variables in nature is difficult to describe. Different studies have been carried out to evaluate the effects of environmental alterations on fish species by measuring a single or a set of biomarkers through acute and chronic exposure to a single toxic substance or a mixture of them. However, the results obtained from these studies are difficult to extrapolate to natural environments and, therefore, lack ecological realism.

Thus, the use of biological indicators emerges as an important water resources management tool in order to assess the health of aquatic systems. Considering that fish species are appropriate end-points for assessing stream integrity and quality due to their aesthetic, ornamental and/or economic importance for citizens, their position in the food chain, and high sensitivity to water quality, in 1981, James Karr developed the first biotic index (IBI, Index of Biological Integrity) entirely based on fish assemblage attributes. The IBI was successfully applied around the world due to its main advantage that it could be adapted to the fish fauna of different geographical areas. Also, this index has the ability to integrate information from individual, population, community, zoogeographic, and ecosystem levels into a single ecologically based index of the quality of a water resource. As a consequence of environmental deterioration, fish species change their abundance and distribution as well as manifest changes in their assemblage attributes. These changes could show different patterns of variation, which could be used as indicators of water quality degradation.

In Argentina, few works attempted to relate changes in fish assemblage composition with variations of physical and chemical water characteristics at field (Bistoni et al. 1999). Consequently, the bioassessment of aquatic systems has been difficult due to the lack of data on fish assemblages as well as information on the life history of most fish species.

Like other basins around the world, the Suquía River Basin suffers from the negative impact of different human activities affecting the inhabiting biota. In order to evaluate the water quality of the basin, Hued and Bistoni (2005) collected information on fish fauna to develop and validate a Biotic Index to assess degradation of the Suquía River Basin.

The sampling sites were selected according to previous studies reporting water quality variations along the watershed (Bistoni et al. 1999; Pesce and Wunderlin, 2000), and according to their location with respect to the most important cities along the watershed. Consequently, areas upstream from cities were identified as sites representative of pristine or near pristine conditions, while areas in or downstream from cities were identified as hypothetically polluted areas. A total of 16 sampling sites were sampled (Fig. 11.1): San Francisco River, Villa Giardino (VG, 31° 3′0.24″ South; 64°30′36.22″ West) and Huerta Grande (HG, 31° 3′54.22″ South; 64°30′51.68″ West), both before La Falda city, Valle Hermoso (VH, 31° 6′59.80″ South; 64°29′36.27″ West) and Molinari (ML, 31°11′33.32″ South; 64°28′35.57″ West), both after the mentioned city; Cosquín River, Cosquín (CQ, 31°13′6.36″ South; 64°28′54.26″ West) and Villa Bustos (VB; 31°15′27.33″ South; 64°27′43.74″ West), before and after the Cosquín city, respectively, Villa Caeiro (VC: 31°17′36.04″ South; 64°27′36.50″ West), after Cosquín city; Yuspe River, RY; San Antonio River, Cuesta Blanca (CB: 31°28′58.52″ South; 64°34′32.71″ West) and San Antonio de Arredondo (SA, 31°28′45.71″ South; 64°31′32.75″ West) before Villa Carlos Paz city and Villa Carlos Paz (VCP, 31°25′14.06″ South; 64°30′34.26″ West) located at the mentioned city; and Suquía River, LC and Saldán (SLD; 31°19′25.47″ South; 64°18′28.07″ West), before Córdoba city, IDP, at the mentioned city, and site BG and VCM located after Córdoba city.

A total of 9375 individual fish were collected along the Suquía River Basin and were classified into 21 fish species in 12 families. As other biotic indexes, the Biotic Index for the Suquía Basin (IBI-SUQUÍA) consists of an aggregation of metrics that are based on fish assemblages, taxonomic and trophic composition, and/or the abundance and health condition of fish. Therefore, Hued and Bistoni (2005) classified the biological parameters according to their sensitiveness or tolerance to the water quality gradient, and proposed candidate metrics based on fish distribution, abundance variations, and position in the water column. Those candidate metrics that were significantly different between water quality characteristics were considered for the index calculation. When a metric did not differ between reference and polluted sites, it was eliminated from further consideration. Once the Biotic Index was developed, it was correlated to water quality characteristics (based on physical and chemical parameters), and statistical analyses were performed in order to determine how well it corresponded to the estimates of environmental conditions.

As a final result, the parameters used for the calculation of the IBI-SUQUÍA were: the abundance of *Astyanax eigenmanniorum*, *Rineloricaria catamarcensis*, *Gambusia affinis*, and *Cnesterodon decemmaculatus*, the proportion of sensitive species richness, and the proportion of tolerant species richness. These metrics clearly distinguished the polluted from the reference sites.

The variation pattern of the IBI-SUQUÍA along the studied basin is shown in Fig. 11.2. The proposed new index, completely based on biological information, identified an environmental gradient, and it was significantly different among sampling areas. Polluted sites did have significantly lower scores than reference sites. Those sites located upstream from Córdoba city were characterized by a high water quality. On the other hand, according to the Biotic Index values, site 3

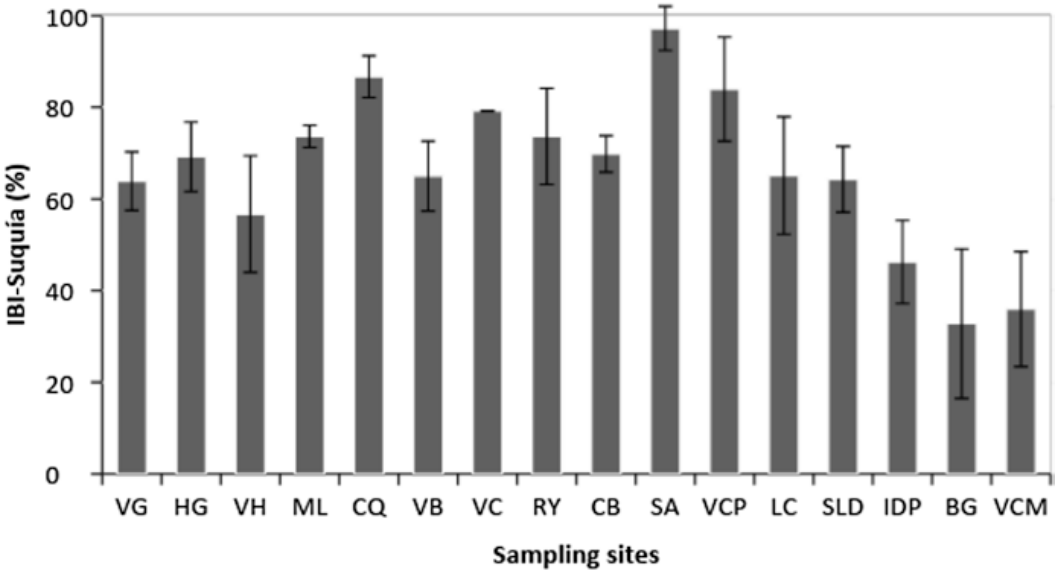

Fig. 11.2 Mean IBI-SUQUÍA values and standard deviation for each sampling site along the Suquía River Basin. VG: Villa Giardino; HG: Huerta Grande; VH: Valle Hermoso; ML: Molinari; CQ: Cosquín; VB: Villa Bustos; VC: Villa Caeiro; RY: Río Yuspe; CB: Cuesta Blanca; SA: San Antonio de Arredondo; VCM: Villa Carlos Paz; LC: La Calera; SLD: Saldán; IDP: Isla de los Patos; BG: Bajo Grande; VCM: Villa Corazón de María

(after La Falda city) and IDP (located in Córdoba city) were classified as moderate altered areas. Finally, those sites located downstream from Córdoba city were the most negatively impacted by pollution (BG and VCM). Fish assemblages from these sites showed the severe alterations of the aquatic environment, which was reflected by the lowest index values (32.81% and 35.94%, respectively).

The proposed Biotic Index version for the Suquía River Basin was sensitive to changes in water quality, and displayed a strong relationship to the water quality characteristics based on physical and chemical parameters measured at the same time of fish collection.

Few years later from the first application of de IBI-SUQUÍA, Merlo et al. (2011) and Maggioni et al. (2012) applied this index again. Of the sites sampled, two were the same as those surveyed by Hued and Bistoni (2005). Both works arrived at the same conclusion: as was registered by Hued and Bistoni (2005), the changes observed in assemblage structure through the index application indicated that the alterations of water quality are severe when the river runs through Córdoba city and receives wastewater discharges from WWTP and the effluents of the industrial zone. However, both groups of authors pointed out that in few years from the first IBI-SUQUÍA application, the water quality worsened at the most contaminated site (Table 11.3), extending the degradation of the river water quality by many kilometers downstream from WWTP, turning the aquatic environment unfavorable for the survival of fish species (Maggioni et al. 2012). So far from Table 11.3, we can see a clear spatial degradation from LC to VCM, which is greatly influenced by Córdoba city, particularly from its WWTP. When performing

Table 11.3 Variation of IBI-SUQUÍA values over time. Note the water quality deterioration over time in the most contaminated site (Villa Corazón de María)

Studies on the Suquía River Basin	La Calera (LC)	Villa Corazón de María (VCM)
Hued and Bistoni (2005)	65.10	35.94
Merlo et al. (2011)	58.30	22.26
Maggioni et al. (2012)	57.28	16.67

a temporal analysis, the conditions are even worse showing a drop in IBI-Suquía values from 2005 to 2012, which is particularly injurious at VCM, downstream from the city WWTP. These results clearly show the lack of an appropriate sanitation policy by the city government, in addition to the lack of control and regulations from the state government. Unfortunately, this is a quite common situation in developing countries, not only in Latin America that needs to be urgently improved to avoid further negative effects on both the water quality and the health of the inhabiting biota, including humans living along the basin.

References

Amé MV, Díaz MP, Wunderlin D (2003) Occurrence of toxic cyanobacterial blooms in San Roque Reservoir (Córdoba – Argentina): a field and chemometric study. Environ Toxicol 18:192–201

Angus RA, McNatt HB, Howell WM, Peoples SD (2001) Gonopodium development in normal male and 11-ketotestosterone-treated female mosquitofish (*Gambusia affinis*): a quantitative study using computer image analysis. Gen Comp Endocrinol 123:222–234

Avigliano E, Schenonea NH, Volpedoa AV et al (2015) Heavy metals and trace elements in muscle of Silverside (*Odontesthes bonariensis*) and water from different environments (Argentina): aquatic pollution and consumption effect approach. Sci Total Environ 506–507:102–108

Ballesteros ML, Bianchi GE, Carranza M, Bistoni MA (2007) Endosulfan acute toxicity and histomorphological alterations in *Jenynsia multidentata* (Anablepidae, Cyprinidontiformes). J Environ Sci Health B 42:351–357

Ballesteros ML, Gonzalez M, Wunderlin DA, Bistoni MA, Miglioranza KSB (2011) Uptake, tissue distribution and metabolism of the insecticide endosulfan in *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). Environ Pollut 159:1709–1714

Ballesteros ML, Miglioranza KSB, Gonzalez M et al (2014) Multimatrix measurement of persistent organic pollutants in Mar Chiquita, a continental saline shallow lake. Sci Total Environ 490:73–80

Ballesteros ML, Hued AC, Gonzalez M, Miglioranza KSB, Bistoni MA (2017) Evaluation of the health status of the silverside (*Odontesthes bonariensis*) at RAMSAR site in South America. Bull Environ Contam Toxicol 99:62–68

Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T (1999) Histopathology in fish: proposal for a protocol to assess aquatic pollution. J Fish Dis 22:25–34

Bistoni MA, Hued AC, Videla MM, Sagretti L (1999) Efectos de la calidad del agua sobre las comunidades ícticas de la región central de Argentina. Rev Chil Hist Nat 72:325–335

Bonansea RI, Amé MV, Wunderlin DA (2013) Determination of priority pesticides in water samples combining SPE and SPME coupled to GC–MS. A case study: Suquía River basin (Argentina). Chemosphere 90:1860–1869

Brandalise MV, Nadal F, Rodriguez MI, Larrosa N, Ruiz M, Halac S, Olivera P, Licera C (2007) Índice de Calidad de Agua para uso Recreativo en Ambientes con Cianobacterias. http://www.ina.gov.ar/pdf/ifrrhh/02_007_Brandalise.pdf. Accessed 05 Dec 2014

Bridges DW, Cech JJ, Pedro DN (1976) Seasonal haematological changes in winter flounder, *Pseudopleuronectes americanus*. Transactions of American Fisheries Society (5):596–600

Cazenave J, Wunderlin DA, Bistoni MA, Amé MV, Krause E, Pflugmacher S, Wiegand C (2005a) Uptake, tissue distribution and accumulation of Microcystin- RR in *Corydoras paleatus*, *Jenynsia multidentata* and *Odontesthes bonariensis*. Aquat Toxicol 75:178–190

Cazenave J, Wunderlin D, Hued A, Bistoni M (2005b) Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae), captured from pristine and polluted water. Hydrobiologia 537:25–33

CCME (2013) Canadian Water Quality Guidelines for the protection of aquatic life. http://st-ts.ccme.ca/en/index.html. Accessed 07 Mar 2019

Çoğun H, Yüzereroğlu TA, Kargin F, Firat Ö (2005) Seasonal variation and tissue distribution of heavy metals in shrimp and fish species from the Yumurtalik coast of Iskenderun Gulf, Mediterranean. Bull Environ Contam Toxicol 75(4):707–715

Contardo-Jara V, Galanti LN, Amé MV et al (2009) Biotransformation and antioxidant enzymes of *Limnoperna fortunei* detect site impact in watercourses of Córdoba Argentina. Ecotoxicol Environ Saf 72:1871–1880

Costa PM, Caeiro S, Lobo J, Martins M, Ferreira AM, Caetano M, Costa MH (2011) Estuarine ecological risk based on hepatic histopathological indices from laboratory and in situ tested fish. Mar Pollut Bull 62:55–65

Cui B, Zhang Q, Zhang K, Liu X, Zhang H (2011) Analyzing trophic transfer of heavy metals for food webs in thenewly-formed wetlands of the Yellow River Delta, China. Environ Pollut 159:1297–1306

de Wit CA (2002) An overview of brominated flame retardants in the environment. Chemosphere 46:583–624

Denslow N, Chow M, Kroll K, Green L (1999) Vitellogenin as a biomarker of exposure for estrogen or estrogen mimics. Ecotoxicology 8:385–398

Doyle CJ, Lim RP (2002) The effect of 17beta-estradiol on the gonopodial development and sexual activity of Gambusia holbrooki. Environ Toxicol Chem 21:2719–2724

Dural M, Göksu MZL, Özak AA, Derici B (2006) Bioaccumulation of some heavy metals in different tissues of *Dicentrarchus labrax* L, 1758, *Sparus aurata* L, 1758, and *Mugil cephalus* L, 1758 from the Camlik Lagoon of the eastern coast of Mediterranean (Turkey). Environ Monit Assess 118:65–74

Erdoğrul Ö, Erbilir F (2007) Heavy metal and trace elements in various fish samples from Sir Dam Lake, Kahramanmaraş, Turkey. Environ Monit Assess 130:373–379

Farag AM, Nimick DA, Kimball BA et al (2007) Concentrations of metals in water, sediment, biofilm, benthic macroinvertebrates, and fish in the Boulder river watershed, Montana, and the role of colloids in metal uptake. Arch Environ Contam Toxicol 52:397–409

Figueiredo-Fernandes A, Ferreira-Cardoso JV, Garcia-Santos S, Monteiro SM, Carrola J, Matos P et al (2007) Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. Pesq Vet Bras 27(3):103–109

Galanti LN, Amé MV, Wunderlin DA (2013) Accumulation and detoxification dynamic of cyanotoxins in the freshwater shrimp *Palaemonetes argentinus*. Harmful Algae 27:88–97

Gómez S (1993) Concentración letal de oxígeno disuelto para *Corydoras paleatus* y *Pimelodella laticeps* (Pisces, Siluriformes). Revista del Museo Argentino de Ciencias Naturales Bernardino Rivadavia 7:31–45

Griboff J (2017) Transferencia de metales y metaloides a través de la cadena trófica y su acumulación en organismos destinados a consumo humano. Estudios de fitoremediación). Tesis doctoral FCQ, UNC

Guyón NF (2013) Biomarcadores específicos de disrupción endocrina. Análisis en peces autóctonos expuestos a contaminantes ambientales. Facultad de Ciencias Exactas, Físicas y Naturales, Córdoba

Heath AG (1995) Water pollution and fish physiology, 2nd edn. CRC Lewis Publishers, Boca Raton/London-New York

Hued A, Bistoni MA (2002) Effects of water quality variations on fish communities in the Central Part of Argentina, South America. Proc Int Assoc Theor Appl Limnol 28:112–116

Hued AC, Bistoni MA (2005) Development and validation of a Biotic Index for evaluation of environmental quality in the central region of Argentina. Hidrobiologia 543:279–298

Hued AC, Oberhofer S, Bistoni MA (2012) Exposure to a commercial glyphosate formulation (Roundup) alters the normal gill and liver histology and affects the male sexual activity of *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes). Arch Environ Contam Toxicol 62(1):107–117

Hued AC, Nostro FLL, Wunderlin DA, Bistoni MA (2013) Reproductive impairment of a viviparous fish species inhabiting a freshwater system with anthropogenic impact (2013). Arch Environ Contam Toxicol 64(2):281–290

Ibelings BW, Chorus I (2007) Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: a review. Environ Pollut 150:177–192

IRAM (2008) Calidad ambiental, calidad del agua. Determinación de la toxicidad letal aguda de sustancias en peces de agua dulce. Método semiestático. IRAM 29112:2008

Jarić I, Višnjić-Jeftić Z, Cvijanović G et al (2011) Determination of differential heavy metal and trace element accumulation in liver, gills, intestine and muscle of sterlet (*Acipenser ruthenus*) from the Danube River in Serbia by ICP-OES. Microchemical J 98:77–81

Jessica D, Robert M, Frederic S, Arnaud B, Delphine L, Jean-Pierre T, Patrick K (2007) Do sewage treatment plant discharges substantially impair fish reproduction in polluted river? Sci Total Environ 372:497–514

Jun X, Min Z (2012) Primary consumers as bioindicator of nitrogen pollution in lake planktonic and benthic food webs. Ecological Indicators 14:189–196

Karr JR (1981) Assessment of Biotic Integrity using fish communities. Fisheries 6(6):21–27

Laura V (2009) Metallothioneins in aquatic organisms: fish, Crustaceans, Molluscs, and Echinoderms. Metal Ions Life Sci 5:99–237

Leusch FDL, Chapman HF, Kay GW, Gooneratne SR, Tremblay LA (2006) Anal fin morphology and gonadal histology in mosquitofish (*Gambusia holbrooki*) exposed to treated municipal sewage effluent. Arch Environ Con Tox 50:562–574

López F (2012) Utilización de Biomarcadores histomorfológicos en *Jenynsia multidentata* para la evaluación de la calidad del agua del río Suquía en su tramo inferior. Facultad de Ciencias Exactas, Físicas y Naturales, Córdoba

Maggioni T, Hued A, Monferrán M, Bonansea R, Galanti L, Amé V (2012) Bioindicators and Biomarkers of Environmental Pollution in the Middle-Lower Basin of the Suquía River (Córdoba, Argentina). Arch Environ Contam Toxicol 63:337–353

Mazej Z, Al Sayegh-Petkovsek S, Pokorny B (2010) Heavy metal concentrations in food chain of Lake Velenjsko jezero, Slovenia: an artificial lake from mining. Arch Environ Contam Toxicol 58:998–1007

Merlo C, Abril A, Amé MV, Argüello GA, Carreras HA, Chiappero MS, Hued AC, Wannaz E, Galanti LN, Monferrán MV, González CM, Solís VM (2011) Integral assessment of pollution in Suquía River (Córdoba, Argentina) as a contribution of lotic ecosystem restoration programs. Sci Total Environ 409:5034–5045

Metcalfe TL, Metcalfe CD (1997) The trophodynamics of PCBs, including mono- and non-ortho congeners, in the food web of North-Central Lake Ontario. Sci Total Environ 201:245–272

Monferrán MV (2018) Metals and metalloids in water and sediment of the Suquía River basin: spatial and temporal changes. In: Handbook of environmental chemistry (vol 62, pp 113–143). Springer International Publishing, Switzerland

Monferrán MV, Galanti LN, Bonanesa RI, Amé MV, Wunderlin DA (2011) Integrated survey of water pollution in the Suquía River basin (Córdoba, Argentina). J Environ Monit 20:1–13

Monferrán MV, Garnero P, Bistoni MA, Anbar AA, Gordon GW, Wunderlin DA (2016) From water to edible fish. Transfer of metals and metalloids in the San Roque Reservoir (Córdoba, Argentina). Implications associated with fish consumption. *Ecological Indicators* 63:48–60

Newman MC, Unger MA (2003) Fundamentals of Ecotoxicology, 2nd edn. Lewis Publishers, CRC Press, Boca Raton, p 458

Nimptsch J, Wunderlin DA, Dollan A et al (2005) Antioxidant and biotransformation enzymes in *Myriophyllum quitense* as biomarkers of heavy metal exposure and eutrophication in Suquía River basin (Córdoba, Argentina). Chemosphere 61:147–157

Orlando E, Davis W, Edwards T, Sulkowski D, Toft G, Guillette L (2005) Mosquitofish as a sentinels of exposure to environmental androgens and estrogens (pp 436–453). In: Uribe MC, Grier HJ (eds) Viviparous fishes. New Life Publications, Florida, p 603

Osinaga-Acosta O, Torres Dowdall J, Martín E, Lascano E (2006) Aves Playeras. In: Bucher EH (ed) Bañados del río Dulce y Laguna Mar Chiquita (Córdoba, Argentina). Academia Nacional de Ciencias, Córdoba, pp 263–275

Pesce SF, Wunderlin D (2000) Use of water quality indices to verify the impact of Córdoba city on Suquía River. Water Res 34:2915–2926

Pesce SF, Cazenave J, Monferrán MV, Frede S, Wunderlin DA (2008) Integrated survey on toxic effects of lindane on neotropical fish: corydoras paleatus and Jenynsia multidentata. Environ Pollut 156:775–783

Pizzolón L, Tracanna B, Silva H, Prósperi C, Fabricius ALM, Emiliani MOG, Otaegui AV, Amalfi M, Labollita H, Santinelli N, Sastre V (1997) Inventario de ambientes dulceacuícolas de la Argentina con riesgo de envenenamiento por cianobacterias (toxic cyanobacterial blooms in Argentinean freshwaters). Ingeniería Sanitaria Ambiental 33:26–34

Ploetz DM, Fitts BE, Rice TM (2007) Differential accumulation of heavy metals in muscle and liver of a marine fish, (King Mackerel, *Scomberomorus cavalla* Cuvier) from the Northern Gulf of Mexico, USA. Environ Contam Toxicol B 78:124–127

Rautenberg GE, Amé MV, Monferrán MV, Bonansea RI, Hued AC (2014) A multi-level approach using G. affinis as a bioindicator of environmental pollution in the middlelower basin of Suquía River. Ecol Indicat 48:706–720

Regoli L, Van Tilborg W, Heijerick D, Stubblefield W, Carey S (2012) The bioconcentration and bioaccumulation factors for molybdenum in the aquatic environment from natural environmental concentrations up to the toxicity boundary. Sci Total Environ 435–436:96–106

Rey Vázquez G, Meijide F, Da Cuña R, Lo Nostro F, Piazza Y, Babay P, Trudeau V, Maggese C, Guerrero G (2009) Exposure to waterborne 4-tert-octylphenol induces vitellogenin synthesis and disrupts testis morphology in the South American freshwater fish *Cichlasoma dimerus* (Teleostei, Perciformes). Comp Biochem Phys C 150:298–306

Rios FS, Kalinin AL, Rantin FT (2002) The effects of long-term food deprivation on respiration and haematology of the neotropical fish Hoplias malabaricus. J Fish Biol 61:85–95

Ruibal Conti AL, Reguerira M, Guerrero JM (2005) Levels of microcystins in two reservoirs used for water supply and recreation: differences in the implementation of safe levels. Environ Toxicol 20:263–269

Ruiz M, Galanti LN, Ruibal AL, Rodriguez MI, Wunderlin DA, Amé MV (2013) First Report of Microcystins and Anatoxin-a Co-occurrence in San Roque Reservoir (Córdoba, Argentina). Water Air Soil Pollut 224:1593

Saint-Paul U (1984) Physiological adaptation to hypoxia of a neotropical characoid fish *Colossoma macropomum*, Serrasalmidae. Environ Biol Fish 11:53–62

Santiago AN, Bonansea RI, Valdés ME (2018) Organic pollutants in the Suquía River Basin. In: Handbook of environmental chemistry (vol 62, pp 145–180). Springer International Publishing, Switzerland

Scarafia ME, Agnese AM, Cabrera JL (1995) *Microcystis aeruginosa*: behavior and toxic features in San Roque Dam (Argentina). Nat Toxins 3:75–77

Schlenk D, Benson W (eds) (2001) Target organ toxicity in marine and freshwater teleosts. Taylor & Francis, Boca Raton/London-New York

SRHN (Subsecretaría de Recursos Hídricos de la Nación) (2017) Niveles Guía Nacionales de Calidad de Agua Ambiente. Subsecretaría de Recursos Hídricos de la Nación, República Argentina https://www.mininterior.gov.ar/obras-publicas/rhcalidad-niveles.php

Storelli MM, Barone G, Storelli A, Marcotrigiano GO (2006) Trace metals in tissues of Mugilids (*Mugil auratus*, *Mugil capito*, and *Mugil labrosus*) from the Mediterranean Sea. Environ Contam Toxicol B 77:43–50

Tao Y, Yuan Z, Xiaona H, Wei M (2012) Distribution and bioaccumulation of heavy metals in aquatic organisms of different trophic levels and potentiall health risk assessment from Taihu lake, China. Ecotoxicol Environ Saf 81:55–64

Turner CL (1941) Morphogenesis of the gonopodium in Gambusia affinis affinis. J Morphol 69:161–185

Turner CL (1947) The rate of morphogenesis and regeneration of the gonopodium in normal and castrated males of *Gambusia affinis*. J Exp Zool 106(2):125–143

UNEP, Stockolm Convention (2011) The 9 new POPs under the Stockholm convention. Available from: http://chm.pops.int/Programmes/New POPs/The9newPOPs/tabid/672/language/en-US/Default.aspx. Accesed 26 June 2012

Val AL, Silva MNP, Almeida-Val VMF (1998) Hypoxia adaptation in fish of the Amazon: a never-ending task. South Afr J Zoo 33:107–114

Valdés ME, Amé MV, Bistoni MA, Wunderlin DA (2014) Occurrence and bioaccumulation of pharmaceuticals in a fish species inhabiting the Suquía River basin (Córdoba, Argentina). Sci Total Environ 472:389–396

van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149

Visnjic-Jeftic Z, Jaric I, Jovanovic L et al (2010) Heavy metal and trace element accumulation in muscle, liver and gills of the Pontic shad (Alosa immaculata Bennet 1835) from the Danube River (Serbia). Microchem J 95:341–344

Wang C, Lu GH, Wang PF et al (2011) Assessment of environmental pollution of taihu lake by combining active biomonitoring and integrated biomarker response. Environ Sci Technol 45:3746–3752

Wood CM (2001) Toxic responses of the gills. In: Schlek D, Benson WH (eds) Target organ toxicity in marine and freshwater teleosts. Taylor & Francis, Boca Raton, pp 1–89

World Health Organization [WHO] (1998) Health criteria and other supporting information. In: Guidelines for drinking-water quality, addendum to vol. 2, 2nd edn. World Health Organization, Geneva, pp 95–110

Wurl O, Obbard JP (2005) Organochlorine pesticides, polychlorinated biphenyls and polybrominated diphenyl ethers in Singapore's coastal marine sediments. Chemosphere 58:925–933

Zambrano MJ, Rautenberg GE, Bonifacio AF, Filippi J, Amé MV, Bonansea RI, Hued AC (2018) Effects of water quality on aspects of reproductive biology of Cnesterodon decemmaculatus. Sci Total Environ 645:10–21

Chapter 12
Effects Induced by the Presence of Metals in Species of Economic and Ecological Importance in Mexican Aquatic Environments

Fernando Alberto Mares Guzmán, Mario Alejandro Muñoz Nájera, Guadalupe Barrera Escorcia, and Patricia Ramírez Romero

12.1 Introduction

Wastewater frequently contains toxic metals and other contaminants that can be accumulated in the organisms that live in Mexico's aquatic environments, which are exploitable resources. Their consumption can transfer contaminants to the highest trophic levels. Their effects on organisms result from direct contact with water, as well as from consumption of contaminated prey. This is also valid for people.

In the world, 97% of the water is marine, and, therefore, freshwater constitutes only a small portion, but it is very important as a potable water source and for other uses (Prescott et al. 2000). Lately, water quality has been compromised by the emission of contaminants. In the marine environment, pollution has increased especially in coastal areas. In continental areas, a variety of activities like mining, agriculture, and industry dump their wastes in the aquatic ecosystems. These toxic compounds have a direct impact on the aquatic organisms, and this depends on their chemical composition. Their effects can be manifested at the short, medium, or large term, which depends on their concentration, dispersion mechanisms, and toxicity level. Effects include physiological and biochemical alterations of the organism functions, increased susceptibility to illness (pathogen agents), as well as a decreased resistance to environmental factors. Contaminants can influence reproductive capacity and therefore species survival and, in severe situations, the result can be the extermination of wildlife populations (Dillon and Lynch 1981; Ramade 1989). Water pollution puts human beings at risk since their survival depends directly on water quality, which can have direct effects on their health and on organisms in general (Carabias and Landa 2006).

F. A. M. Guzmán (✉) · M. A. M. Nájera · G. B. Escorcia · P. R. Romero
Laboratorio de Ecotoxicología, Departamento de Hidrobiología, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Unidad Iztapalapa, Mexico City, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_12

Water pollution studies have become relevant due to the growing demand for this resource, the economic activities that accompany human demographic growth, and the growing shortage of this resource stemmed from climate changes. As a consequence, there has been an increment in the exploration to create new wells for water supply and to increase storage through dams (Schmitz 1995).

A serious and recurring problem is the entry to aquatic ecosystems of domestic and industrial untreated wastewaters, which adds nutrients like phosphorus, nitrogen, and organic matter. All this modifies the ecological processes and may cause dissolved oxygen depletion, pH alterations, and introduction of coliform bacteria, among other consequences. Wastewaters frequently contain toxic metals and pesticides that are deposited and accumulated in the receptor aquatic system (Valdez and Vázquez 2003).

Toxic metals commonly found in aquatic ecosystems include lead, cadmium, chromium, and copper. The first two are not essential, and their toxic effects can hardly be compensated at the metabolic level. On the other hand, chromium and copper are essential, cells require small quantities, and therefore, there is some tolerance toward them. However, above certain quantities, these too can be toxic.

Lead is used in paints, ceramics, printing, batteries, gasoline, and other products. The main intake routes are pulmonary and gastrointestinal. It is distributed through the erythrocytes in which it binds to hemoglobin; it is accumulated in bone, kidney, liver, muscle, and brain tissues. Its toxic effects at the cellular level include the inhibition of ATPase activity, DNA and RNA syntheses, cellular respiration, and neural processes interference (Albert 2011).

Cadmium is used for alloys, welding, galvanizing, electroplating, paints, ceramics, batteries, pesticides, and fertilizers. It is absorbed through the pulmonary route, as well as gastrointestinal and dermal (Albert 2011). It is transported in the blood bound to proteins like metallothioneins (MT), hemoglobin, and albumin. It is deposited in the renal cortex, kidney, and testicles. Cd causes renal alterations, cellular damage, and tumors. Its toxic effects include the inhibition of cellular respiration, protein synthesis, and iron absorption; and it interferes the zinc and calcium metabolism. When Cd is incorporated into the cell, this stimulates the metallothioneins synthesis (Mencías and Mayero 2000).

Chromium is an abundant element in the earth's crust, and it is used to generate stainless steel, metallic and plastic surface electrolytic covering, ceramic materials manufacturing, food supplements, pesticides, and leather dyes, among other things (Moreno 2003). Cr is absorbed through pulmonary, dermal, and oral routes. It is transported in transferrins, hemoglobin, and metallothioneins. Some of the effects that Cr can cause are gastrointestinal irritation and bleeding with nausea, vomit, diarrhea, aerial track irritation, and dermatitis (Mencías and Mayero 2000; Moreno 2003).

Copper is an essential nutrient widely distributed in nature. Humans use it to manufacture utensils, nutrients, coins, fertilizers, and pesticides, among other things. Cu is an important component of multiple enzymes, like oxidases which play a fundamental part in the cell's energy metabolisms. It also a part of the superoxide dismutase enzyme, responsible for the reduction of the superoxide radical to

peroxide hydrogen, a detoxification process that protects the cell against oxidation. Excess copper can cause an intoxication characterized by loss of function of the previously mentioned enzymes, as well as necrosis and hepatic lesions. The main absorption route is oral, and this metal is transported bound to albumin and metallothioneins (Moreno 2003).

Quantification of water contaminant levels is fundamental in environmental analysis, especially in those cases where there is legislation to protect aquatic and terrestrial ecosystems; however, determination of pollutants concentrations in the environment does not directly inform about the risks to populations in a particular site, and therefore, it is necessary to know the contaminant's effects on the organisms that persist in the system. These effects can be evaluated through a series of responses that as a group is known as biomarkers.

Biomarkers allow the estimation of early damage; they gather responses at different levels of organization, which permits a holistic vision of the toxic effects. The information that they provide is beyond the simple quantification of environmental concentrations (McCarthy et al. 1991). Biomarkers express in a quick, easy, and cheap way the consequences of exposure to one or more toxic agents impacting an environment and can be measured in the organisms (Zapata-Pérez et al. 2002; Gold-Bouchot and Zapata-Pérez 2004). Biomarkers evaluation can be done at different levels: biochemical and molecular (effects on enzymes, proteins, DNA, RNA, and others), cellular (membrane changes and damages), and histological and individual (effects on growth).

Biomarkers are classified into three categories:

(a) Exposure: these allow the detection and measurement of chemical compounds and their metabolites, or register the interaction between a xenobiotic agent with certain molecule or cell, and are evaluated in an organism compartment.
(b) Effect: these measure biochemical, physiological, and other modifications in tissues or fluids, which can be recognized and related to possible alterations in the health status of the organisms.
(c) Susceptibility: these indicate the loss or acquisition of capacities to respond to changes caused by a specific toxic. These include genetic factors and changes in the susceptibility to such exposure (Van der Oost et al. 2003).

Biomarkers responses are generally considered as an intermediate reaction between the exposure to a xenobiotic and evident clinical effects in the individual. When these compensatory effects are activated, the organism' survival probability declines and it will depend on its capability to adapt to environmental changes (Van Der Oost et al. 2003).

An example of an exposure biomarker associated with the effects of metals is metallothionein (MT). These are low molecular weight proteins that contain cysteine; they can bind a variety of biological important metals like zinc and copper. MT can also bind metals that do not have any known biological function; this is the case of Cd, Pb, and Hg. The concentration of these proteins rises when the concentration of metals increases in organs and tissues like the liver and muscle, among others. That is why it is possible to establish a direct relationship between metal exposure

and MT concentration, which in turn allows for the determination of exposure in cases when there are no detectable metal levels in the tissues (Hamilton and Mehrle 1986; Dallinger et al. 1997).

The acetylcholinesterase enzyme activity is a biomarker of effect which is usually used to evaluate neurological effects of diverse toxins like pesticides and metals like lead. This enzyme regulates nerve impulse transmission by hydrolyzing the accumulated acetylcholine in the cholinergic synapsis (Brewer et al. 2000). The inhibition of this enzyme can cause muscular paralysis, which in fish is manifested as disorientation, erratic swimming, and reflex lethargy (Dembele et al. 2000), which in turn causes hematomas due to the animal colliding with the rocks of the aquatic environment.

The study of biomarkers is carried out in test organisms that can be laboratory models (like the zebrafish), as well as in those that are found in contaminated sites (biomonitors). Biomonitor organisms represent an important tool to evaluate the consequences associated with the presence of toxins in the aquatic environment. The accumulation of some toxins occurs in specific organs like lungs, kidneys, and liver and in specific tissues like adipose, muscular, and bones. Pollutants accumulation analysis in organisms is a good indicator of the biological impact since it automatically takes into account their availability to living beings (Borgmann 2000; Chapman 1996).

Biomonitors can be used at the individual, community, and ecosystem level, while the damage they may present can be evaluated through biomarkers at the biochemical, histological, physiological, anatomical, genetic, and reproductive level (Capó 2002; Rosenberg et al. 2008; Prat et al. 2009). Biomonitors should be chosen carefully since the results of their investigation should allow inferring what happens with most of the species that inhabit a specific ecosystem. This type of analysis is denominated as ecotoxicological (Capó 2002). Tataruch and Kierdorf (2003) proposed the following characteristics for a biomonitor species: it should be distributed in one or more big areas so it can be considered representative; its collections should not represent a big effort, its manipulation and identification should be easily performed. Its population density should be high enough so that its sampling does not represent a problem. Its size should be enough to perform the necessary measurements and tissue and organ biopsies. It should be sensitive but at the same time resistant to the contaminants of the environment, so that it can manifest effects but without compromising its descendants. A long life cycle is convenient so that it can accumulate the contaminant and show evident effects along the time. Its importance as an exploitable resource will evidence if there is a risk for human health. An aquatic biological indicator is an organism whose presence and abundance signal a process or the state of the system in which it inhabits (Roldán 1999). Previous knowledge of the species function under normal circumstances is necessary, including life cycle, seasonality, and its natural variations, in a way that it is possible to compare before and after an environmental perturbation.

In Mexico, some species have the previously mentioned characteristics; such is the case of mollusks and fish that live in different environments, particularly of the

oyster *Crassostrea virginica* and the tilapia *Oreochromis niloticus* which are two species that have been used as bioindicators.

The oyster *Crassostrea virginica* has demonstrated to be a good laboratory study model, due to the changes it manifests when exposed to contaminants, like behavioral, physiological, biochemical, and genetic modifications. *C. virginica* can be considered a good biomonitor since it has a wide distribution, it is a benthic sedentary organism in adult life, it resists contaminants, and it can accumulate metals (Baqueiro et al. 2007). Its role in the ecosystem includes the reduction of organic matter. In Mexico, it is distributed in the Gulf of Mexico, where it is extracted and has great commercial importance so that there are two close seasons per year established for its protection in the nation's laws: NOM-015-SAG/PESC-2016 (SEGOB 2016). The uptake of metals in *C. virginica* occurs through direct contact of the animal with the metal in water, or through the oral route. This last route is more important when there are solid particles in the media and these are contaminated (Roesijadi 1996; Levine et al. 2006). Once inside the organism, the contaminated particles can interfere with a variety of processes. Metals can be accumulated, but in most benthic filtering organisms, like *C. virginica*, they hardly reach a dynamic equilibrium, resulting in a long-term presence of the contaminant in its tissues (Mok et al. 2015; Soto-Jiménez 2011). During stressful environmental conditions, the closing of the valves can last many hours, which implies that the filtration process is stopped. This represents a mechanism that minimizes the contact with the toxic. Additionally, when the metal is already in the organism, there are detoxification mechanisms like the induction of proteins specialized in the sequestering of metals, known as metallothioneins (Lemus et al. 2016).

The tilapia *O. niloticus* is an organism that was introduced to Mexico, and despite not being native, it has adapted itself to the conditions of the country, and it is an important food resource nowadays. There are three species of the *Oreochromis* genera in the territory: *O. mossambicus*, *O. aureus*, and *O. niloticus* (SAGARPA 2012). Its life cycle consists of various stages: egg, fry, young, juvenile, and adult. It develops between 20 °C and 30 °C. Males mature sexually between 4 and 6 months, while females do it between 3 and 5 months. When they are 6–12-month old, they can weigh between 250 and 500 g, with a length of 12–15 cm. Tilapia is highly accepted in the market due to its high nutritional value. Most commercialized fish in Mexico weigh around 150 and 500 g, and for fillet and exportation around 800 g to 1 kg (SAGARPA 2006, 2012; Saavedra 2006). A variety of toxic compounds affects its quality as a food resource and represents a human health risk; such is the case of Cd, Pb, Cr, and Cu. Tilapia is ideal to evaluate toxic effects because of its accelerated growth rate, continuous reproductive periods, high fecundity, high resistance to illnesses, its omnivore habits, as well as its tolerance to temperature variations, which is why it is distributed in semiwarm and warm waters. Like other fishes, tilapia can bioaccumulate metals in target organs and muscle and will manifest effects like morphometric, physiological, reproductive, and genetic modifications (Ramírez and Mendoza 2008). Numerous toxicological and biological studies have been performed in tilapia.

The objective of this chapter was to integrate information of effects caused by some metals present in Mexican aquatic ecosystems, on two ecologically and commercially important bioindicators, the oyster *Crassostrea virginica* (Gmelin 1791) and tilapia *Oreochromis niloticus* (Linnaeus 1758).

12.2 Method

The collection of oysters was carried out in the Gulf of Mexico (Tecolutla River, Veracruz, while tilapia was obtained from an artificial inland dam (Tenango, Puebla). The procedures applied for each species are described below.

12.2.1 C. virginica *as a Bioindicator of Metal Trophic Transfer*

Specimens of *C. virginica* were extracted from the Tecolutla estuary (20°28′48″ N, 97°06′40″ W), which is located in the state of Veracruz. This area has a beach expanding 64 km^2; the weather is warm and humid with rains during the summer, the median annual temperature is 22 °C, and the average precipitation is 60 mm (H. Ayuntamiento de Tecolutla 2016). The primary economic activities of the region are agriculture, ranching, fishing, and tourism. The Tecolutla River provides most of the organisms that are locally commercialized, including shrimp (*Litopenaeus* sp.), fishes, crustaceans, and mollusks. The estuary is very important since it is associated with a mangrove forest that helps in the reproduction of the commercialized organisms (INEGI 2016).

Organisms were obtained from the pier of the Tecolutla River (Fig. 12.1, Site 2). In this site and other five located along the river, physicochemical parameters were registered in situ: pH, dissolved oxygen (DO), and temperature with a HANNA potentiometer (HI98128) and a YSI analog oximeter (model 54A). Salinity was measured with an ATC hand refractometer. Additionally, superficial water samples were taken at the same sites to analyze Cu and Cd concentrations.

Water samples were taken manually in 1 L plastic bottles previously washed with 10% HNO_3. Samples were fixed to <2 pH with 0.5 mL of nitric acid (reagent grade) and put in a cooler at 4 °C, to be transported to the Ecotoxicology Laboratory in Mexico City where they were frozen to – 20 °C until metal analysis was performed.

Organisms were collected also manually, put in a black plastic bag, and transported to the laboratory in a cooler containing ice, where they were maintained in a water system that mimics the characteristics of its habitat, until the experimental phase.

When the organisms arrived at the laboratory, each oyster was washed under tap water with the help of a brush with plastic bristles to remove sediment, organic matter,

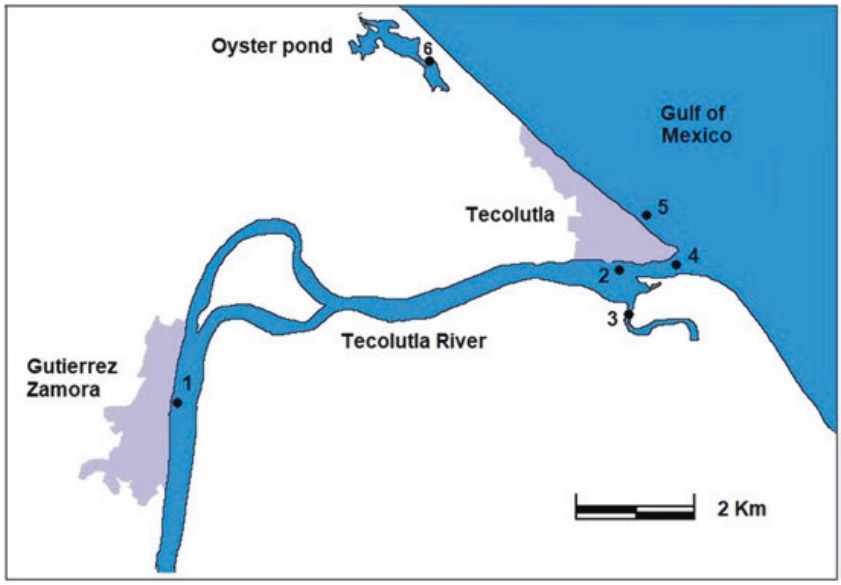

Fig. 12.1 Study Site: Tecolutla, Veracruz. Sampling sites: (1) Gutiérrez Zamora, (2) pier, (3) estuary, (4) mouth, (5) sea, (6) oyster lagoon

and epibionts adhered to the shell; then, the oysters were submerged in a 70% alcohol solution for 10 min and rinsed with artificial seawater.

The maintenance system contained 800 L of artificial seawater (Kent marine), ripen a month in advance, that flowed continuously through 14 interconnected aquariums (to guarantee aeration). The system had a physical filter for the removal of particles, and an activated carbon filter, as well as a skimmer for the removal of proteins. Physicochemical parameters (pH, DO, temperature, and salinity) were evaluated every day. Initially, water salinity was prepared to be similar to that registered in the field, to avoid osmotic stress in the organisms. Later it was slowly increased to 22 ups.

The study of the effects of the metals on the oyster was performed in two phases. The first was to secure a *Chlorella* sp. culture to feed to the oysters as Cd- or Cu-contaminated food. It was necessary to test the conditions in which the algae could grow exposed to the metals, in order to generate the necessary cell concentration for feeding and, also, to guarantee metal accumulation. In the second phase, the oysters were exposed to contaminated food to evaluate the biomarkers.

Chlorella culture was prepared in a mineral medium with a pH of 6 in artificial seawater (5 ups, Kent marine). Cell concentration counts were done using a Neubauer chamber and a Zeiss optical microscope. 0.01 mg of reagent grade (J.T. Baker) copper or cadmium salts ($CuSO_4 \cdot 5H_2O$ and $CdCl_2 \cdot 2\ 1/2\ H_2O$, respectively) was added to the medium, which implied a Cu concentration of 0.0254 mg/L or a Cd concentration of 0.0493 mg/L, as suggested by Cordero et al. (2005). *Chlorella* population growth was monitored every 24 h, until the exponential phase and a density of 30×10^6 cells per mL was reached (110 h). Culture media was centrifuged 3 minutes at 500 RPM in a Solbat centrifuge to separate the cell from

the mineral medium. The supernatant was eliminated, and the cells were stored in 50 mL Falcon tubes added with artificial salty water at 5 ups, without a mineral medium, to feed the oysters.

Seventy-five organisms that had been fed every 24 h (30×10^6 cells/mL) were exposed either to clean food, Cu-contaminated food, or Cd-contaminated food. Biomarkers (lysosomal membrane stability and metallothioneins) evaluation in oysters was performed every 24 h for a 96 h period; for each time and concentration, five organisms were selected using an aleatory numbers table to avoid bias; morphometric data of each oyster were taken and its condition index calculated.

Lysosomal membrane stability was measured through the neutral red retention technique (Lowe et al. 1995). Slides were previously prepared with 10 µL of a solution containing 15 µL of poly-L-lysine (SIGMA-Aldrich) and 135 µL of distilled sterile filtered water, dried in a humid and dark chamber for 30 min. To extract hemolymph of each animal, its valves were moved away leaving the visceral mass exposed; then with a hypodermic needle containing 0.1 mL of saline physiological solution 0.9 NaCl (PISA), 0.1 mL of hemolymph was extracted from the venous sinus (heart). The needle was removed from the syringe, and the hemolymph was transferred to a silicone Eppendorf tube containing poly-L-lysine. Later 40 µL of the hemolymph and saline solution were dispensed over the slides to proceed to 30 min incubation in a wet chamber, to allow the cells to adhere to the slide. The excess solution was dried with a paper towel, and 40 µL of neutral red solution was added to each slide. The neutral red solution was prepared by diluting 20 mg of neutral red in 1 mL dimethyl sulfoxide; then, 10 µL of this mixture was diluted with 990 mL of saline solution.

Slides were placed in a humid dark chamber, and to observe the coloration, a cover glass was added before reviewing them under an optical microscope at 40×. Observations were done every 15 min during the first hour and then every 30 min for two more hours. To avoid the drying of the samples that can result in dye crystals formation, observation lasted less than 1 min.

Metallothionein analysis (Scheuhammer and Cherian 1986) required the previous hemolysis of heparinized rabbit's blood; to this end, 10 mL of blood with 20 mL of KCl 1.15% were centrifuged at 500G for 5 min at 10 °C to sediment erythrocytes, which were resuspended with 20 mL of heparinized 1.15% KCl and centrifuged again under the same conditions twice more. Erythrocytes lysis was achieved by resuspending with 15 mL of a Tris 30 mM pH 8 buffer for 10 min. The supernatant was recovered in 1 mL Eppendorf tubes, which were preserved at −85 °C until their use.

MT analysis was performed in the digestive gland of the oysters, which was dissected from each organism analyzed using plastic knives, previously washed with 10% HNO_3. The tissue samples were placed in Eppendorf tubes over ice. For each wet gram of tissue, 4 mL of cold sucrose 0.25 M solution was added, followed by a slow homogenization with Teflon pestle, to try to avoid bubble formation that could oxidize the tissues. Later the tissue was centrifuged at 2000*g* for 20 min at 4 °C. The supernatant was recovered and stored in 2 mL Eppendorf tubes. 375 µL of the supernatant was mixed with 825 µL glycine buffer to obtain a final volume close to

1200 µL. 500 µL of silver solution was added to each sample and incubated in darkness for 20 min, followed by the addition of 200 µL of hemolyzed rabbit's blood to sweep along those metals not trapped by metallothioneins. Samples were then put in a double boiler for 2 min to eliminate undesirable molecules; then they were centrifuged at 4000 RPM. The supernatant was recovered and centrifuged at 13,000 RPM for 5 min. All samples were kept frozen at – 85 °C in a REVCO freezer until further analysis. MT concentration (µg/g wet weight) was obtained through the detection of silver (Ag) in atomic absorption spectroscopy with Varian AA20 equipment.

12.2.2 **O. niloticus** *as a Bioindicator of the Presence of Metals in the Environment*

The tilapia study took place in the Tenango dam (20°12′13″ N y 97°59′27″ W) which is located on the north mountain range of the State of Puebla, close to Tenango de Las Flores town in the Huachinango municipality (Fig. 12.2). It is located 1472 m ASL and it is part of the Necaxa River watershed. The dam belongs to the Necaxa hydroelectric system and has two main water sources the Acatlan and the Nexapa dams, which are connected through tunnels. In turn, the Tenango dam supplies water to the Necaxa dam for the generation of hydroelectric energy (INE-UACH 2007). There are touristic activities in the Tenango dam, like boat rides, camping, handicrafts, and ornate plants (chrysanthemums, gardenias, carnations, roses, poinsettias, orchids, cempasuchil, etc.) cultures; these last require the use of chemical fertilizers and pesticides. Among the fertilizers used are blue Nitrofosca, Chilean Nitrate, Urea 94515, Triple 20, and Multi-npk. In regard to the pesticides, Benomilo©, Folicure©, Talstar©, Tecto 60©, and Amistar are used to fight fungus,

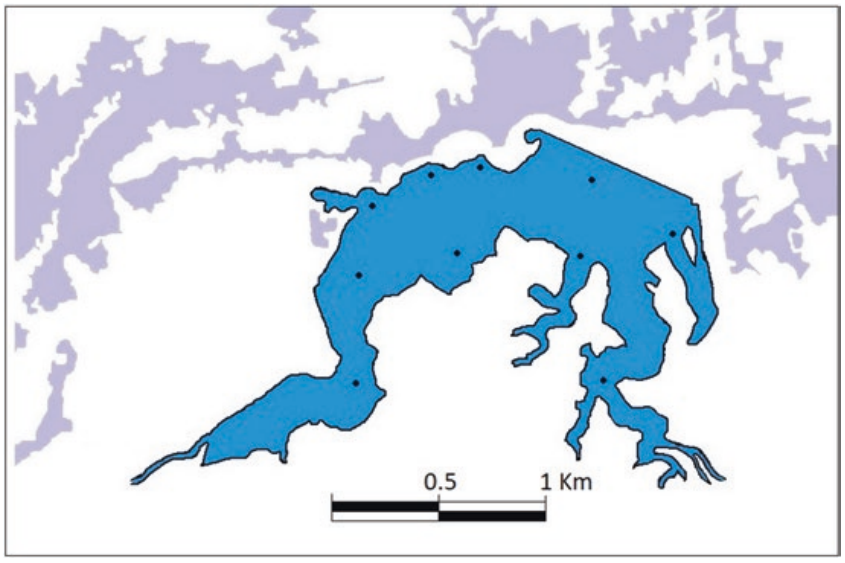

Fig. 12.2 Tenango dam sampling sites

while Furadan©, Dioxinon©, Foley©, and Compo© are used to fight insects and Nemacur© and Etoprop© to fight nematodes. These pesticides are applied year round, but Benomilo© and Furadan© are the most used in the area. There is also raw wastewater deposition, coming from the homes located in the dam's periphery; this is due to the lack of a drainage system. Solid wastes (bottles, clothing, food leftovers, and tires) are also deposited on the dam shores.

The study in Tenango took place along 1 year during which five field trips were accomplished (January, April, June, September, and November) in 2015. Water samples encompassed the whole dam; for metal quantification, APHA (1992) recommendations were applied: samples were taken in plastic bottles that were previously washed with Extran, followed by a 24 h soak in 10% HNO_3 and a distilled water rinse. Samples pH was adjusted between 1 and 2 using a Hanna field pH meter (model HI981) and analytic grade Fermont HNO_3. Afterward, samples were refrigerated at 4 °C and later transported in a cooler to the ecotoxicology laboratory where they were preserved at −20 °C until further processing.

With regards to fish, 30 tilapias were captured with the help of fishermen and a nylon monofilament net. Morphometric data (weight and length) of each specimen were taken, and a visual inspection was performed checking eye and gills appearance, wounds, lesions, hematomas, deformities and tumors presence, skin color, and general appearance as described in SAGARPA's production of tilapia manual (2006), where quality standards for tilapia as a consumption product sold to restaurants and the general public are explained.

Muscle and liver samples were obtained from each specimen; one portion was for metals quantification and another for metallothionein evaluation. These were cut using plastic instruments to avoid metals traces contamination. Samples were placed in separate glass containers preciously washed as explained before (APHA 1992; Lozada-Zarate et al. 2006). Samples were transported in a cooler to the Ecotoxicology laboratory where they were frozen to −20 °C until further analysis.

Water samples were analyzed for the presence of Pb, Cd, Cr, and Cu with a Varian 220 FS atomic absorption spectrometer, following the technique explained in NMX-AA-051-SCFI-2001 (Secretaría de Economía 2001). Metal concentrations were compared with Mexican water quality criteria (QWC) for urban use and for the protection of aquatic life (CONAGUA 2016).

In Pb, Cd, Cr, and Cu analysis in fish tissues, liver and muscle samples were weighted and dried at 40 °C in a stove until constant weight was achieved. 0.5 g (dry weight) of each sample was placed in a Teflon cup, and 5 mL of analytic grade HNO_3 was added before digesting the sample in a CEM MARSX5 microwave oven at 170 °C for 20 min (EPA 1995). The resulting sample was diluted to a volume of 30 mL with deionized water and analyzed in a Varian 220 FS atomic absorption spectrometer (Lozada-Zarate et al. 2006). Tissues metal levels were compared with the maximum allowed concentrations in norm NOM-242-SSA1-2009 (Secretaría de Salud 2011) and with Brazilian legislation for food for human consumption (LBMP 2017).

Metallothionein production was evaluated with Scheuhammer and Cherian (1986) technique previously described. In this case, tilapia tissues were homoge-

nized with 4 volumes of 0.25 M sucrose (weight/volume = 1:4); the supernatant was frozen at – 85 °C in a REVCO freezer until further analysis.

Results were statistically analyzed with IBM SPSS Statistics 23 and NCSS 2007 software; comparisons were examined with ANOVA or Kruskal-Wallis if data were not normal (Kolmogorov-Smirnov test) and had no homoscedasticity (Levene test). To analyze the variables relationships, Pearson and Spearman's simple correlations were used according to each case (Sokal and Rohlf 2012). The significant level considered was $P < 0.05$ (Marques de Cantú 1991).

12.3 Results

12.3.1 Metal Trophic Transfer in C. virginica

The analyzed biomarkers responses indicated harmful effects due to Cu and Cd exposure through ingestion of contaminated *Chlorella*.

Lysosomal stability was lost, which was observed through the reduction in neutral red dye retention times (NRRT) in lysosomes (Fig. 12.3). Control organisms did not show changes in NRRT throughout the essay with a maximum retention time of 210 min. Adverse effects were observed since the first 24 h. By 72 h, organisms fed with Cu-contaminated cells presented a NRRT close to 24 min. By the end of the assay, those oysters fed with Cd-contaminated algae showed a NRRT of 9 min.

MT analysis in oyster's digestive gland also showed deleterious effects after 24 h exposure to contaminated algae food. In the Cu assay, there was an increase in MT production with a subsequent reduction after 48 h. For Cd, the increase happened after 24 h of exposure and reached a maximum value at 72 h followed by a decrease at 96 h. Control oysters presented concentrations close to 60.0 μgMT/g tissue throughout the assay, except at 48 h where there was a significant increase (88.5 μgMT/g tissue, $P < 0.05$, Kruskal-Wallis test). Even when this concentration was higher than those obtained in the other days, the experimental groups (Fig. 12.4;

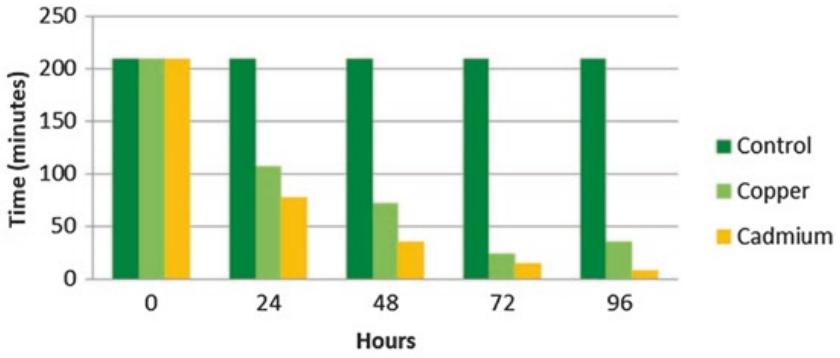

Fig. 12.3 Neutral red dye lysosomal retention time in *C. virginica* fed with metal-exposed *Chlorella*

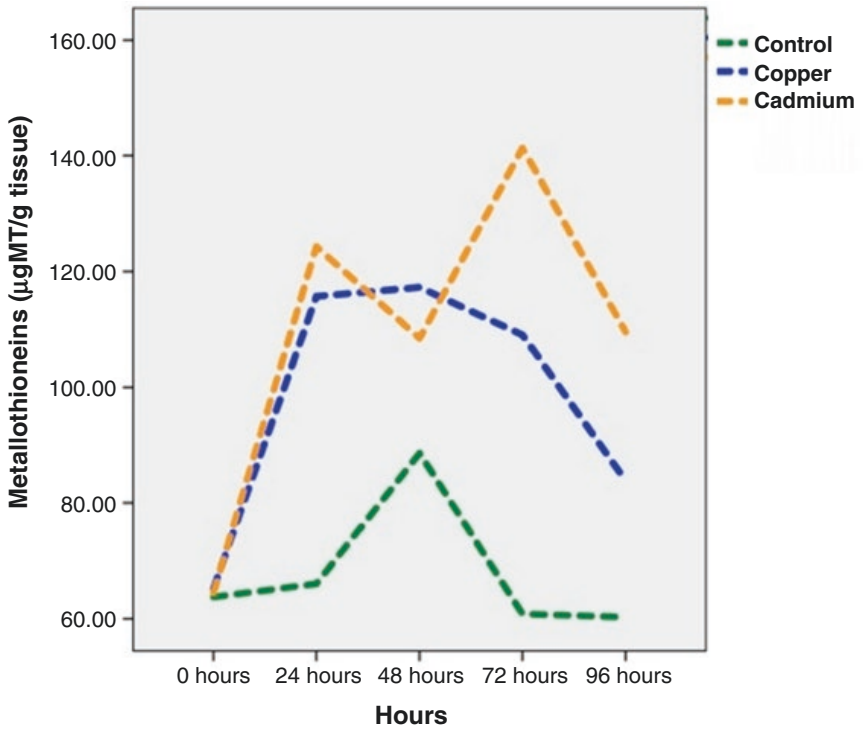

Fig. 12.4 Metallothionein average induction in oyster *C. virginica* fed with Cu- and Cd-contaminated algae

Table 12.1) showed significant differences between them and controls; the MT were manifested in Cu-exposed animals after 24 h (115.6 µgMT/g tissue), and in Cd-exposed oysters, bigger concentration was at 72 h (115.6 µgMT/g tissue).

Other different values were not significantly different from controls. The absence of difference between exposed and control organisms may be due to wide data dispersion, which may mask the possible differences (Fig. 12.5). Standard deviations were always higher in exposed organisms, except at 96 h. In spite of not been able to demonstrate differences, the maximum values registered at 24 h indicate an important induction in oysters exposed to both metals, since they reached their highest values: 172.0 µgMT/g tissue for Cu, and 202.8 µgMT/g tissue for Cd, while the control only reached 107.4 µgMT/g tissue at 96 h.

Water metal pollution in Tenango dam showed significant differences among all collections (Table 12.2).

According to Mexican laws, Pb was the metal that exceeded in more occasions the WQC which occurred in four out of the five collections (January, April, June, and September) (Fig. 12.6). Cr exceeded the WQC in January, April, and September. Cu and Cd had similar behavior, exceeding the WQC for urban use and aquatic life protection in the month of June, while in November they only exceeded the WQC for aquatic life protection.

A total of 150 specimens of *O. niloticus* from the Tenango dam was analyzed; the morphological attributes considered for the visual quality inspection were fulfilled by all organisms, so they were suitable for commercialization (SAGARPA 2006).

Table 12.1 Metallothionein quantitation (μgMT/g tissue) in *C. virginica* digestive gland

Hours		Control	Con Cu	Con Cd
0	$\bar{x}$ ± SD Min-Max	63.7 ± 5.0 57.8–68.0	65.2 ± 11.7 51.8–77.5	64.4 ± 7.8 56.5–74.0
24	$\bar{x}$ ± SD Min-Max	66.0 ± 18.6 51.5–97.0	115.6 ± 31.8* 97.3–172.0	124.0 ± 51.6 68.9–202.8
48	$\bar{x}$ ± SD Min-Max	88.5 ± 3.2 85.3–93.7	117.2 ± 32.7 81.8–170.3	108.4 ± 23.6 78.9–139.8
72	$\bar{x}$ ± SD Min-Max	60.8 ± 26.7 19.0–88.9	109 ± 41.3 60.0–167.9	141.2 ± 34.3* 110.6–194.9
96	$\bar{x}$ ± SD Min-Max	60.2 ± 30.0 35.5–107.4	83.8 ± 28.8 56.2–114.8	109.5 ± 14.0 93.8–128.3

$\bar{x}$ ± *SD* average and standard deviation, *Min* minimum, *Max* maximum
*Significant differences ($P < 0.05$)

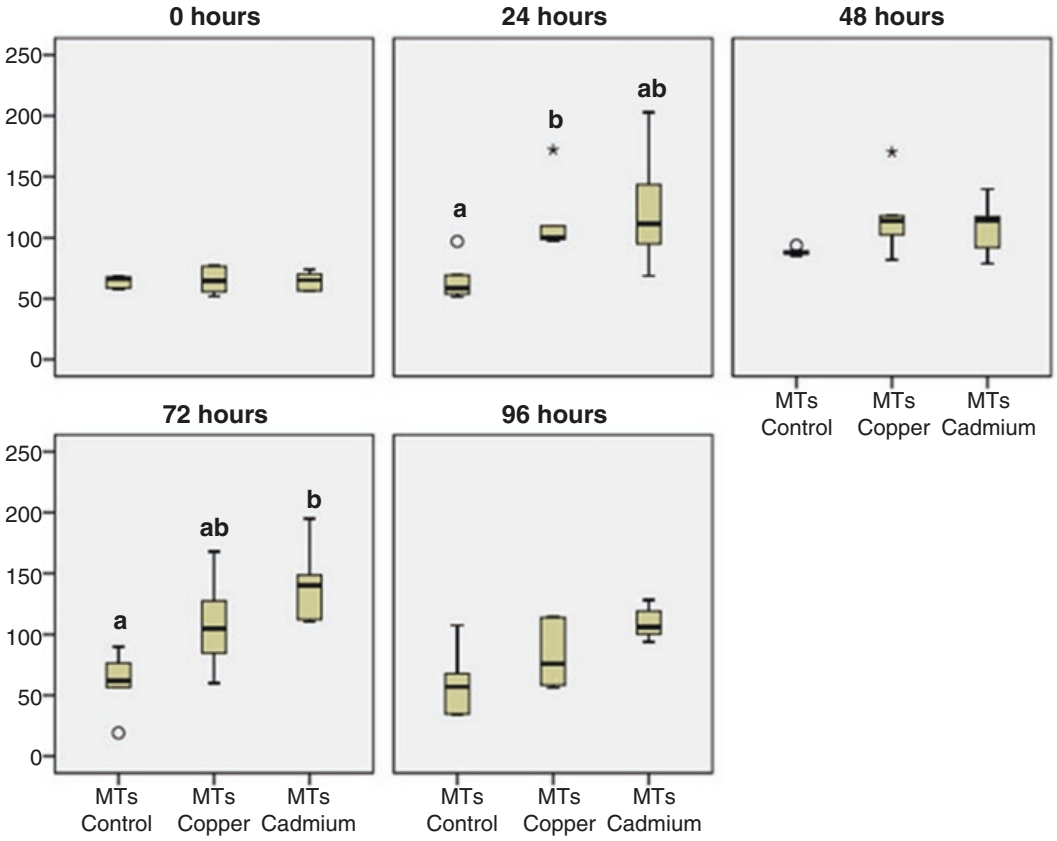

Fig. 12.5 Metallothionein expression in oysters fed with Cu- and Cd-contaminated *Chlorella* cells for 96 h. Metals present in *O. niloticus* and their possible relationship with morphological parameters and biomarkers. Different letters indicate statistically significant differences ($P < 0.05$)

Table 12.2 Tenango dam water average metal concentrations (mg/L) and standard deviations

	January	April	June	September	November	Limit
*Pb	**3.20 ± 1.05**	**2.93 ± 1.50**	**0.40 ± 0.15**	**0.04 ± 0.03**	0.03 ± 0.02	0.05[a] 0.03[b]
*Cr	**0.42 ± 0.13**	**0.16 ± 0.11**	0.01 ± 0.01	**0.11 ± 0.06**	0.02 ± 0.01	0.05[a,b]
*Cu	<0.01	<0.01 ± 0.00	**1.37 ± 0.62**	<0.01	**0.25 ± 0.03**	1.00[a] 0.05[b]
*Cd	0 ± 0	0 ± 0	**0.13 ± 0.05**	<0.01	**0.01 ± 0.01**	0.01[a] 0.004[b]

*Statistically different between collections ($P < 0.01$)
[a]Maximum limit allowed for urban use
[b]Maximum limit allowed for the protection of aquatic life (CONAGUA 2016)

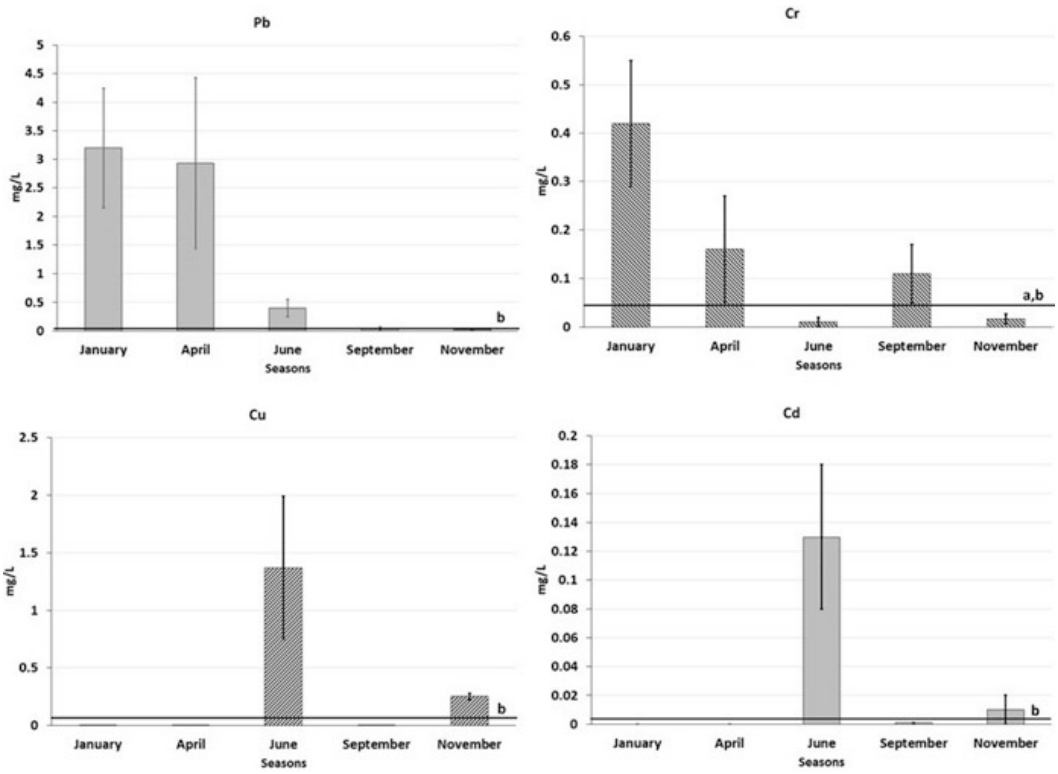

Fig. 12.6 Metals average concentrations for each sampling. WQC (**a**) for urban use, 0.05 mgCr/L, and (**b**) for protection of aquatic life, 0.03 mgPb/L, 0.05 mgCr/L, 0.05 mgCu/L, 0.004 mgCd/L (CONAGUA 2016)

That is, eyes had black pupils and a crystalline cornea and were convex; gills were bright, pelvic and pectoral fins were present, and the animals did not show cuts, lesions, deformations, or mucus over the body; flesh was firm and elastic and finger pressure did not leave imprints; skin was red, gray, or black or combined as expected; scales loss was less than 15% on the body's surface. Only in January and April, 4% of the specimens presented hematomas.

There were significant differences in length among collections, and in April and November, Tilapia size was less than acceptable for commercialization. Weight did

Table 12.3 Morphometry and liver and muscle metal concentrations in *O. niloticus* from Tenango dam (average and standard deviation)

Morphometry	January	April	June	September	November	Ideal values
*Total length (cm)	18.7 ± 2.7	**15.6 ± 1.4**	18.4 ± 2.2	18.4 ± 1.1	**16.4 ± 4.1**	18–25[a]
Weight (g)	**120 ± 42**	**101 ± 21**	**122 ± 45**	**112 ± 33**	**112 ± 27**	150–300[b]
Concentration (mg/kg)						MAL
*Pb – Liver	11.12 ± 3.63	11.13 ± 0.42	0.11 ± 0.04	0.13 ± 0.02	0.08 ± 0.01	0.5[c]
*Pb – Muscle	6.82 ± 4.18	3.68 ± 1.06	0.10 ± 0.04	0.13 ± 0.02	0.09 ± 0.02	0.5[c]
*Cd – Liver	1.62 ± 0.98	1.26 ± 0.51	0.01 ± 0.00	0.01 ± 0.002	0.01 ± 0.008	0.5[c]
*Cd – Muscle	1.89 ± 0.89	2.06 ± 0.47	0.01 ± 0.00	0.008 ± 0.00	0.01 ± 0.001	0.5[c]
*Cr – Liver	1.49 ± 0.76	1.34 ± 0.10	0.07 ± 0.02	0.08 ± 0.05	0.06 ± 0.02	0.1[c]
*Cr – Muscle	0.89 ± 0.43	0.53 ± 0.02	0.06 ± 0.01	0.06 ± 0.04	0.06 ± 0.01	0.1[c]
*Cu – Liver	0.45 ± 0.06	0.16 ± 0.02	0.18 ± 0.04	4.01 ± 0.04	0.35 ± 0.11	30[d]
*Cu – Muscle	0.17 ± 0.04	0.13 ± 0.03	0.12 ± 0.02	0.03 ± 0.02	0.32 ± 0.11	30[d]

MAL maximum allowable limit
*Statistically different between collections ($P < 0.01$)
[a]Total length interval for sale to the public and restaurants (SAGARPA 2006)
[b]Ideal weight interval for sale to the public and restaurants (SAGARPA 2006)
[c]MAL for fresh, refrigerated, and frozen fishery products NOM-242-SSA1-2009 (Secretaria de Salud 2011)
[d]MAL for food intended for human consumption (LBMP 2017)

not present significant differences; however, in all samplings, the organisms presented lower than acceptable values (Table 12.3). Metal concentrations in the liver and muscle presented significant differences among collections.

Pb presented the highest concentration in liver and muscle in January and April; these values exceeded the acceptable concentration in fishing products for human consumption, in accordance with Mexican law NOM-242-SSA1-2009 (Fig. 12.7). Cd presented the second highest concentrations and behavior similar to that of Pb, with higher concentrations in January and April and unacceptable values for human consumption. Cr also presented high values, exceeding the 0.1 mg/kg acceptable level for human consumption, also in January and April. Since Mexican laws don't include Cu limits, Brazil's regulation was used to evaluate its presence in fish tissues. It's worth mentioning that neither FAO's CODEX Alimentarius nor the European Union include Cu as a substance worth controlling in fishing products for human consumption. Cu was present in small concentrations, below the 30 mg/kg acceptable limit.

Metallothionein concentrations in liver and muscle showed significant differences among collections. The highest concentrations of these proteins were observed in livers extracted in January, September, and November. The muscle MT concentrations presented a similar pattern with higher values in the previously mentioned months; however, these values were smaller than those in the liver, except for November where they were similar (Table 12.4).

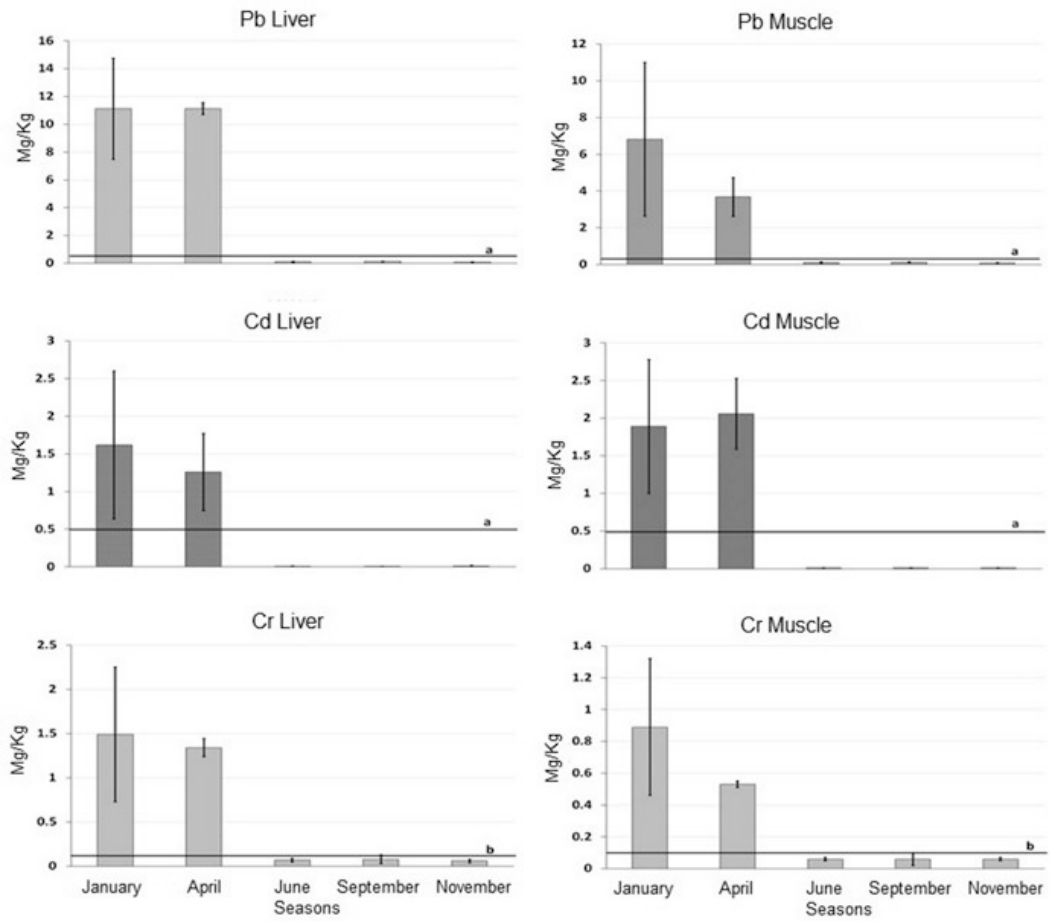

Fig. 12.7 Metal concentrations in tilapia liver and muscle for each sampling. (**a**) Maximum limit (0.5 mg/kg) for fishery products; (**b**) maximum limit (0.1 mg/kg) for fishery products according to NOM-242-SSA1-2009 (Secretaría de Salud 2011)

Table 12.4 Metallothioneins in the liver and muscle of *O. niloticus* (average and standard deviations)

Concentration (μgMT/g tissue)	January	April	June	September	November
*Liver	125.43 ±48.04	80.46 ±11.45	79.26 ±6.18	108.26 ±36.88	106.35 ±46.37
*Muscle	79.31 ±44.65	40.56 ±7.90	6.59 ±2.05	68.80 ±34.9	102.24 ±47.25

*Statistically different between collections ($P < 0.01$)

O. niloticus weight and length had a significant correlation ($P < 0.05$) with tissue metal concentration; that is, the higher the size and weight, the higher the liver and muscle metal concentration. Likewise, MT levels and tissue metal concentration had a positive correlation, which implies that the higher the metal concentration in liver and muscle, the higher their MT concentrations. This was confirmed in all

Table 12.5 Correlation between metal concentrations in tissues and morphometric variables and metallothionein concentrations

		Liver				Muscle			
		Pb	Cd	Cr	Cu	Pb	Cd	Cr	Cu
Morphometric variables									
January	Weight	0.84	0.97	0.96	0.93	0.91		0.94	0.81
	Length	0.79	0.94	0.9	0.87	0.83		0.88	0.77
April	Weight					0.93		0.77	
	Length					0.82		0.7	
June	Weight	0.82	0.96	0.84	0.95	0.82	0.95	0.84	0.97
	Length	0.76	0.89	0.77	0.85	0.79	0.89	0.77	0.89
September	Weight	0.9	0.9	0.9	0.91	0.89	0.87	0.86	0.91
	Length	0.92	0.97	0.91	0.78	0.89	0.9	0.96	0.79
November	Weight	0.86	0.9	0.91	0.89	0.83		0.84	0.92
	Length	0.71		0.83	0.8	0.65			
Metallothioneins in the liver									
January		0.9	0.95	0.96	0.9				
April									
June		0.89	0.97	0.89	0.9				
September		0.95	0.98	0.91	0.78				
November		0.77	0.66	0.92	0.87				
Metallothioneins in the muscle									
January						0.88	0.51	0.94	0.76
April						0.85		0.67	
June						0.92	0.98	0.83	0.94
September						0.87	0.95	0.97	0.86
November						0.89		0.6	0.75

collections, except for April, where only muscle Pb and Cr had a positive correlation with MT (Table 12.5).

12.4 Discussion

Mexico has legislation to limit toxic substances concentrations, including some metals, in fresh and refrigerated fish that will be consumed by humans: NOM-242-SSA1-2009 (Secretaría de Salud 2011), and water quality criteria for various uses, including the protection of aquatic life (CONAGUA 2016). In spite of this, the degradation observed in many aquatic ecosystems demonstrates that these laws are not enforced. This situation includes freshwater bodies, as well as estuarine systems, where alimentary resources like Tilapia and oysters are found and caught. Metals presence in water is a determinant factor in their bioaccumulation, but the trophic transfer has not been analyzed in depth.

In the present study, trophic transfer of Cu and Cd in the oyster *C. virginica* resulted in deleterious effects on the evaluated biomarkers. Even though lysosomal stability is considered a nonspecific biomarker, because the lysosomal membrane can be affected by a variety of toxics, the results of this study demonstrate that the decrease in NRRT is related with the ingestion of contaminated *Chlorella* cells, which was evident after 24 h of exposure. This biomarker has been evaluated in different mollusk species. Matozzo et al. (2001) exposed *Tapes philippinarum* to Cu and Cd and observed that their highest concentrations (110 μgCu/L and 450 μgCd/L) resulted in the lowest NRRT, which were close to 5 min compared to 60 min in controls; even their lowest concentration (60 μg/l) reduced the NRRT to 30 min.

On the other hand, studies with *C. angulata* have shown that as Cu concentration increases, the digestive gland can eliminate the excess metal, when its uptake is through the trophic route. This is because this organ's lysosomes sequester, neutralize, and eliminate contaminants (Rodríguez de la Rua et al. 2005). Lysosomes are the main cell organelle in charge of receiving and eliminating materials, either foreign or metals transported by MT for sequestering and elimination (Petrovic et al. 2001).

The differences in NRRT observed in the present study in *C. virginica* can be attributed to the type of metal (essential vs. nonessential). Cu is an essential metal that once inside the organism activates diverse processes related to homeostasis in the cells. In adequate amounts, it helps with the immune system maintenance, the generation of oxide-reduction enzymes, and the formation of molecules associated with genetic expression's regulatory mechanisms (ATSDR 2016). However, excess Cu (in the food provided in the present study) causes a loss of balance and toxic effects appear in the lysosomes; which in this study were measured as a decrease in NRRT after 48 h. A similar effect was observed after 24 h in the Cd exposure. Lysosomal stability loss in this last experiment was due to the fact that Cd is not essential and is bioaccumulated rapidly, which damages the lysosomes during the first 24 h of exposure. Reduction in retention time has been linked to high levels of metal accumulation in the lysosomes which can cause oxidative stress and lead to an inhibition of the proton pump, in charge of maintaining acid in the internal lysosomal environment (Nassiri et al. 2000).

Metallothioneins are considered exposure biomarkers and according to Capó (2002), they offer a fast answer to detect metal pollution in disturbed environments, which makes them very useful for environmental analysis. These proteins constitute a primary defense mechanism to avoid damage on the cell components in the presence of metals like enzyme-specific sites, structural proteins, lipid membranes, and even DNA (Giguére et al. 2003). They also comprise protection against oxidation and free radicals (Coyle et al. 2002).

MT role in the cells is to mobilize essential metals, regulating and directing them to the lysosome for deposition or elimination; therefore, a detoxification role is also attributed to them (Tanguy et al. 2001; Moulis 2010). Due to MT affinity to metals, when organisms are exposed to high concentrations, these proteins can be increased. In bivalve mollusks like *C. virginica* and *C. gigas* and other invertebrates, MT have been proven to help transport, regulate, and control Cu and Zn levels, which are essential elements, but also Cd that is not essential (Ettajani et al. 2001). Many of

these investigations evaluate MT levels associated with metal concentrations in the water where these mollusks inhabit. In a study with *Mytilus edulis*, MT induction was observed in the digestive gland when exposed to low Cu concentrations for short periods of time; under these conditions, MT support metal transport. However, when Cu concentrations are raised (above 10 μg/L), or the exposure is prolonged, these proteins concentration gradually decreases, which prevents them to fulfill their functions (Perić et al. 2017). Research with *Mytilus* (Brown et al. 2004; Pytharopoulou et al. 2011; María and Bebianno 2011) demonstrated that under acute Cu exposure (50–100 μg/L), this metal is accumulated in the digestive gland, while MT induction is reduced. In this work, MT induction through metal trophic transfer was evident after 24 h and in the case of Cu, there was a significant reduction after 72 h. Serafim and Bebianno (2009) have related a MT reduction with a break in the MT/Cu complex, which indicates that when there is depuration, MT decrease. In the case of MT induction due to Cd-contaminated food, the highest concentrations were observed after 72 h (with a 141.2 μgMT/g tissue average and a maximum of 194.9 μgMT/g tissue). Decrease after 96 h (average of 109.5 μgMT/g tissue) did not reach levels as low as those associated with Cu feeding (average 83.8 μgMT/g tissue) and even less than those of controls (60.2 μgMT/g tissue). Amiard et al. (2006) and Ivanina et al. (2008) demonstrated that MT are expressed under stress conditions caused by Cd in *C. virginica*. When this metal is incorporated through trophic route, it is captured by the digestive gland, where MT act with other proteins, to capture and detoxify this metal. This is why MT can be expressed in a short time in exposed organisms; and, when Cd decreases in the individuals, MT are also reduced (Perić et al. 2017). Unlike these previously mentioned studies, in the present work, MT Cd induction occurred later than that of Cu.

MT response in different tissues is not the same; their induction is faster when exposure is through the gills since this organ is sensitive to environmental changes (Coyle et al. 2002). MT are more active in the gill than in the digestive gland (Serafim and Bebianno 2009). On the other hand, the digestive gland is an organ that under normal circumstances is in constant renewal of its functional units (Coyle et al. 2002). Barrera (2006) documented MT concentrations in *C. virginica* up to 471 μgMT/g tissue in digestive gland and 275 μgMT/g tissue in gills on oysters exposed to 110 μg/L and 211 μg/L of Cd. This is almost double than the levels detected in gills. In another work (Ivanina et al. 2008), where oysters were exposed to Cd, digestive gland MT levels after 4 h were double than the concentrations found in the gills. While Cd concentration increases in the medium, MT in the digestive gland are very effective at capturing most metal molecules; therefore, depuration is considered effective, and as a consequence gills MT report lower concentrations. Blackmore and Wang (2004) pointed out that once a particle moves in the digestive gland, intra- and extracellular digestion takes place in the tubules, so an excess of metallic particles can damage this organ compromising its function. Petrovic et al. (2001) mentioned that when metals are incorporated into the cell, MT spring into action, particularly if metal concentrations are high. In this case, the detoxification process is carried out by sequestration and transport of metals to the digestive gland lysosomes. These processes explain the obtained results since there

was a MT increment in the digestive gland of *C. virginica*, more evident after 24 h of exposure for both metals, and highest after 72 h for Cd. In addition, NRRT decreased after 24 h and continued diminishing until the end of the experiment (96 h), which indicates that lysosomes were destabilized. Regardless of the metal type (essential or not essential), when MT transport metals to the lysosomes, these organelles' integrity is compromised.

C. virginica is a species that lives in brackish environments, where metals tend to precipitate, and therefore, its uptake through particle matter is possible, as well as through trophic transfer; in contrast, freshwater species are more exposed to dissolved metals. *O. niloticus* is very well adapted to Mexican environments, and due to the lack of control over wastewater discharges to dams and other aquatic systems, it is convenient to find out if this fishing resource is affected by pollution.

To study in *O. niloticus* the relationship between morphological parameters and biomarkers in the presence of metals, monitoring was carried out in the Tenango dam during an annual cycle.

Metal concentrations in the Tenango dam appeared to be related to the management of the water that is allowed to enter and exit the system. The increase in Pb and Cr in January and April may be explained assuming that during these months the dam received water from the Nexapa dam when gates were open as a precautionary measure to lower the water level in this last dam after a period of intense rains, registered in the area during the samplings. This could have dragged metallic traces, detected as higher concentrations in these months. Cd and Cu presented higher concentrations in June, which is when rains started, and they probably dragged materials from the tourism and agricultural areas that border the dam. These activities discard batteries, tires, plastic bottles, fertilizer, and pesticide containers that may contain metallic traces in their manufacturing (Moreno 2003). Combined with the former, there are entries of residual waste water from the houses located in the periphery of the study site. These are characterized by their high metal content (González-Ramírez et al. 2009), so these discharges also contribute to the pollution of the site.

High water metal concentrations vary along the samplings and could have caused a variety of toxic effects on the local fishes, including *O. niloticus*. Pb reached an average of 3.2 mg/L and 2.9 mg/L in January and April, respectively. These concentrations are 100 times higher than the Mexican WQC for the protection of aquatic life. These values may cause deleterious effects like reduction in the fishes movement ability, oxidative stress, and erythrocyte damage, as has been demonstrated in previous studies of lead toxicity in tilapia and other fish species, at exposure concentrations of 0.8–1.6 mg/L (El-Badawi 2005; Ercal et al. 2001; Hou et al. 2011). Cr also presented elevated concentrations (eight times above the WQC) in the same months, 0.42 mg/L in January and 0.16 mg/L in April. Exposure to this metal may cause excess mucus secretion, respiratory and osmoregulatory capacity reduction, blood vessel congestion, and spermatozoid hypertrophy, as has been demonstrated in studies where tilapia and trout have been exposed to Cr concentrations of 0.01, 0.10, and 0.35 mg/L (Ackermann 2008; Arillo and Melodio 1988). Tilapia bioconcentration reflected the observed Pb and Cr increment, since their muscle concentrations were the highest in January and April (6.82mgPb/kg, 3.68 mgPb/kg, 0.89 mgCr/

kg and 0.53 mgCr/kg). These were 14 and 9 times higher than the acceptable limits for Pb and Cr, respectively. It is necessary to emphasize that fish consumption with Pb concentrations like the ones found in this work has been associated with intoxications characterized by a decrease in protoporphyrins, erythrocyte pigmentation, abdominal pain, headaches, constipation, and nausea (Valle 2000). The registered Cr concentrations can be linked to gastrointestinal irritation, abdominal pain, vomit, and diarrhea (Mencías and Mayero 2000).

Cd concentrations in water were 30 times higher than WQC in June (0.13 mg/L average). Exposures to 0.1 mg Cd/L cause blood vessel congestion, hemoglobin concentration reduction, and cellular inflammation in tilapia (Dyk et al. 2007). Cu water concentrations were also 30 times higher than QWC in June (1.37 mg/L average). 0.02 mg Cu/L causes variation in methionine, histidine, and cysteine concentrations in the aquatic organism, due to the generation of oxygen reactive species (Harris and Githlin 1996; Grosell and Wood 2002). High Cd concentrations in tilapia did not coincide with high water concentrations; in contrast, Pb and Cr did match. High concentrations of Cd were observed in January and April (average 1.89 mg/kg and 2.06 mg/kg, respectively) and were four times higher than muscle acceptable levels. Concentrations like these have been related to renal tube small alterations in fish consumers (Pérez and Azcona 2012). In contrast, Cu concentrations did not exceed the limits defined by the criteria.

Except for Cd, metal concentrations were higher in the liver than in the muscle in *O. niloticus*, which coincides with Abdulali et al. (2012) results.

Regarding tilapia quality, the morphological characteristics required for their sell did not show evident imperfections; therefore, from this point of view, tilapia has good quality for commercialization and consumption in the Tenango dam. The only characteristics out of criteria were length and weight, which were lower than desired. This may be a consequence of an excessive fishing effort on this resource that does not allow its recovery. Small size implies that young specimens are extracted. However, other studies point out that having a small size may be due to the deleterious effects of the presence of metals like Cd, Cr, and Cu, since growth and development problems have been reported in fish exposed to these metals (Shiau and Ning 2003; Abbas et al. 2007; Reid 2011).

Even though Cu concentrations exceeded WQC in June, tilapia's levels did not surpass the criteria for their consumption in any specimen.

With regard to the observed statistical relationships, metal concentration-weight and metal concentration-length coincide with Authman's study (2008), where tilapia metal uptake occurs gradually; so, bigger organisms tend to present the highest metal concentrations. This was the case even for Cu, which was not bioaccumulated in high concentrations. In addition, authors like Canli and Atli (2003) and Evans et al. (1993) stated that metal uptake in fish is favored by feeding and respiration, and in some cases metabolic regulation decreases as animals grow old and therefore, they tend to accumulate more metals.

Many metals can induce MT synthesis to regulate the concentrations of these molecules inside the organism; and, the higher the metal concentration, the higher MT concentration Atli and Canli 2003; Roesijadi 1992; Hamilton and Mehrle

1986). This was also observed in the present study since a direct relationship was found, indicating that MT were induced by the presence and the concentration of the metals. The highest metals and MT concentrations were found in the liver, which resembles the results of Gülüzar and Canli (2008), Hauser-Davis et al. (2014), Lim et al. (1998), Chatterjee et al. (2016), and Chandrasekera et al. (2008). In these studies, the liver accumulated more metals, and more MT were induced, with respect to other tissues.

At the international level, *O. niloticus* has been used in numerous studies to analyze human health risks due to fish consumption; to accomplish this, metal concentrations were quantified in fish muscle (Adazabra et al. 2014; Authman 2008; Cleoni Dos Santos et al. 2012; Muhammad et al. 2013; Mohamed and Osman 2014; Mulu and Mehari 2013; Pezo et al. 1992; Taweel et al. 2011; Yilmaz 2009).

In Mexico, tilapia (*O. niloticus*) has been analyzed in many studies. It was used as a biomonitor in the Metztitlan lagoon (Hidalgo State) to quantify metal concentrations (Lozada-Zarate et al. 2006); fish from the Fernando Hirirart Balderrama dam (also in Hidalgo State) were used to evaluate arsenic toxicity (Báez 2001). In the State of Tamaulipas, metals were quantified in specimens from the Laguito de Nuevo Laredo (Ramos et al. 2004); and in Chiapas State, metals and their effects were evaluated through biomarkers (Gold-Bouchot et al. 2006).

The importance of biomarkers studies in mollusks and fish from Mexican aquatic ecosystems relies on the fact that they are considered good-quality foods. If bad quality is demonstrated, even when morphological characteristics are acceptable, their consumption may imply a health risk. These types of studies are scarce in Mexico, which shows the importance of continuous monitoring in aquatic ecosystems to guarantee food quality and the ecosystem's health, which today is not done.

12.5 Conclusions

Metal trophic transfer cause damages in the oyster *C. virginica* similar to those caused by water exposure.

The observed lysosomal membrane destabilization in the oyster indicated that there was oxidative stress related to trophic exposure to both metals after 24 h. Similarly, MT were expressed in the same period, in higher quantities in those organisms exposed to Cd. We propose that the higher activity of these proteins caused the rapid degradation of the lysosomes.

Metal water concentrations in the Tenango dam may be due to the entry of water from other dams that are connected to this study site; they can contribute with metal traces. Pb and Cr increases support this statement.

Cd and Cu increases in the dam's water were related to potential runoffs, caused by rains, from the agricultural areas that may contain metal traces from fertilizers and pesticides.

Metal concentrations in the water of the Tenango dam prevent its exploitation for urban use and represent a risk for aquatic life.

The tilapia *O. niloticus* was a good biomonitor since it bioaccumulated the metals present in the dam's water, but also it exhibited the deleterious effects expected from metals exposure.

Visually, tilapia fulfilled the quality specifications for its sale to the public; however, metal concentrations in the muscle tissue make it undesirable for human consumption. It is probable that the effects of the water metal concentrations are the reason for the fish small size and weight.

Pb and Cu are the metals that represent the highest risk for tilapia in the dam, since they contribute to the reduction of its quality as a food resource; in the future it could affect the fishermen's economy.

The biomarkers evaluated in both species work out adequately and showed the exposure and effect associated with exposure to metals, either through trophic transfer or through contact with the contaminated water.

This study points to fact that the evaluated fishing resources are at risk due to metal exposure, and suggests that this may be the case of other Mexican aquatic ecosystems.

References

Abbas HHH, Hammada MM, Miller JD (2007) Vitamin C and cadmium toxicity in fish *Oreochromis niloticus*. Online J Vet Res 11(1):54–74

Abdulali KA, Shuhaimi-Othman M, Ahmad AK (2012) Analysis of heavy metal concentrations in Tilpia fish (*Oreochromis niloticus*) from four selected markets in Selangor, Peninsular Malaysia. J Biol Sci 12(3):138–145

Ackermann C (2008) A quantitative and qualitative histological assessment of selected organs of Oreochromis mossambicus after acute exposure to cadmium, chromium and nickel. University of Johannesburg, South Africa, M. Sc. dissertation

Adazabra AN, Kombat EO, Fletcher JJ (2014) Parameterization of non-essential heavy metals concentration in different tissues of inland commercial fish *Oreochromis niloticus* from Vea Dam, Bolgatanga, Northern Ghana. Int J Curr Res Acad Rev 2(7):247–258

Agencia para Sustancias Tóxicas y el Registro de Enfermedades (ATSDR) (2016) Reseña Toxicológica del Cobre. Atlanta: GA. Departamento de Salud y Servicios Humanos de EE. UU., Servicio de Salud Pública. Recuperado el 10 mayo del 2018. Disponible en https://www.atsdr.cdc.gov/es/phs/es_phs132.html

Albert LA (2011) Curso Básico de toxicología ambiental. Limusa, México

American Public Health Association (APHA) (1992) Standard methods for the examination of water and wastewater, 18th edn. American Public Health Association, Washington, D. C.

Amiard J, Amiard-Triquet C, Barka S, Pellerin J, Rainbow P (2006) Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat Toxicol 76(2):160–202

Arillo A, Melodio F (1988) Effects of hexavalent chromium on trout mitochondria. Toxicol Lett 44:71–76

Atli G, Canli M (2003) Natural occurrence of metallothionein-like protein in liver of fish *Orechromis niloticus* and effects of cadmium, lead, copper, zinc, and iron exposures on their profiles. Bull Environ Contam Toxicol 70:619–627

Authman MMN (2008) *Orechromis niloticus* as a biomonitor of heavy metal pollution with emphasis on potential risk and relation to some biological aspects. Glob Vet 2(3):104–109

Báez ROA (2001) Toxicidad del arsénico de fuentes subterráneas naturales de agua potable y presa Fernando Hirirart Balderrama de Zimapán, Hgo. en Oreochromis niloticus. Tesis de maestría. Instituto Politécnico Nacional (IPN), D.F. México

Baqueiro CER, Borabe L, Goldaracena ICG, Rogríguez NJ (2007) Los moluscos y la contaminación. Una revisión. *Revista Mexicana de Biodiversidad* Suplemento 78:1–7

Barrera EG (2006) Toxicidad del Cromo y Cadmio en ostión Crassostrea virginica de la laguna de Mandinga, Ver. Tesis de Doctorado en Ciencias Biológicas. Universidad Autónoma Metropolitana. México, D.F. 229 pp.

Blackmore G, Wang W-X (2004) The transfer of cadmium, mercury, methylmercury, and zinc in an intertidal rocky shore food chain. J Exp Mar Biol Ecol 307(1):91–110

Borgmann U (2000) Methods for assessing the toxicological significance of metals in aquatic ecosystems: bioaccumulation-toxicity relationships water concentrations and sediment spiking approaches. *Aquat Ecosyst Health Manag* 3:277–289

Brewer SK, Little EE, Delonay AJ, Beauvais SL, Jones SB, Ellersieck MR (2000) Behavioral dysfunctions correlate to altered physiology in rainbow trout (*Oncorhynchus mykiss*) exposed to cholinesterase-inhibiting chemicals. Arch Environ Contam Toxicol 40:70–76

Brown RJ, Galloway TS, Lowe D, Browne MA, Dissanayake A, Jones MB, Depledge MH (2004) Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. Aquat Toxicol 66(3):267–278

Canli M, Atli G (2003) The relationships between heavy metals (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ Pollut 121(1):129–136

Capó MA (2002) Principios de Ecotoxicología. In: Diagnóstico, tratamiento y gestión del medio ambiente. España, McGraw-Hill

Carabias J, Landa R (2006) Agua, medio ambiente y sociedad. Colegio de México, México

Chandrasekera LWHU, Pathiratne A, Pathiratne KAS (2008) Effects of water borne cadmium on biomarker enzymes and metallothioneins in Nile tilapia, *Oreochromis niloticus*. J Natl Sci Found 36(4):315–322

Chapman D (1996) Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring. Chapman Hill, Londres, p 626

Chatterjee S, Datta S, Das TK, Veer V, Mishra D, Chakraborty A, Chattopadhyay B, Datta S, Mukhopadhyay KS, Gupta KD (2016) Metal accumulation and metallothionein induction in *Orechromis niloticus* grown in waster fed fishponds. Ecol Eng 90(16):405–416

Cleoni Dos Santos C, Aline BV, Sobreiro SH, Gaeta EE, Narciso FM (2012) Biomarker responses as indication of contaminant effects in *Orechromis niloticus*. Chemosphere 89:60–69

Comisión Nacional del Agua (CONAGUA) (2016) Ley federal de derechos. Disposiciones aplicables en materia de aguas nacionales. Diario Oficial de la Federación, México. Noviembre 13

Cordero J, Guevara M, Morales E, Lodeiros C (2005) Efecto de metales pesados en el crecimiento de la microalga tropical *Tetraselmis chuii (Prasinophyceae)*. Rev Biol Trop 53:325–330

Coyle P, Philcox JC, Carey LC, Rofe AM (2002) Metallothionein: the multipurpose protein. Cell Mol Life Sci 59(4):627–647

Dallinger R, Egg M, Kock G, Hofer R (1997) The role of metallothionein in cadmium accumulation of Arctic char (*Salvelinus alpinus*) from high mountain lakes. Aquat Toxicol 38:47–66

Dembele K, Haubruge E, Gaspar C (2000) Concentration effects of selected insecticides on brain acetylcholinesterase in the common carp (*Cyprinus carpio L.*). Ecotoxicol Environ Saf 45:49–54

Dillon TM, Lynch MP (1981) Physiological response with determinative stress in marine and estuarine organisms. In: Barret W, Rosemberg R (eds) Stress effect on natural ecosystems. Wiley, Chichester, pp 56–134

Dyk JCV, Pieterse GM, Van Vuren JHJ (2007) Histological changes in the liver of *Oreochromis mossambicus* (Cichlidae) after exposure to cadmium and zinc. Ecotoxicol Environ Saf 66:432–440

El-Badawi AA (2005) Effect of lead toxicity on some physiological aspects of Nile tilapia fish, Oreochromis niloticus. In: International conferences of the veterinary research division. NRC, Cairo

EPA (1995) Method 3015A. Microwave assisted acid digestion of aqueous sample an extract. https://www.epa.gov/sites/production/files/2015-12/documents/3015a.pdf

Ercal N, Gurer-Orhan H, Aykin-Burns N (2001) Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem 1:529–539

Ettajani H, Berthet B, Amiard JC, Chevolot L (2001) Determination of cadmium partitioning in microalgae and oyster's contribution to the assessment of trophic transfer. Arch Environ Contam Toxicol 40:209–221

Evans DW, Dodoo DK, Hanson PJ (1993) Trace elements concentrations in fish livers implications of variations with fish size in pollution monitoring. Mar Pollut Bull 26(6):329–334

Giguére A, Couillard Y, Campbell PG, Perceval O, Hare L, Pinel-Alloul B, Pellerin J (2003) Steady-state distribution of metals among metallothionein and other cytosolic ligands and links to cytotoxicity in bivalves living along a polymetallic gradient. Aquat Toxicol 64(2):185–200

Gold-Bouchot G, Zapata-Pérez O (2004) Contaminación, ecotoxicología y manejo costero. In: Rivera Arriaga E, Villalobos GJ, Adeath IA, May FR (eds) El Manejo Costero en México, vol 654. Universidad Autónoma de Campeche, SEMARNAT, CETYS-Universidad, Universidad de Quintana Roo, pp 277–286

Gold-Bouchot G, Zapata-Pérez O, Rodríguez-Fuentes G, Ceja-Moreno V, Rio-García M, Chan-Cocom E (2006) Biomarkers and pollutants in the Nile Tilapia, *Orechromis niloticus*, in four lakes from San Miguel, Chiapas, Mexico. Int J Environ Pollut 26(1–3):129–141

González-Ramírez CA, Prieto-García F, Prieto-Méndez J, Román-Gutiérrez AD (2009) Contaminación y fitotoxicidad en plantas por metales pesados provenientes de suelos y agua. *Trop Subtrop Agroecosyst*. Sin mes:29–44

Grosell M, Wood CM (2002) Copper uptake across rainbow trout gills: mechanisms of apical entry. J Exp Biol 205:1179–1188

Gülüzar A, Canli M (2008) Responses of metallothionein and reduced glutathione in a freshwater fish *Oreochromis niloticus* following metal exposures. Environ Toxicol Pharmacol 25:33–38

H. Ayuntamiento de Tecolutla 2014–2017 (2016). Disponible en http://www.tecolutla.gob.mx

Hamilton SJ, Mehrle PM (1986) Metallothionein in fish: a review of its importance in assessing stress from metal contaminants. Trans Am Fish Soc 115:596–609

Harris ZL, Githlin JD (1996) Genetic and molecular basis for cooper toxicity. Am J Clin Nutr 63:836S–841S

Hauser-Davis RA, Bastos FF, Tuton B, Chávez RR, Saint PT, Ziolli LR, Arruda MAZ (2014) Bile and liver metallothionein behavior in copper-exposed fish. J Trace Elem Med Biol 28:70–74

Hou JL, Zhuang P, Zhang LZ, Feng L, Zhang T, Lium JY (2011) Morphological deformities and recovery, accumulation and elimination of lead in body tissues of Chinese sturgeon, *Acipenser sinensis*, early life stages: a laboratory study. J Appl Ichthyol 27:514–519

Instituto Nacional de Ecología-Universidad Autónoma de Chihuahua (INE-UACH) (2007) Estudio de Ordenamiento Ecológico Territorial de las Cuencas Hidrológicas de los ríos Necaxa y Laxaxalpa. https://www.agua.org.mx/biblioteca-tematica/manejo-de-cuencas/95%2D%2Dsp942/676-ordenamiento-de-las-cuencas-de-los-rios-necaxa-y-laxaxalpa. Consultado el 15 de mayo de 2017

Instituto Nacional de Estadística y Geografía (INEGI) (2016) Catálogo Nacional de Indicadores. Disponible en: http://www3.inegi.org.mx

Ivanina AV, Cherkasov AS, Sokolova IM (2008) Effects of cadmium on cellular protein and glutathione synthesis and expression of stress proteins in eastern oysters *Crassostrea virginica* Gmelin. J Exp Biol 211(4):577–586

Legislación brasileña de metales pesados (LBMP) (2017) Metales pesados en materia de alimentos. http://paguicidas.comercio/MetalPesado.pdf. Consultado el 15 de mayo de 2017

Lemus M, Salazar R, Lapo B, Chung K (2016) Metalotioneínas en bivalvos marinos. Lat Am J Aquat Res 44(2):202–215

Levine JF, Law M, Corsin F (2006) Bivalvos. In: Lewbart GA (ed) Medicina de los invertebrados. Editorial Acribia, S.A. Zaragoza, España, pp 111–140

Lim LK, Wai KP, Ka-Yee J, Ming CK (1998) Metal toxicity and metallothionein gene expression estudies in common Carp and Tilapia. Mar Environ Res 46(1–5):563–566

Lowe DM, Soverchia C, Moore MN (1995) Lysosomal membrane responses in the blood and digestive cells of mussels experimentally exposed to fluoranthene. Aquat Toxicol 33(2):105–112

Lozada-Zarate EJ, Monks S, Pulido-Flores G, Gordillo-Martínez AJ, Prieto-García F (2006) Determinanción de metales pesados en Oreochromis niloticus en la laguna de Metztitlan, Hidalgo, México. https://www.researchgate.net/profile/Alberto_Martinez15/publication/209827913_Determinacion_de_metales_pesados_en_Cyprinus_carpio_en_la_Laguna_de_Metztitlan_Hidalgo_Mexico/links/0046353bd88c3a19d8000000/Determinacion-de-metales-pesados-en-Cyprinus-carpio-en-la-Laguna-de-Metztitlan-Hidalgo-Mexico.pdf. Consultado el 24 de abril de 2018

María VL, Bebianno MJ (2011) Antioxidant and lipid peroxidation responses in *Mytilus galloprovincialis* exposed to mixtures of benzo (a) pyrene and copper. Comp Biochem Physiol 154(1):56–63

Marques de Cantú MJ (1991) Probabilidad y estadística para ciencias quimicobiológicas. McGraw-Hill, México, 657 p

Matozzo V, Ballarin L, Pampanin DM, Marin MG (2001) Effects of copper and cadmium exposure on functional responses of hemocytes in the clam, *Tapes philippinarum*. Mar Arch Environ Contam Toxicol 41:163–170

McCarthy JF, Halbrook RS, Shugart LR (1991) Conceptual strategy for design, implementation, and validation of a biomarker-based biomonitoring capability. Oak Ridge National Lab, Oak Ridge

Mencías RE, Mayero FLM (2000) Manual de toxicología básica. Díaz de Santos. Madrid, Madrid

Mohamed EH, Osman AR (2014) Heavy metals concentration in water, muscles and gills of *Orechromis niloticus* collected from the sewage-treated water and the White Nile. Int J Aquac 4(6):36–42

Mok JS, Yoo HD, Kim PH, Yoon HD, Park YC, Lee TS, Kwon JY, Son KT, Lee HJ, Ha KS, Shim KB, Kim JH (2015) Bioaccumulation of heavy metals in oysters from the southern coast of Korea: assessment of potential risk to human health. Bull Environ Contam Toxicol 94(6):749–755

Moreno GMD (2003) Toxicología ambiental. México, McGraw Hill

Moulis JM (2010) Cellular mechanisms of cadmium toxicity related to the homeostasis of essential metals. Biometals 23(5):877–896

Muhammad JL, Shehu RA, Bilbis LS, Dangoggo SM (2013) Estimation of some heavy metals and mineral elements in tissues of *Orechromis niloticus* collected from Goronyo Dam and its two tributaries in North-Western sub-Sahara Nigeria. J Environ Sci Toxicol Food Technol 3(5):46–52

Mulu BD, Mehari MW (2013) Distribution of trace metals in two commercially important fish species (Tilapia *zilli* and *Oreochromis niloticus*) sediment and water from lake Gubdahri, Eastern Tigrar of Northern Ethiopia. Int J Sci Res Publ 3(9):1–7

Nassiri Y, Rainbow PS, Amiard-Triquet C, Smith BD, Rainglet F (2000) Trace-metal detoxification in the ventral caeca of *Orchestia gammarellus* (Crustacea: Amphipoda). Mar Biol 136(3):477–484

Pérez GPE, Azcona CMI (2012) Los efectos del cadmio en la salud. Revista de especialidades Médico-Quirúrgicas 17(3):199–205

Perić L, Nerlović V, Žurga P, Žilić L, Ramšak A (2017) Variations of biomarkers response in mussels *Mytilus galloprovincialis* to low, moderate and high concentrations of organic chemicals and metals. Chemosphere 174:554–562

Petrovic S, Ozretic B, Krajnovic-Ozretic M, Bobinac D (2001) Lysosomal membrane stability and metallothionein in digestive gland of mussels (*Mytilus galloprovincialis* Lam.) as biomarkers in a field study. Mar Pollut Bull 42(12):1373–1378

Pezo DR, Paredes AH, Bedayán ANY (1992) Determinación de metales pesados bioacumulables en especies ícticas de consumo humano en la Amazonia peruana. Folia Amazonica 4(2):171–181
Prat N, Ríos B, Acosta R, Rieradevall M (2009) Los macroinvertebrados como indicadores de calidad de las aguas. In: Domínguez E, Fernández HR (eds) Macroinvertebrados bentónicos sudamericanos. *Sistemática y biología*. Fundación Miguel Lillo, Tucumán, pp 631–654
Prescott LM, Harley JP, Klein DA (2000) Microbiologia. McGraw Hill, España, pp 683–702
Pytharopoulou S, Grintzalis K, Sazakli E, Leotsinidis M, Georgiou CD, Kalpaxis DL (2011) Translational responses and oxidative stress of mussels experimentally exposed to Hg, Cu and Cd: one pattern does not fit at all. Aquat Toxicol 105(1–2):157–165
Ramade F (1989) The pollution of the hydrosphere by global contaminants and its effects on aquatic ecosystems. In: Boudou A, Rybeyre F (eds) Aquatic ecotoxicology: fundamental concepts and methodologies. CRC Press Inc, Boca Raton, pp 152, 352 p–183
Ramírez RP, Mendoza CA (2008) Ensayos toxicológicos para la evaluación de sustancias químicas en agua y suelo. La experiencia en México. SEMARNAT-INE, México
Ramos O, Guevara N, Macías B, Ortiz Y (2004) Evaluación de riesgo a la salud por la presencia de metales pesados en pescado. Toxicología 1:2–4
Reid SD (2011) Molybdenum and chromium. Academic Press, New York
Rodríguez de la Rua A, Arellano J, González M, Blasco J, Sarasquete C (2005) Acumulación de cobre y alteraciones histopatológicas en el ostión *Crassostrea angulata*. Cienc Mar 31(3):455–466
Roesijadi G (1992) Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat Toxicol 22:81–114
Roesijadi G (1996) Environmental factors: response to metals. In: Kennedy VS, Newell RIE, Eble AF (eds) The eastern oyster *Crassostrea virginica*. Maryland Sea Grant College, College Park, pp 515–537
Roldán PG (1999) Los macroinvertebrados y su valor como indicadores de la calidad del agua. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 23(88):375–387
Rosenberg DM, King RS, Resh VH (2008) Use of aquatic insects in biomonitoring. In: Merritt RW, Cummins KW, Berg M (eds) An introduction to the aquatic insects of North America, 4th edn. Kendall/Hunt, Dubuque, pp 123–137
Saavedra MA (2006) Manejo del cultivo de tilapia. CIDEA-Coastal Resources Center, Nicaragua
Scheuhammer AM, Cherian MG (1986) Quantification of metallothionein by silver saturation methods. Toxicol Appl Phamarcol 82(3):417–425
Schmitz RJ (1995) Introduction to water pollution biology. Gulf Publishing Co., Huston
Secretaria de Agricultura, Ganadería, Desarrollo regional, Pesca y Alimentación (SAGARPA) (2012) Criterios técnicos y económicos Para la producción sustentable de Tilapia en México. SAGARPA, México
Secretaría de Agricultura, Ganadería, Desarrollo rural, Pesca y Alimento (SAGARPA) (2006) Manual de producción de tilapia con especificaciones de calidad e inocuidad. SAGARPA, México
Secretaría de Economía (2001) NMX-AA-051-SCFI-2001. In: Determinación de metales por absorción atómica en aguas naturales, potables, residuales y residuales tratadas. México, Diario oficial de la federación
Secretaría de Gobernación (SEGOB) (2016) NOM-015-SAG/PESC-2016. Para regular el aprovechamiento de ostión (Crassostrea virginica) en los sistemas lagunarios estuarinos del Estado de Tabasaco. Diario Oficial de la Federación, México
Secretaria de Salud (2011) NOM-242-SSA1-2009. Productos y servicios. Productos de la pesca frescos, refrigerados, congelados y procesados. Especificaciones sanitarias y métodos de prueba. Diario Oficial de la Federación, México
Serafim A, Bebianno MJ (2009) Metallothionein role in the kinetic model of copper accumulation and elimination in the clam *Ruditapes decussatus*. Environ Res 109(4):390–399
Shiau SY, Ning YC (2003) Estimation of dietary copper requirements of juvenile tilapia *Oreochromis niloticus* & *O. aureus*. J Anim Sci 77:287–292

Sokal RR, Rohlf FJ (2012) Biometry: the principles and practice of statistics in biological research, 4th edn. W. H. Freeman and Co, New York

Soto-Jiménez MF (2011) Transferencia de elementos traza en tramas tróficas acuáticas. Hidrobiológica 21(3):239–248

Tanguy A, Mura C, Moraga D (2001) Cloning of a metallothionein gene and characterization of two other sequences in the Pacific oyster *Crassostrea gigas* (CgMT1). Aquat Toxicol 55:35–47

Tataruch F, Kierdorf H (2003) Mammals as bioindicators. In: Bioindicators and biomonitors: principles, concepts and applications. Elsevier, Oxford

Taweel A, Shuhaimi-Othman M, Ahmad AK (2011) Heavy metals concentration in different organs of tilapia fish (*Orechromis niloticus*) from selected areas of Bangi, Selangor, Malaysia. Afr J Biotechnol 10(55):11562–11566

Valdez C, Vázquez GA (2003) Ingeniería de los sistemas de tratamiento y disposición de agua residuales. Fundación ICA, A.C., México

Valle VP (2000) Toxicología de Alimentos. Instituto Nacional de Salud Pública, México

Van der Oost R, Beyer J, Vermeulen NP (2003) Fish bioaccumulation and biomarkers in environmental risk. Assess Environ Toxicol Pharmacol 13:57–149

Yilmaz F (2009) The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three economically important fish (*Anguilla anguilla*, *Mugil cephalus* and *Orechromis niloticus*) inhabiting Koycegiz Lake-Mugla. Turk J Sci Technol 4(1):7–15

Zapata-Pérez O, Gold-Bouchot G, Ortega A, López T, Albores A (2002) Effect of pyrene on hepatic cytochrome P450 1A (CYP1A) expression in Nile tilapia (*Oreochromis niloticus*). Arch Environ Contam Toxicol 42:477–485

Chapter 13
Environmental Pollution by Hydrocarbons in Colombia and Its Impact on the Health of Aquatic Ecosystems

Yohana M. Velasco-Santamaría, Wilson Corredor-Santamaría, and Alexander Torres-Tabares

13.1 Introduction

Hydrocarbons are fossil fuels widely used around the world as fundamental generators of various forms of energy (Velásquez 2017); these are a nonrenewable natural resource that originated from the anaerobic degradation for long periods of time of organic matter under conditions of high temperature and pressure, converting it into a natural gas, crude, and multiple derivatives, which are formed by a complex and variable mixture of organic compounds, ranging in molecular weight from methane gas to the high molecular weights of tars and bitumens (Acuña-González et al. 2004). Petroleum-derived hydrocarbons are classified into three major groups, alkanes, alkenes, and aromatics; saturated alkanes or hydrocarbons are the major constituents of petroleum-derived products (Widdel and Rabus 2001). The toxicity of petroleum hydrocarbons, both aliphatic and aromatic, is variable, but in general, those of lower molecular weight exert acute toxicity (Lawal 2017). Many polycyclic aromatic hydrocarbons (PAHs) have toxic, mutagenic, and/or carcinogenic properties. PAHs are highly lipid-soluble and therefore readily absorbed in the gastrointestinal tract being rapidly distributed in a wide variety of tissues with a marked tendency to localize in body fat (Abdel-Shafy and Mansour 2016). One of the undesired characteristics of oil production is the possibility that in its extraction, contamination is generated in water and soils due to constant accidental or provoked spills, which are very common in producing countries (Zabbey and Olsson 2017).

Y. M. Velasco-Santamaría (✉) · W. Corredor-Santamaría · A. Torres-Tabares
Grupo de Investigación en Biotecnología y Toxicología Acuática y Ambiental – BioTox, Escuela de Ciencias Animales, Facultad de Ciencias Agropecuarias y Recursos Naturales, Universidad de los Llanos, Villavicencio, Meta, Colombia
e-mail: ymvelascos@unillanos.edu.co

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_13

PAHs are classified according to their origin as petrogenic and pyrogenic. Petrogenics HAPs come from oil-related sources including drilling and extraction activities, as well as spills and pollution from industrial sites and refineries. Most petrogenics have two to four rings such as naphthalene, anthracene, phenanthrene, and chrysene, which are associated with acute toxicity and genotoxicity (Manzetti 2013), and can be found in different matrices such as water, air, soil, and sediments and even in biological samples by absorption from environment (Lima et al. 2005). Pyrogens HAPs that originate from different pyrolysis substrates, such as fossil fuels and biomass, include four or more rings derived from combustion processes, represented by traffic pollution, industrial activities, incineration, and volcanic eruptions. Pyrene, benzo[a]pyrene, benzoanthracene, and others are related to mutagenicity and carcinogenicity (Manzetti 2013), which are closely associated both physically and chemically with sediments and soils (Lima et al. 2005).

Hydrocarbons have played an important role in the productive sector for the development of Colombia in recent years. This sector has become a determining activity for the economic balance and productivity of the country. The production of oil and natural gas, two of the hydrocarbons with the highest domestic production, grew at rates of 7% and 5% on average annually, respectively, during the last 8 years according to information from the Ministerio de Minas y Energía and the Agencia Nacional de Hidrocarburos from Colombia (EIA 2019). Likewise, the contributions of the hydrocarbon sector to the Central National Government represent more than 20% of its current income and the generation of royalties, equivalent to more than 2.2% of GDP (WBG 2016).

Unfortunately, Colombia is one of the few countries in the world where criminal actions are carried out against the oil infrastructure for political and terrorist purposes. There are reports that for every 100 barrels of crude oil spilled as a consequence of terrorist attacks, only 25–30% are recovered. One of the main centers of hydrocarbon exploitation in Colombia is the Caño Limón-Coveñas pipeline, which in around 19 years of operation has been affected by thousand attacks and which have caused the spill of more than three million barrels of crude oil that have ended up in tropical basins and ecosystems.

13.2 Environmental Context

The environmental impact generated in Colombia due to the oil extraction and spill accidents previously stated includes the contamination of water sources, mortality of fauna and flora, or changes in the dynamics of natural ecosystems.

One of the most important is the effects on soil fertility as a result of direct toxicity on organisms, reduction in moisture or nutrient retention, compaction, as well as changes in pH and salinity. It is important to highlight that in the case of soil contamination with hydrocarbons, factors such as the type of soil sand, silt, and clay and the amount of existing organic matter determine the fate of the hydrocarbons and the extent of the damage (Yu et al. 2013).

Pollution by hydrocarbons exerts indirect adverse effects on plants leading to a deterioration of soil structure, loss of organic matter content, and loss of nutrients such as potassium, sodium, sulfate, phosphate, and nitrate, essential for their development (Serrano et al. 2013). Hydrocarbons also tend to accumulate and form a hydrophobic layer, inducing fragmentation of aggregates and causing reduction and (or) inhibition of vegetation cover and modification of microbial populations of the edaphic environment (Díaz-Martínez et al. 2013). The fate of PAHs is related to their solubility, bioavailability, biodegradability, adsorption, and desorption. PAHs are less prone to environmental degradation due to the strong nature of aromatic bonds, so they can persist and accumulate in the environment and biota (Soclo et al. 2008). Once accumulated in the soil, they can be transported to surface waters or groundwater through precipitations and discharges or be emitted to the atmosphere by volatilization (Srogi 2007); their lipophilic nature confers them with very low solubility in water, and as a consequence of their hydrophobicity in aquatic environments, they tend to associate rapidly with particulate matter that ends up in sedimentation (Qiu et al. 2009).

Derivatives of hydrocarbons such as gasoline, kerosene, oils, fuels, paraffins, and asphalt, among many others, not only impact the surface layer of the soil, they can also move to groundwater generating pollution due to the characteristics of the soil, which may allow different degrees of filtration (Cubillos et al. 2014).

In a hydrocarbon contamination, most volatile components are eliminated by evaporation. Depending on their molecular weight, hydrocarbons can be oxidized by solar radiation and can dissolve in water to become potential pollutants of sediments (Mendelssohn et al. 2012). Unsolved hydrocarbons can be transported by runoff, increasing the environmental damage even more, especially in surface waters because they tend to float on the surface due to the difference in density with respect to water, e.g., crude oil can trigger a blockage in the penetration of light and gas exchange, which leads to a decrease in oxygen concentrations necessary for the survival of aquatic ecosystems (Behera et al. 2018). González et al. (2011) have reported lethal and sublethal effects of petroleum hydrocarbons on fish. Also, Saadoun (2015) reported the negative effects of oil pollution of crustaceans, turtles, and some species of coastal vertebrates such as sea ducks and otters, which is highly relevant taking into account the effects of bioaccumulation of heavy metals and some hydrocarbons, and their high residuality in trophic chains.

Another important focus of hydrocarbon pollution is related to substances derived from petroleum that do not have an exclusive origin in petroleum activities such as the dumping of residues from the change of oil from vehicles, the cans of lubricant, and domestic oil (Ssempebwa and Carpenter 2009). Oil, for example, being a petroleum derivative, can cause damage to the environment through various mechanisms, including toxicity associated with ingestion or absorption through the skin or respiratory system, affecting gas exchange, temperature regulation, and oxygen depletion by microbial processes associated with oil degradation (Mendelssohn et al. 2012).

Petroleum companies have become a potential source of contamination due to the large volume of water used to extract petroleum through injection at high pressure generating something called produced water. High varieties of xenobiotics, like polycyclic aromatic hydrocarbons (PAHs), metals, phenols, etc., are eliminated through the produced water. This water coming from crude oil can change its chemical composition in relation to the type of the oil well and type of crude oil (Bakke et al. 2013; Schifter et al. 2015), and although this water has different purifying treatments, its quality is not ideal, being important to determine its impact on aquatic organisms. Considering the large number of oilfields in Colombia, large volumes of discharges of its wastewater are generated, leading to disturbances in the rivers; this situation, along with the domestic waste and agricultural pesticides become a mixture of contaminants directly discharged into water sources and increases their environmental impact (Vera-Parra et al. 2011; Calderón-Delgado et al. 2019).

Finally, it is important to mention the social damage caused by environmental pollution by hydrocarbons. Many of people who live in these regions live directly from their natural resources, and when an oil spill occurs negative impacts are generated on the biota and soil fertility, which directly affect the economy and food security of agricultural producers, who choose to leave their lands to survive, generating processes of migration, colonization and transculturation in the areas of influence of oil projects, creating a social environment dominated by poverty and unemployment.

Based on the above, for almost a decade, researchers from the BioTox research group of the Universidad de los Llanos have been concerned to evaluate the impact of oil extraction in the department of Meta, Colombia, using aquatic organisms as bioindicators of contamination through the evaluation of different biomarkers, which have allowed to understand the effects that both PAHs and produced waters have on these organisms. The main results obtained to date about the impact of wastewater and PAHs in fish and algae are described below.

13.3 Impact of Wastewater from Petroleum Industry on Native Fish

Fishes are considered as one of the main bioindicators for assessing the quality of aquatic ecosystems because they are ubiquitous in most aquatic environments exposed to pollutants and for their ecological relevance due to their influence on the structure of the food web, in the nutrient cycle, and in the transfer of energy. These characteristics facilitate the chronic exposure to complex mixtures of substances with the resulting imbalance on the physiological and biochemical parameters, which can be monitored through the evaluation of the antioxidant response and the tissue architecture, among others (Van der Oost et al. 2003).

Colombian and Venezuelan Orinoquia region has a remarkable hydric wealth, which unfortunately has been negatively affected over the years without proper control of wastewater discharges that have impacted the populations of aquatic

organisms, in particular fish, affecting their position in the trophic chain. Case studies have evaluated different biomarkers on sentinel fish, which show the utility and advantage of using fish as bioindicators of contamination.

13.3.1 *Biomarkers of Oil Pollution on* Aequidens metae *and* Astyanax gr. bimaculatus *Caught in the Ocoa River*

Ocoa River located in the Colombian Orinoco region whose basin has an extension of 282.9 Km^2 is in the central part of the municipality of Villavicencio, Meta, receiving regularly wastewater discharges from different city sources. Fish from each species, *Astyanax gr. bimaculatus* (Characidae) and *Aequidens metae* (Cichlidae), were caught at each monitoring site in dry and rainy seasons. Both fish and water samples were collected at two sites along the river: (1) Nacimiento, the site before entering Villavicencio city; and (2) dumping of produced water (DPW) site, where the sewage waters from the petroleum industry are discharged. Additionally, a reference site (R) with low probability of contamination called Negro River was monitored (Table 13.1).

In *A. metae* a significant increase in the hepatosomatic index (HSI) was observed in fish caught at DPW site when compared to the reference site ($p < 0.05$). Similarly, *A. gr. bimaculatus* from the DPW site showed an increase in the HSI compared to the reference site. This HSI increase was consistent with the higher occurrence of histopathological lesions in the liver in both species (data not shown). Fish samples of *A. metae* were not found in Nacimiento site; therefore, no data are reported in that site.

Table 13.1 Physicochemical parameters monitored in the Ocoa River, Villavicencio, Colombia

Water physicochemical parameters	Dry			Rainy		
	Nacimiento	DPW	Reference	Nacimiento	DPW	Reference
Temperature (°C)	23.5[a]	31.3[a]	28.7	22.2[a]	28.7	30.5
pH	5.1	6.5	5.9	4.8	6.1	5.2
Dissolved oxygen (mg/L)	7.3	4.1[a]	5.9	6.5	4.5	6.8
Alkalinity (mg/L)	17.1	31.4	22.8	17.1	34.2	22.8
Hardness (mg/L)	54.1	52.2	31.3	62.7	55.6	22.8
Total dissolved solids (g/L)	89	180.2[a]	31.9	97.8	173.1[a]	37
Conductivity (μS/cm)	121.0[a]	478.5[a]	52.8	71.5[a]	397.1[a]	44.4
Total ammonia (mg/L)	0.1	0.8[a]	0.1	0.1	0.6[a]	0.1
Water chemical analysis						
Cadmium (mg Cd/L)	<LD	–	<LD	–	<LD	–
Mercury (μg Hg/L)	–	<LD	–	<LD	3.51	<L.D.
Surfactants (mg SAAM/L)	<LD	0.078	<LD	0.049	<LD	0.037
Total hydrocarbons (mg/L)	4.61	5.18	<LD	3.39	0.08	0.05

[a]Indicates significant differences with respect to the reference site ($p < 0.05$). Nacimiento, area before the Ocoa River enters Villavicencio city; dumping of produced water (DPW), where sewage water from petroleum industry is discharged

13.3.1.1 Hematological Biomarkers

Hematology analysis is a tool widely used in ecotoxicological studies because it is a reproducible, noninvasive technique and could be applied to a large number of organisms.

The decrease in the red cell count in *A. metae* in the DPW site in the rainy season in comparison to the reference site could be caused by a reduction in hematopoiesis due to the presence of heavy metal contaminants, among others, caused by intrasplenic and intrahepatic hemorrhage (Zaghloul et al. 2007). These results are comparable to those observed in this study with the greatest presence of hepatic congestion during the rainy season at the dumping of produced water (DPW) site (Table 13.2).

The decrease in the concentration of hemoglobin and in the percentage of the hematocrit in *A. gr bimaculatus* in the DPW site at the dry season (Table 13.2), can occur in environments with a high presence of ammonium and in the presence of toxic metals such as mercury (Ishikawa et al. 2007), diesel and drilling fluid (Kayode and Shamusideen 2010), or copper (Kumar and Nandan 2014). The possible hemolytic effect of toxic metals such as cadmium or pyrethroid insecticides can be associated with the inhibition of Na+K+ – ATPase activity, which generates an increase in the influx of sodium to the cells, causing disturbances in the ion exchange, affecting the cellular permeability, inducing swelling, and finally, causing rupture of membranes (Assis et al. 2009).

The decrease in the mean corpuscular volume observed in *A. gr. bimaculatus* at dry season is due to the reduction in the percentage of hematocrit, which is caused by the decrease of circulating erythrocytes (Ishikawa et al. 2007). Similarly, a reduction in the concentration of mean corpuscular hemoglobin in the DPW site when compared to the reference site in *A. gr bimaculatus* at the dry season was observed. Similar results were reported by Kayode and Shamusideen (2010) who exposed nilotic tilapia (*Oreochromis niloticus*) to sublethal concentrations of diesel (23.4 mg/L) and drilling fluid (492 mg/L) for 28 days.

The reduction in erythrocyte count, hemoglobin concentration, and percentage of hematocrit found in this study is related to inflammatory-type (congestion) and growth-related lesions (lamellar and interlamellar hyperplasia, aneurysms, and epithelial detachment), due to induced hypoxia by contaminants capable of inducing interference in gas exchange capacity such as those observed by Moharram et al. (2011) in *Siganus rivulatus* exposed to different concentrations of sea water adjacent to a drain from the Egyptian Mediterranean coast and also by Elahee and Bhagwant (2007) in *Scarus ghobban* exposed to the polluted waters of the Bain Des Dames lagoon, Mauritius.

Regarding the thrombocyte count, a measurement which has been associated with the cellular defense response in teleost fish (Tavares-Dias et al. 2007), a decrease count in fish from the DPW site was found at dry season, possibly due to the fact that the concentration of pollutants at this season could be a cause of an inhibition in the immune response (Jerônimo et al. 2009; Tavares-Dias et al. 2008).

Table 13.2 Hematological parameters of *Astyanax gr. bimaculatus* and *Aequidens metae* caught in two sites of the Ocoa River and a reference site (Negro River), Villavicencio—Meta, Colombia, during dry and rainy season

Hematological parameters	*Astyanax gr. bimaculatus*						*Aequidens metae*			
	Dry			Rainy			Dry		Rainy	
	Nacimiento	DPW	Reference	Nacimiento	DPW	Reference	DPW	Reference	DPW	Reference
Erythrocytes (x106x µl-1)	1.83 ± 0.1	2.00 ± 0.1	2.18 ± 0.2	1.56 ± 0.0	1.08 ± 0.1	1.55 ± 0.1	1.90 ± 0.1	2.38 ± 0.1	1.47 ± 0.1[a]	2.26 ± 0.1
Leukocytes (x103x µl-1)	62.26 ± 4.6	51.55 ± 3.3	57.48 ± 4.2	32.46 ± 1.8	47.50 ± 4.5	39.53 ± 1.2	68.02 ± 5.3	60.11 ± 5.5	34.11 ± 0.9	32.32 ± 1.6
Thrombocytes (x103x µl-1)	62.82 ± 7.0	30.91 ± 5.2[a]	50.93 ± 7.4	28.65 ± 2.2	40.55 ± 3.8	32.41 ± 1.2	55.41 ± 3.4	57.36 ± 7.0	31.22 ± 1.1	26.73 ± 0.9
Hemoglobin (g/dl)	10.87 ± 0.4	6.62 ± 0.0[a]	10.08 ± 0.4	10.79 ± 0.2	9.51 ± 0.3	10.36 ± 0.2	8.33 ± 0.2	10.03 ± 0.3	8.42 ± 0.1	8.61 ± 0.5
PVC (%)	24.27 ± 0.7	18.60 ± 0.9[a]	25.30 ± 0.9	22.66 ± 0.9	20.20 ± 1.2	22.94 ± 1.1	20.70 ± 0.6	23.63 ± 1.0	20.91 ± 0.5	26.0 ± 1.0
MCV (fL)	144.34 ± 6.8	111.62 ± 14.8[a]	133.50 ± 9.3	145.71 ± 3.1	187.19 ± 3.4	152.43 ± 5.7	127.34 ± 9.3	105.04 ± 8.2	150.12 ± 4.9	119.73 ± 5.8
MCH (pg)	70.45 ± 6.3	43.04 ± 7.2[a]	56.78 ± 5.2	71.93 ± 3.0	89.32 ± 3.1	71.83 ± 4.7	52.64 ± 4.4	44.33 ± 2.9	63.34 ± 3.5	35.56 ± 5.5
MCHC (%)	46.75 ± 2.7	35.76 ± 2.5	41.57 ± 2.6	49.97 ± 2.3	47.90 ± 2.0	47.09 ± 2.5	40.74 ± 1.2	43.0 ± 1.3	41.32 ± 1.2	28.30 ± 3.0
Lymphocytes (%)	70.81 ± 0.5	63.90 ± 0.9	70.09 ± 0.7	70.25 ± 0.6	64.20 ± 1.2	69.76 ± 0.8	69.70 ± 1.2	75.38 ± 1.0	69.16 ± 1.2	73.93 ± 1.1
Neutrophils (%)	20.23 ± 0.7	27.75 ± 1.0	20.00 ± 0.6	19.38 ± 0.6	24.70 ± 1.0	19.47 ± 0.8	28.43 ± 1.0	23.75 ± 0.9	28.86 ± 0.8	23.0 ± 1.0
Monocytes (%)	5.65 ± 0.5	5.10 ± 0.5	5.61 ± 0.5	5.78 ± 0.5	6.00 ± 0.7	6.35 ± 0.6	4.27 ± 0.4	2.38 ± 0.3	3.00 ± 0.3	2.79 ± 0.3
Basophils (%)	0.42 ± 0.1	0.35 ± 0.1	0.48 ± 0.1	0.53 ± 0.1	0.50 ± 0.2	0.47 ± 0.1	0.62 ± 0.1	0.44 ± 0.1	0.38 ± 0.1	0.64 ± 0.1
Eosinophils (%)	0.46 ± 0.1	0.00 ± 0.0	0.35 ± 0.1	0.44 ± 0.1	0.00 ± 0.0	0.35 ± 0.1	0.10 ± 0.0	0.38 ± 0	0.30 ± 0.1	0.36 ± 0.1

DPW dumping of produced water

[a]For the same season of year, indicate statistically significant differences ($p < 0.05$) between the reference and Nacimiento sites

Table 13.3 Frequency of micronuclei and other nuclear abnormalities in peripheral blood of *Astyanax gr. bimaculatus* and *Aequidens metae* sampled in two sites of Ocoa River and a reference site (Negro River), Villavicencio—Meta, Colombia, during dry and rainy season

	Astyanax gr. bimaculatus						*Aequidens metae*			
	Dry season			Rainy season			Dry season		Rainy season	
Nuclear abnormality	Nacimiento	DPW	Reference	Nacimiento	DPW	Reference	DPW	Reference	DPW	Reference
Micronuclei	0.07 ± 0.01	0.13 ± 0.02[a]	0.12 ± 0.01	0.09 ± 0.01	0.20 ± 0.02[a]	0.09 ± 0.02	0.11 ± 0.01	0.06 ± 0.01	0.22 ± 0.04[a]	0.09 ± 0.02
Lobed	0.09 ± 0.02	0.68 ± 0.05[a]	0.09 ± 0.02	0.09 ± 0.01	0.70 ± 0.05[a]	0.11 ± 0.02	0.34 ± 0.09[a]	0.08 ± 0.01	0.38 ± 0.01[a]	0.09 ± 0.01
Blebbed	0.11 ± 0.02	0.55 ± 0.03[a]	0.05 ± 0.01	0.08 ± 0.01	0.67 ± 0.07[a]	0.10 ± 0.01	0.31 ± 0.01[a]	0.07 ± 0.01	0.37 ± 0.09[a]	0.11 ± 0.02
Notched	0.08 ± 0.01	0.11 ± 0.06	0.08 ± 0.01	0.07 ± 0.01	0.23 ± 0.09[a]	0.12 ± 0.01	0.14 ± 0.05	0.12 ± 0.02	0.26 ± 0.01[a]	0.15 ± 0.03
Binucleated cells	0.10 ± 0.02	0.12 ± 0.02[a]	0.07 ± 0.01	0.08 ± 0.01	0.13 ± 0.03[a]	0.11 ± 0.02	0.11 ± 0.01	0.09 ± 0.02	0.12 ± 0.05[a]	0.08 ± 0.02

DPW dumping of produced water

[a]For the same season of year, indicate statistically significant differences ($p < 0.05$) between the Nacimiento and reference site and the DPW site

Likewise, hematological alterations in this study were associated with disturbances in hepatic morphology such as the presence of pyknotic nuclei, vacuolation, and lymphocyte infiltration, such as those found by Maceda-Veiga et al. (2010) in *Barbus meridionalis* caught in different sites of the Ripoll River which receives high volumes of wastewater, showing higher liver alterations and lower hemoglobin concentrations and percentages of hematocrit in sites with higher concentrations of toxic metals and deficient conditions of water quality.

13.3.1.2 Genotoxicity Biomarkers

The lipid peroxidation occurrence and histopathological alterations in the liver of the two fish species were evaluated; the results showed that the specimens of *A. gr bimaculatus* from the dumping of produced water (DPW) site had the highest levels of liver damage during the two seasons; these findings were accompanied by the greater genotoxic damage evidenced by the presence of micronuclei, lobed nuclei, and binucleated erythrocytes (Table 13.3). In this regard, several studies have found a direct relationship between the occurrence of LPO and the manifestation of DNA damage due to the appearance of MN, possibly because the lipid damage of the cell membranes leads to the genetic material is exposed and can undergo alterations that trigger irreversible mutations caused by the subtraction of hydrogen from the DNA, the extraction reaction being greater than the capacity of the mechanisms of repair to restore DNA. One study evaluated the impact of the oil spill on a body of water 5 years after the event and found similar results (Katsumiti et al. 2013). These evidences show that the area of influence of this site exerts harmful effects on the organisms that inhabit this contaminated areas.

Additionally, the rainy season has been related to increases in the manifestation of micronuclei (Ossana and Salibián 2013); similar results were found in this study, revealing that in the rainy season, there is a greater availability of genotoxins.

On the other hand, a possible specific relationship has been reported between the exposure to effluents from oil refineries and the generation of lobed nuclei in peripheral blood of fish exposed (Çavas and Ergene-Gözükara 2005); in this study a higher frequency of this abnormality in the DPW site was found, which is exposed to wastewater of petroleum industry; in addition *A. metae* showed significant differences compared to the reference site, showing that *A. gr bimaculatus* may have greater sensitivity to the formation of this type of nuclear abnormalities.

Finally, the presence of binucleated erythrocytes has been associated with contact with wastewater from thermal power plants in *Labeo bata* and *Oreochromis mossambicus* (Talapatra et al. 2007) and also in *Oreochromis niloticus*, *Oreochromis aureus*, and *Tilapia zilli* exposed in situ to waters with domestic and industrial residuals that are discharged into the Nile River (Ali et al. 2008); therefore, the induction of this abnormality has been considered an indicator of cytotoxicity (Çavaş et al. 2005). The Ocoa River site that receives industrial wastewater showed the highest incidence of binucleated erythrocytes, indicating greater exposure of biota to cytotoxic contaminants that can exert genotoxic effects at different concentrations (García-Medina et al. 2011).

13.3.1.3 Oxidative Stress Biomarkers

The effects observed in the morphology of the liver are corroborated by the liver SOD activity, which was reduced in *A. gr bimaculatus*, at two seasons of the year, possibly because the stimulus exerted by the xenobiotics present in the water induced a negative feedback inhibiting the activity of the enzyme. Similar results were found by Carvalho et al. (2012) in *Oreochromis niloticus* exposed to the waters of the Moholinho River in São Carlos-Brazil, which receives large amounts of industrial effluents and urban waste.

Likewise, the liver of *A. gr bimaculatus* recorded high levels of cell damage in terms of the occurrence of lipid peroxidation during the two seasons of the year, with an increase in the DPW site (Table 13.4); the specimens from this site also showed lesions of the degenerative degree in the liver and genotoxic damage due to the presence of micronuclei.

Regarding the SOD activity in gills, testicle, and ovary of *A. gr. bimaculatus* and *A. metae*, a reduction was observed between seasons, being lower in the rainy season, which could indicate an inhibition in the enzymatic activity on the part of substrates forming free radicals present in the water; similar results were obtained by Achuba and Osakwe (2003), who exposed the catfish *Clarias gariepinus* to crude oil dispersed in water and found a reduction in the activity of the enzyme that was accentuated over time.

On the other hand, the reduction in CAT activity in the DPW site at rainy season in *A. metae* was similar to that reported by Camargo and Martinez (2006), who exposed in situ the fish *Prochilodus lineatus* to various sites of an urban river with different levels of contamination, demonstrating that in the greater presence of contaminants, CAT activity decreased more in the rainy season.

Likewise, a reduction in the GST antioxidant response during the rainy season in the liver of *A. metae* was observed. This reduction of GST activity can be caused by an inhibition in the response induced by toxic substances such as herbicides which finally reached the water bodies as a result of agricultural activities. GST plays a fundamental role in the biotransformation processes of xenobiotics, so its use as a biomarker in ecotoxicological studies is relevant (Hellou et al. 2012). One study showed that the oxyfluorfen herbicide could inhibit the GST activity of tilapia *Oreochromis niloticus* when exposed chronically (Peixoto et al. 2006). In this study, the greatest DNA damage was found in the sites that showed significant reduction of the GST activity compared to the control. Those results suggest a possible decrease in the biotransformation capacity of xenobiotics due to restriction in GST activity.

On the other hand, lipid peroxidation in *A. metae* exhibited the highest occurrence in the DPW site in the rainy season, which agrees with the greater presentation of histopathological alterations in gills at this season. This finding suggests an interaction between the two biomarkers, demonstrating the importance of using different types of biomarkers. Likewise, one study pointed the high interaction between the occurrence of LPO, the histopathological alterations, and the concentration of mercury in tissues of fishes in the Amazonian water (Silva et al. 2010). Similarly, in *A. gr bimaculatus* the occurrence of LPO presented the highest values at the DPW site in the rainy season, confirming that in this period, the concentrations of pollutants increase compared to the dry season (Table 13.5).

Table 13.4 Activity of different antioxidant enzymes and lipid peroxidation in gills and liver of *Astyanax gr. bimaculatus* and *Aequidens metae* caught in two sites of Ocoa River and a reference site (Negro River), Villavicencio—Meta, Colombia, during dry and rainy season

			Astyanax gr. Bimaculatus				*Aequidens metae*			
Organ	Season	Monitoring site	SOD	CAT	GST	LPO	SOD	CAT	GST	LPO
Gill	Dry	Reference	14.59 ± 1.42	139.83 ± 19.53	2.83 ± 0.50	8.0 ± 0.73	12.44 ± 0.85	149.64 ± 18.21	2.57 ± 0.17	6.13 ± 0.53
		Nacimiento	11.19 ± 1.21	108.77 ± 9.07	1.76 ± 0.15	7.77 ± 0.57	–	–	–	–
		DPW	15.06 ± 1.87	123.71 ± 11.23	1.93 ± 0.27	9.28 ± 0.76	12.43 ± 0.59	165.66 ± 9.55	2.39 ± 0.14	8.83 ± 0.48
	Rainy	Reference	9.55 ± 1.08	138.61 ± 14.94	2.13 ± 0.27	7.06 ± 0.83	11.82 ± 0.77	218.71 ± 21.77	2.55 ± 0.27	7.03 ± 0.67
		Nacimiento	9.86 ± 0.58	100.13 ± 9.69	2.26 ± 0.31	6.53 ± 0.77	–	–	–	–
		DPW	9.57 ± 0.72	120.19 ± 11.93	2.21 ± 0.25	7.34 ± 0.80	10.71 ± 0.66	164.88 ± 17.92[ab]	2.34 ± 0.18	9.98 ± 0.65
Liver	Dry	Reference	8.80 ± 0.43	114.52 ± 9.81	2.23 ± 0.26	5.98 ± 0.46	7.49 ± 0.79	102.59 ± 8.33	2.56 ± 0.30	4.93 ± 0.55
		Nacimiento	10.41 ± 1.24	110.86 ± 14.11	2.16 ± 0.21	5.76 ± 0.39	–	–	–	–
		DPW	7.81 ± 0.68[a]	70.47 ± 8.16[ab]	1.88 ± 0.15	6.00 ± 0.57	7.46 ± 0.51	120.51 ± 7.92	2.53 ± 0.28	5.98 ± 0.33
	Rainy	Reference	9.10 ± 0.56	107.05 ± 7.0	2.31 ± 0.27	5.37 ± 0.55	11.88 ± 1.10	118.95 ± 13.63	2.29 ± 0.33	5.23 ± 0.23
		Nacimiento	7.16 ± 0.27	110.68 ± 8.25	1.60 ± 0.26	4.79 ± 0.33	–	–	–	–
		DPW	7.07 ± 0.78	89.06 ± 7.85	1.80 ± 0.14	5.66 ± 0.43	7.07 ± 0.78[a]	89.06 ± 7.85[a]	1.80 ± 0.14[a]	5.66 ± 0.43

[a]Indicates statistical significant differences ($p < 0.05$) between dumping of produced water (DPW) site and reference site (Negro River) for the same season
[b]Indicates statistical significant differences ($p < 0.05$) between dumping of produced water (DPW) site and the Nacimiento site for the same season
SOD superoxide dismutase: U/ml/min/mg protein, *CAT* catalase: μmol/min/mg protein, *GST* glutathione S-transferase: nmol/min/mg protein, *LPO* lipid peroxidation: mmol hydroperoxide/mg protein

Table 13.5 Activity of different antioxidant enzymes and lipid peroxidation in gonads of *Astyanax gr. bimaculatus* and *Aequidens metae* caught in two sites of Ocoa River and a reference site (Negro River), Villavicencio-Meta, Colombia, during dry and rainy season

			Astyanax gr. bimaculatus				*Aequidens metae*			
Organ	Season	Monitoring site	SOD	CAT	GST	LPO	SOD	CAT	GST	LPO
Testicle	Dry	Reference	12.82 ± 1.2	151.16 ± 30.7	1.94 ± 0.2	7.76 ± 0.8	7.43 ± 1.0	92.41 ± 17.9	1.59 ± 0.2	5.40 ± 0.5
		Nacimiento	10.52 ± 0.5	176.29 ± 22.7	2.92 ± 0.7	7.46 ± 0.7	–	–	–	–
		DPW	8.68 ± 1.6[a]	88.39 ± 12.1[bc]	1.58 ± 0.3	8.42 ± 1.5	6.34 ± 0.3	105.37 ± 10.6	1.33 ± 0.1	5.43 ± 0.3
	Rainy	Reference	8.60 ± 1.1	146.70 ± 26.7	1.54 ± 0.2	8.95 ± 1.1	6.11 ± 0.4	62.90 ± 6.3	1.28 ± 0.3	5.47 ± 0.8
		Nacimiento	6.83 ± 1.4	86.91 ± 14.0	4.09 ± 0.9	7.92 ± 2.3	–	–	–	–
		DPW	9.77 ± 1.3	120.50 ± 17.0	1.73 ± 0.3	9.70 ± 1.3	11.24 ± 1.0[a]	125.57 ± 11.7[a]	3.64 ± 0.6	9.66 ± 0.8[a]
Ovary	Dry	Reference	10.26 ± 2.9	106.85 ± 18.4	1.73 ± 0.4	5.97 ± 1.4	14.33 ± 2.7	213.96 ± 40.4	2.19 ± 0.5	4.25 ± 0.8
		Nacimiento	8.41 ± 1.4	77.26 ± 10.1	2.55 ± 0.4	5.91 ± 1.0	–	–	–	–
		DPW	6.59 ± 1.5[ac]	82.29 ± 8.6	1.10 ± 0.1	6.57 ± 0.7	16.51 ± 2.1	184.18 ± 22.3[a]	2.06 ± 0.4	4.92 ± 0.6
	Rainy	Reference	10.22 ± 1.0	104.15 ± 6.9	1.91 ± 0.2	5.27 ± 0.6	6.34 ± 0.9	74.00 ± 5.2	0.78 ± 0.1	3.33 ± 0.4
		Nacimiento	9.39 ± 1.9	121.90 ± 24.4	2.37 ± 0.5	4.63 ± 1.1	-	-	–	–
		DPW	9.46 ± 0.5	103.23 ± 9.9	2.08 ± 0.8	4.74 ± 0.2	5.55 ± 0.6	66.02 ± 7.9	0.96 ± 0.1	3.31 ± 0.7

[a]Indicates statistical significant differences ($p < 0.05$)

[b]Indicates highly statistical significant differences ($p < 0.001$) between dumping of produced water (DPW) site and reference site (Negro River) at the same season

[c]Indicates statistical significant differences ($p < 0.05$) between DPW site and the Nacimiento site for the same season

SOD superoxide dismutase: U/ml/min/mg protein, *CAT* catalase: μmol/min/mg protein, *GST* glutathione S-transferase: nmol/min/mg protein, *LPO* lipid peroxidation: mmol hydroperoxide/mg protein

The greater availability of pollutants generated by resuspension of sediments by increasing the flow of the river Ocoa in the rainy season, together with the increase in the leaching processes of products applied in agricultural activities (herbicides, insecticides, pesticides) and other by-products of human activities such as toxic organic compounds, heavy metals, and particulate material, may promote the inhibition of the antioxidant response due to a greater possibility of contact with aquatic biota.

In conclusion, the results obtained show that at the rainy season, the greatest negative impact was observed where the dumping of produced water site exerts a harmful effect on the monitored fish.

13.3.1.4 Histopathological Biomarkers

The greatest occurrence of gill alterations was observed in *A. gr bimaculatus* and *A. metae*, caught in DPW site in both seasons. In the same way, in the case of *A. metae* in both rainy and dry seasons, a greater occurrence of liver lesions was observed in DPW site (Figs. 13.1 and 13.2).

In the same case of *A. gr. bimaculatus* during the rainy season, an increased occurrence of liver lesions was observed at DPW site (Fig. 13.2). On the other hand, the increases in the HSI values in the DPW site in both species with respect to the reference site indicate alterations in the liver that are associated with disturbances in the liver structure, increasing the size of the organ due to degenerative lesions, especially by vacuolization (Costa et al. 2010) and inflammatory type (Barber et al. 2007; Carvalho et al. 2012). These alterations can modify the liver architecture by increasing the size of the organ (Diniz et al. 2005).

The alteration in the structure and function in gills of native fish to rivers and other contaminated water bodies is due to various environmental factors, such as pH, heavy metals, agrochemicals, and other pollutants (Abdel-Moneim et al. 2012; Lukin et al. 2011; Santos et al. 2006). The epithelial uplift in secondary lamellae

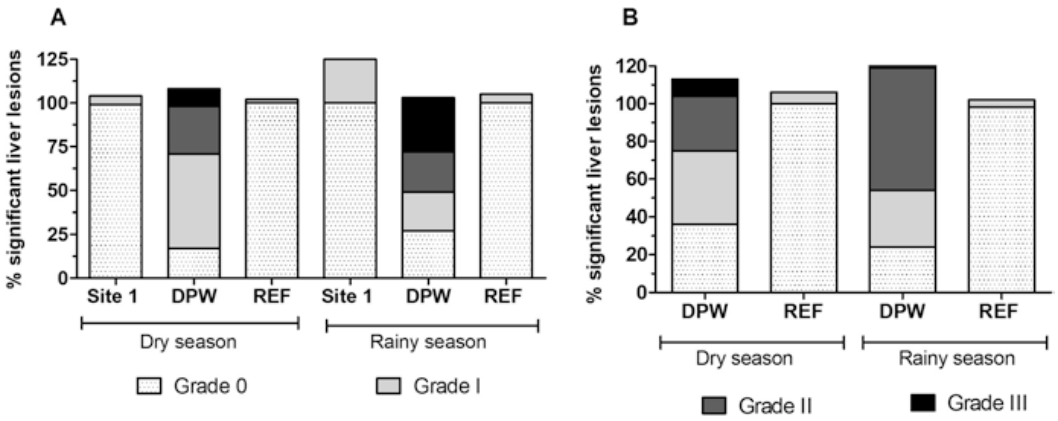

Fig. 13.1 Significant histopathology alterations in the liver of (**a**) *Astyanax gr. bimaculatus* and (**b**) *Aequidens metae* caught at dumping of produced water (DPW) site of Ocoa River and reference site on dry and rainy season, Villavicencio, Colombia

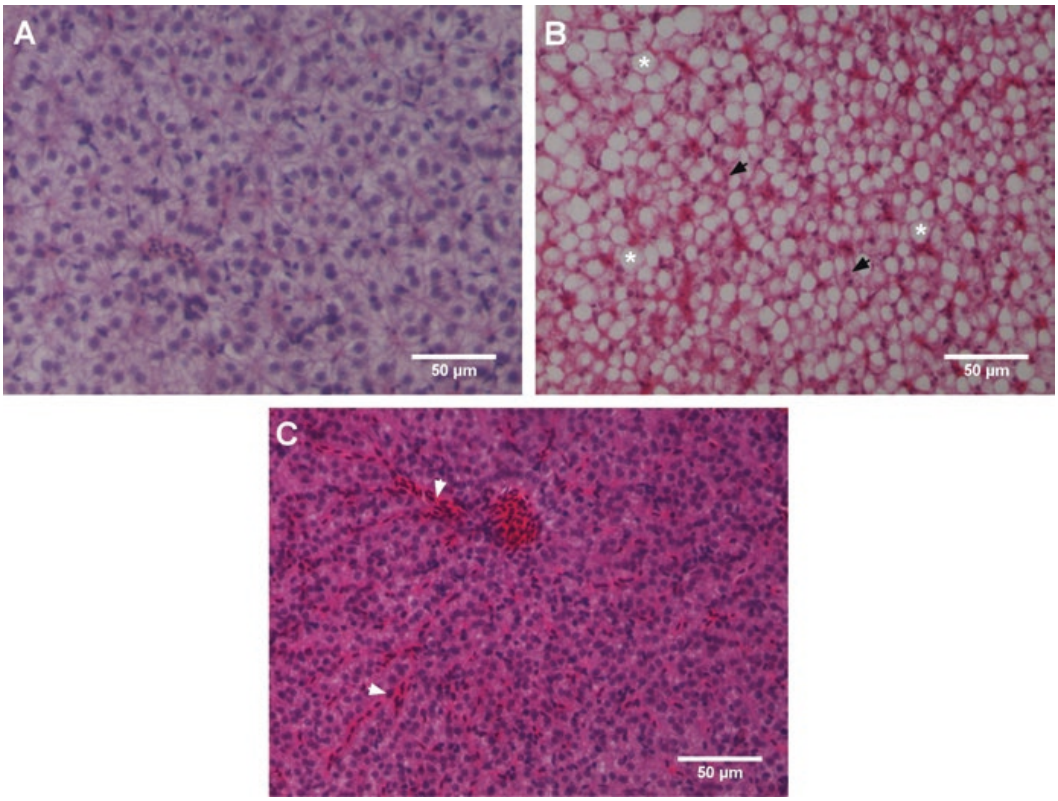

Fig. 13.2 Histopathology changes in the liver**:** (**a**) Control, hepatocyte from reference site in *A. gr bimaculatus* (**b**) Vacuolization (∗) and nuclear degeneration (black arrow) in *A. metae* (**c**) Sinusoidal congestion (short arrow) in *A. gr bimaculatus* caught at dumping of produced water (DPW) site of Ocoa River. H&E staining

due to hyperplasia shows that the gills have been exposed to dangerous chemical products or physical agents. This change could be a response to increase the exchange area between blood and available oxygen, which has been related to hypoxia states (Liu et al. 2010). Interlamellar hyperplasia is a consequence of excess mucus production and the exposure to contaminants stimulates in the epithelium of secondary lamellae an increase in the number of mucus cells. Hyperplasia together with excess mucus causes lamellar fusion that reduces gas exchange capacity (Kumari et al. 2012). These lesions were observed in the fish caught from the dumping of produced water site at both times of the year (Figs. 13.3 and 13.4).

Regarding the water analysis data, it is important to highlight that although those are below the limits allowed by Colombian environmental legislation, these minimum concentrations exceed those of other countries such as those of the European Union and the USA. These countries use them as criteria for the selection of permitted limits for discharges of industrial and domestic wastewater into water bodies, and research reports are constantly carried out on the natural water bodies of each country (Hansen et al. 2001). This is one of the reasons why this study and the other studies done by the research group BioTox at Universidad de los Llanos have considerable relevance when providing the first data on the effect of the contamination on the Ocoa River.

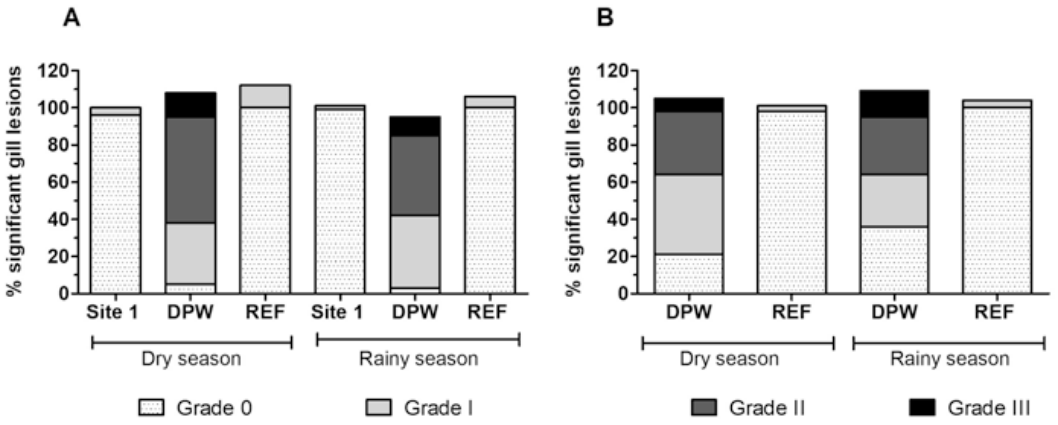

Fig. 13.3 Significant histopathology alterations in gill of (**a**) *Astyanax gr. bimaculatus* and (**b**) *Aequidens metae* caught at Nacimiento and dumping of produced water (DPW) sites of Ocoa River and reference site on dry and rainy season, Villavicencio, Colombia

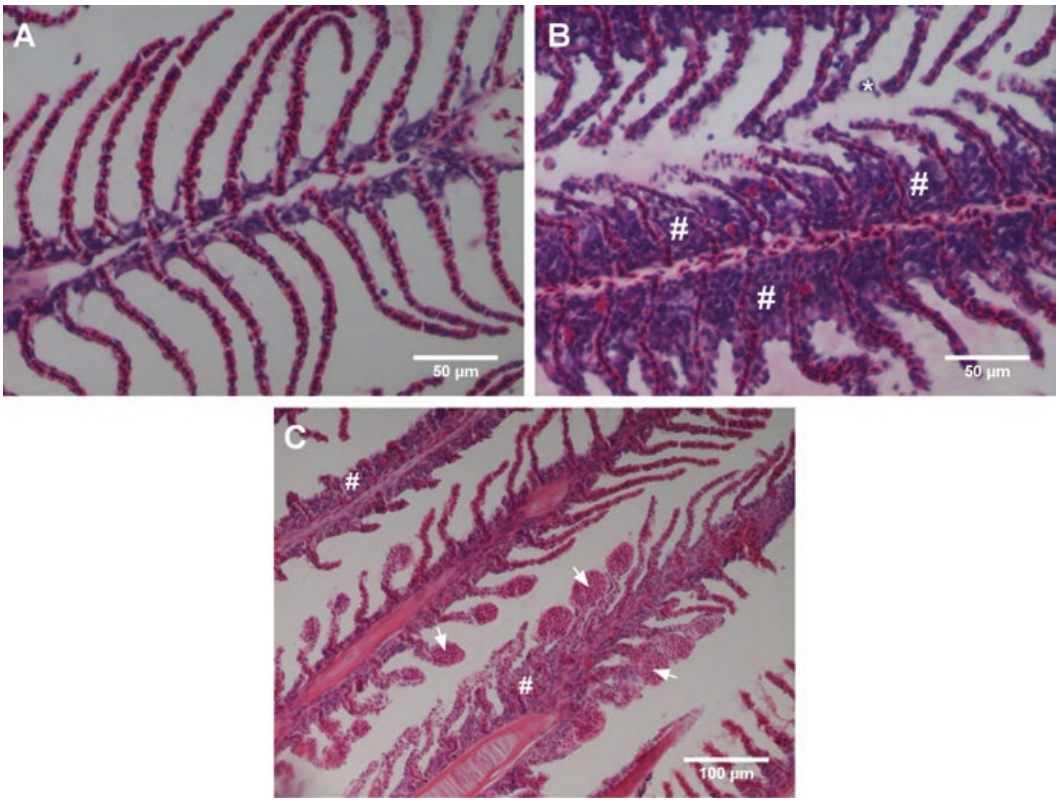

Fig. 13.4 Histopathology changes in the gill: (**a**) Control gill from a reference site *A. gr. bimaculatus* (**b**) epithelial lifting (∗), lamellar partial fusion (#) in *A. metae* and (**c**) lamellar aneurysm (white arrow), lamellar partial fusion (#) in *A. gr bimaculatus* caught at dumping of produced water (DPW) site of Ocoa River. H&E staining

On the other hand, the levels of ammonium found in DPW site in the Ocoa River can exert deleterious effects on freshwater fish, taking into account that concentrations from 0.44 mg/L of NH_3 in the presence of acidic pH are considered toxic for some teleosts such as *Rhamdia quelen* (Miron et al. 2008). In this regard, the concentrations of nonionized ammonium registered in the river during the monitoring

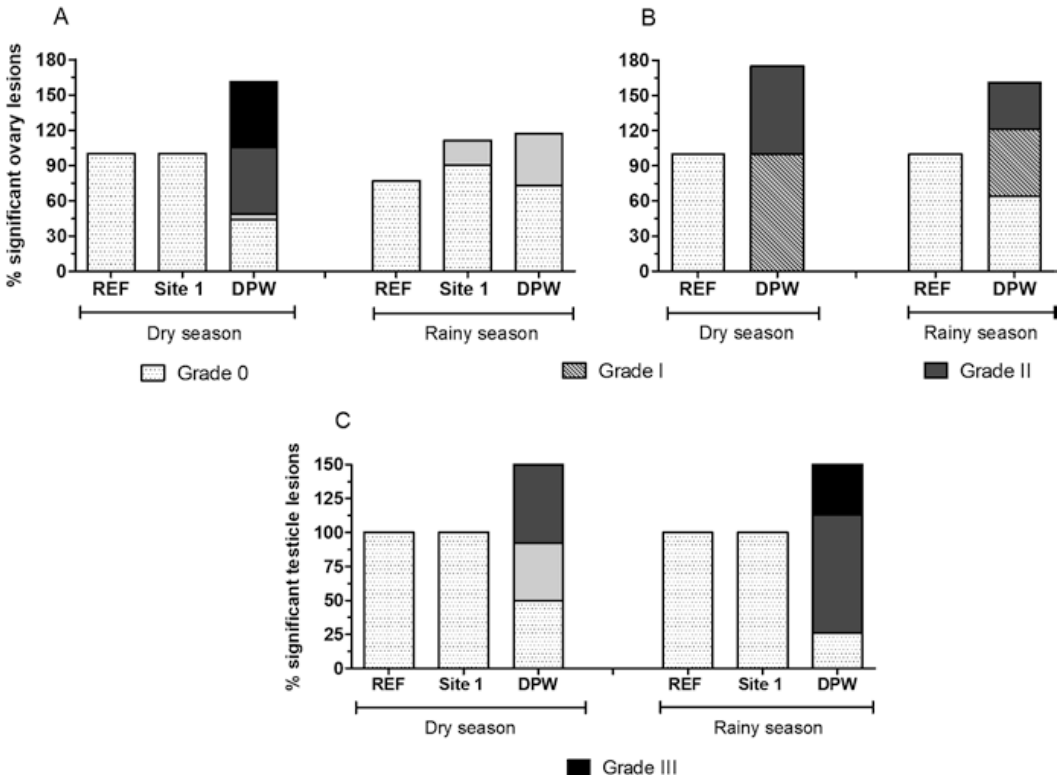

Fig. 13.5 Significant histopathology alterations in (**a**) ovary of *Astyanax gr. bimaculatus*, (**b**) ovary of *Aequidens metae*, and (**c**) testicle of *Astyanax gr. bimaculatus* caught at Nacimiento (site 1) and dumping of produced water (DPW) sites of Ocoa River and reference site on dry and rainy season, Villavicencio, Colombia. (Adapted from Velasco-Santamaría et al. (in press))

reveal that the levels of organic matter present in this body of lotic water are high and therefore can exert harmful effects on gas exchange, damage the branchial epithelium, and interrupt the osmoregulatory activity due to alteration of the blood vessels, affecting the liver and kidneys, among other organs (Camargo and Alonso 2006).

Studies about endocrine disruption in fish in Colombia are limited despite the significant relevance of this kind of studies. Recently, Velasco-Santamaría et al. (in press) showed that in fish caught at site that receives produced water, alterations such as moderate ovarian atresia, atresia of perinucleolar oocytes, hyperplasia of perifollicular cells, interstitial fibrosis, and decrease in the postovulatory follicles with higher association to the rainy season were reported. Likewise, in males of *A. gr. bimaculatus* during rainy season, histological alterations such as increase in the spermatogonia, hypertrophy in Leydig cells, and interstitial fibrosis were observed (Figs. 13.5 and 13.6).

13.4 Experimental Approach to Carry Out Studies in Microalgae Exposed to HAPs or Oil-Produced Water

There are different methodological approaches to carry out studies with microalgae aimed to understand the effects of pollutants on these aquatic bioindicators. The OECD guidelines (Test No. 201: Freshwater Alga and Cyanobacteria, Growth

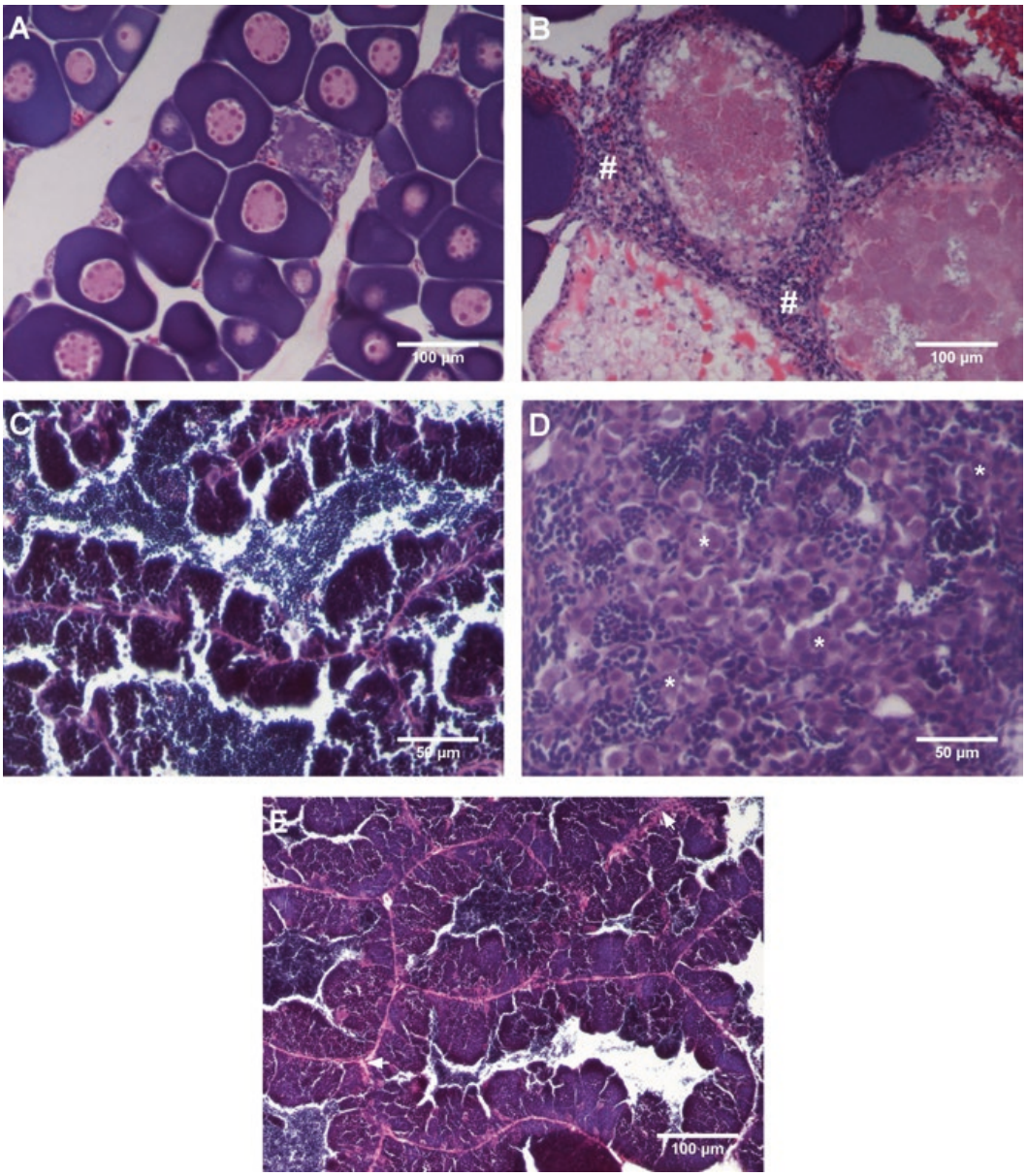

Fig. 13.6 Histopathology alterations in gonads: (**a**) Control ovary from *Aequidens metae* caught at a reference site; (**b**) Ovary interstitial fibrosis in *Aequidens metae* (#); (**c**) Control testicle from *A. gr. bimaculatus* caught at Nacimiento site of Ocoa River; (**d**) increase in spermatogonial cells (asterisk) in testicle of *A. gr. bimaculatus*; (**e**) Interstitial cell aggregates (white arrow) in testicle of *A. gr. bimaculatus* caught at dumping of produced water (DPW) site of Ocoa River, Villavicencio, Colombia

Inhibition Test) and the EPA guidelines (OCSPP 850.4500 Algal Toxicity test) are good options to follow the experimental and systematic procedures for evaluating xenobiotics compounds in algae; however, some methodological adaptations can be done depending on the local conditions, always keeping in mind the reproducibility of the data.

In the case of microalgae toxicity test evaluating oil or produced water, these studies have been carried out in glass containers with a capacity of 0.4 L or 3 L, previously sterilized at a temperature of 121 °C. The culture medium used has

been a sterilized complex inorganic fertilizer (NPK REMITAL® M-17-6-18) at a rate of 1 g/L composed of 17% of total nitrogen, 6% assimilable phosphorous, 18% water-soluble potassium, 2% magnesium, 1.6% total sulfur, 0.2% boron and 0.1% zinc. The microalgae are keep under controlled laboratory conditions with cool and permanent fluorescent light at an irradiance of 36.8 ± 4.2 μmol photons m^{-2} s^{-1}, temperature from 18 to 20 °C, and permanent aeration supplied by a blower or under permanent shaking orbital condition, keeping the algae culture until it reaches the exponential phase. The culture period is maintained until the microalgae reached the exponential phase. Subsequently, the algae *C. vulgaris* or *Scenedesmus* sp. are exposed to different concentrations of xenobiotics, at least five concentrations per each compound. In the studies carry out in the Orinoquia region, different approaches have been done. The first one aimed to understand the effect of nominal concentrations of some polycyclic aromatic hydrocarbons such us phenanthrene (Otero-Paternina et al. 2013; Calderón-Delgado et al. 2020). Second approach has been aimed to understand the effect of the exposure to water dilutions collected at the site of oil dumping well (Aguilar-León 2011; Calderón-Delgado et al. 2019).

In the case of evaluating the PAH exposure, acetone (0.05%) has been used to dilute the PAH and therefore used also as a solvent control (Otero-Paternina et al. 2013; Calderón-Delgado et al. 2020). In the studies with oil-produced water, the control treatment used was water with inorganic culture medium (Calderón-Delgado et al. 2019). In addition, in some of the studies, microalgae are also exposed to a positive control containing crude oil coming from the same oil well than the produced water was generated and extracted (Calderón-Delgado et al. 2019).

The exposure type can fluctuate based on the parameters aimed to evaluate, including acute (72 hours), subacute (5 or 7 days), or chronic exposure (15 days) (Otero-Paternina et al. 2013; Calderón-Delgado et al. 2019). At least five replicates of each treatment must be evaluated (Calderón-Delgado et al. 2019) in order to guarantee data reproducibility.

The biomarkers evaluated include growth parameters such as cell density, average growth rate, total biomass and percent inhibition of biomass, pigment concentration such as chlorophyll *a* and chlorophyll *b* concentration, algae morphology, and antioxidant enzymes such catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST).

13.4.1 Main Results Obtained in Microalgae Exposed to HAPs or Oil-Produced Water

13.4.1.1 Growth Rate Effects

In *Chlorella vulgaris* exposed for 7 days to concentrations from 0.1 to 10.000 μg/L phenanthrene (PHE), a significantly lower cell density compared to the control was observed (Otero-Paternina et al. 2013; Calderón-Delgado et al. 2020).

Table 13.6 Growth rate of *Chlorella vulgaris* exposed to different phenanthrene concentrations at different durations of exposure

Treatment	Growth rate of *Chlorella vulgaris*	
	7 days of exposure (Calderon-Delgado et al. 2020)	3 days of exposure (Otero-Paternina et al. 2013)
Control	0.190 ± 0.005	0.429 ± 0.008
Solvent control	0.150 ± 0.005[a]	ND
0.1 µg/L	0.141 ± 0.005[a]	ND
1.0 µg/L	0.147 ± 0.005[a]	0.403 ± 0.006
10 µg/L	0.145 ± 0.001[a]	0.379 ± 0.006
100 µg/L	0.135 ± 0.006[a]	0.354 ± 0.007[a]
1000 µg/L	0.132 ± 0.006[a]	0.307 ± 0.01[a]
10000 µg/L	ND	0.207 ± 0.013[a]

Values are expressed as mean ± SEM
ND not determined
[a]Indicates significant differences compared to the control ($p < 0.05$)

A progressive decrease in the growth rate of *C. vulgaris* exposed to PHE concentrations has been observed, where the duration of exposure significantly affects the response. For instance, the growth of algae exposed to the lowest concentrations (0.1 µg/L) had a significant lower growth rate at 7 days of PHE exposure; in contrast this response was not observed at 3 days of exposure (Table 13.6).

13.4.1.2 Chlorophyll Concentration

Chlorophyll concentration can be measured by a traditional cell spectrophotometer or in a microplate spectrophotometer reader with results varying slightly, with microplate reader being the more sensitive method. In this regard, chlorophyll *a* in *C. vulgaris* was not affected by the different phenanthrene concentrations during 72 hours of exposure when measured in the traditional way showing high variability on the data despite some trend of reduction observed (Fig. 13.7a). Interestingly, in subsequent study done by Calderón-Delgado et al. (2020), the chlorophyll *a* concentration decreased significantly at 72 hours of exposure from 0.1 µg/L PHE (Fig. 13.7b), being in this study the chlorophyll concentration measured in a lector multimodal.

13.4.1.3 Cell Size

The cellular diameter or cell size is another important variable to determine the effect of xenobiotics on microalgae. In this regard, differences in this variable depend considerably on the microalgae species (Fig. 13.8). For instance, a significant reduction in *Chlorella vulgaris* cell diameter has been observed after 7 days of exposure to HAPs (from 1 to 10.000 µg/L phenanthrene) or after 5 days of exposure

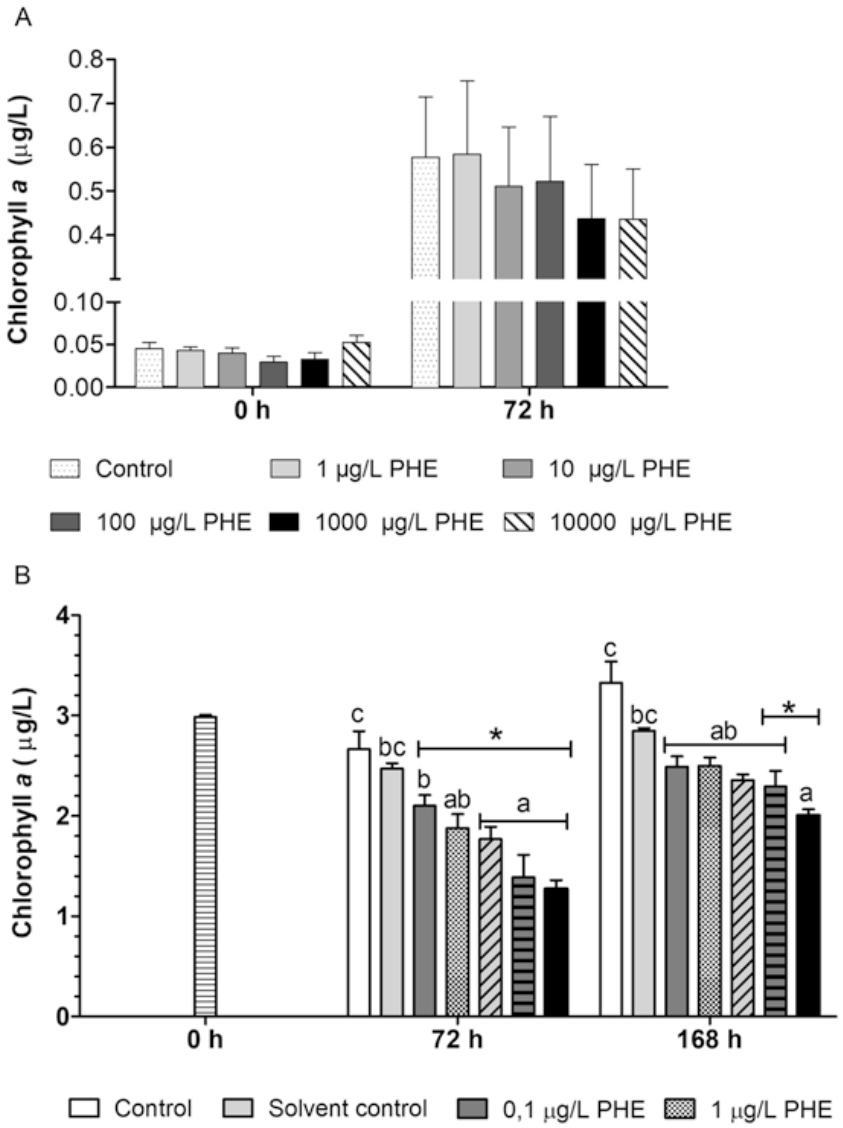

Fig. 13.7 Chlorophyll a concentration in *C. vulgaris* exposed to phenanthrene with measurement done in (**a**) a cell spectrophotometer and (**b**) in a lector multimodal. The values are expressed as mean ± SEM. [a, b, c, d]At the same time, bars with different letters indicate significant differences ($p < 0.05$). (Adapted from Otero-Paternina et al. (2013), Calderón-Delgado et al. (2020))

to different concentrations of produced water. These results are related to the reduction in the chlorophyll content stated previously, when the HAP or the produced water reduced the concentration of photosynthetic pigments in algae, leading to physiological responses such as decrease in cell diameter, which is a probable adaptive response since the smaller diameter leads to an increase in specific microalgae surface (bigger volume/surface ratio) to absorb xenobiotics and biotransform them effectively (Calderón-Delgado et al. 2019). In contrast with the results in *C. vulgaris*, an increase in cell size has been observed in *Scenedesmus* sp. exposed to produced water with more than 20% concentration.

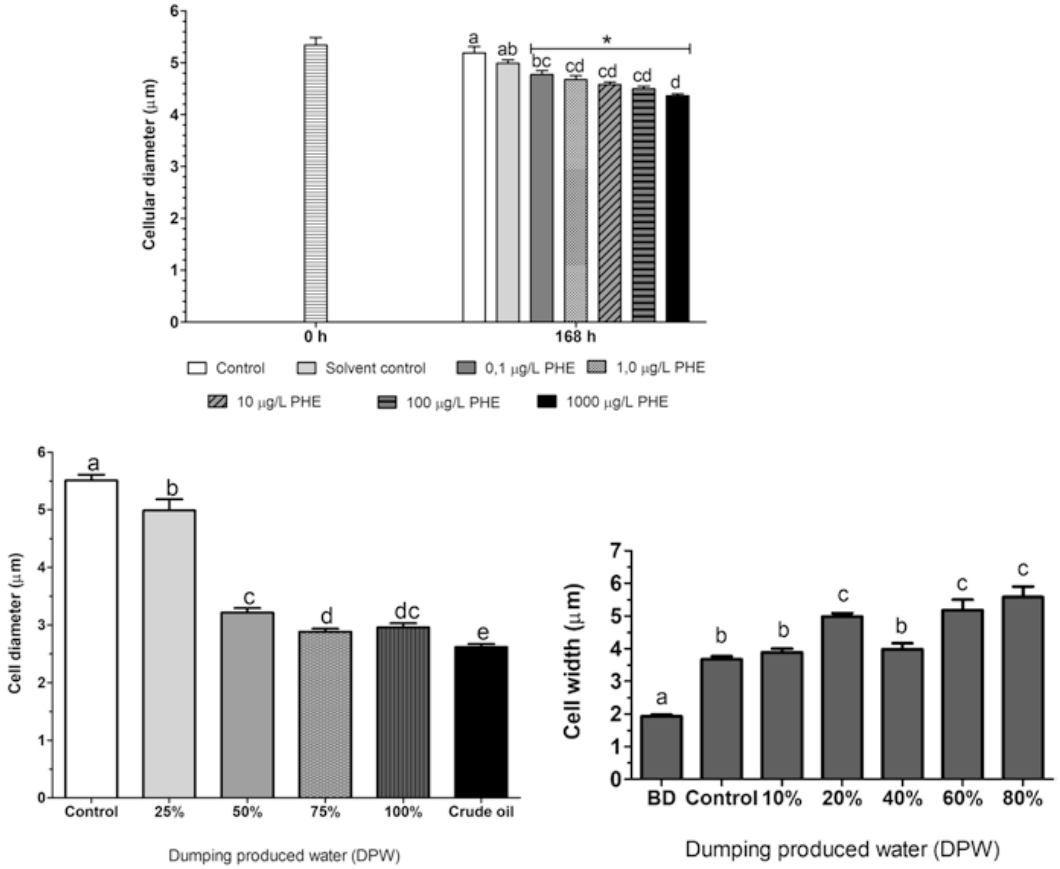

Fig. 13.8 (**a**) Cell diameter in *C. vulgaris* exposed to phenanthrene for 7 days, (**b**) cell diameter in *C. vulgaris* exposed to different percentages of produced water for 5 days, and (**c**) cell width of *Scenedesmus* sp. exposed to different percentages of produced water. [a, b, c, d] Bars with different letters indicate significant differences ($p < 0.05$). (Adapted from (Otero-Paternina et al. (2013), Aguilar-León (2011), Calderón-Delgado et al. (2019))

13.4.1.4 Enzymatic Activity

In plants, the main enzyme that counteracts H_2O_2 is catalase (CAT), and the main complementary systems to counteract ROS is the SOD/CAT route and ascorbate-glutathione cycle (Dazy et al. 2009); therefore, the determination of these enzyme activities constitutes a good biomarker of the health microalgae status. A variation of CAT and SOD activity in *C. vulgaris* exposed to different produced water concentrations has been observed with a different pattern throughout 5 days of exposure, which results in a toxicological response of the produced water (Calderón-Delgado et al. 2019).

Regarding CAT activity in *Chlorella vulgaris* exposed to phenanthrene, a significant decrease was observed at 72 hours and 7 days of exposure as the concentration increased. Unlike CAT, the SOD activity of microalgae exposed to phenanthrene did not show significant differences at 72 hours of exposure; however, at 7 days of exposure, all phenanthrene concentrations showed significantly lower levels than the control (Calderón-Delgado et al. 2020) (Table 13.7). These results demonstrate that microalgae enzyme activity associated with oxidative stress is an important complementary tool in ecotoxicological studies.

Table 13.7 Superoxide dismutase activity (U/mg protein) and catalase activity (mmol/min/mg protein) of *C. vulgaris* exposed to different produced water concentrations during 5 days

Exposure		Control	25%	50%	75%	100%	Crude oil
0 hours	SOD	34.52 ± 1.98					
	CAT	10.59 ± 0.74					
24 hours	SOD	39.22 ± 0.06^{a}	38.34 ± 0.23^{a}	62.27 ± 0.04^{*b}	61.25 ± 0.02^{*b}	63.71 ± 1.09^{*b}	64.35 ± 1.35^{*b}
	CAT	13.41 ± 1.41	14.38 ± 1.37	13.78 ± 2.93	18.91 ± 1.62*	20.25 ± 0.88*	21.96 ± 2.55*
72 hours	SOD	37.72 ± 0.22	38.46 ± 0.36	41.55 ± 2.17	47.31 ± 0.81*	36.54 ± 0.32	37.74 ± 0.62
	CAT	10.54 ± 0.68^{a}	25.06 ± 0.96^{*b}	24.87 ± 1.47^{*b}	24.06 ± 4.62^{*ab}	22.46 ± 1.88^{*ab}	24.94 ± 2.20^{*b}
120 hours	SOD	37.40 ± 0.27^{a}	21.11 ± 0.14*b	22.48 ± 1.04b	24.56 ± 0.20b	21.79 ± 0.81*b	21.51 ± 2.12b
	CAT	11.50 ± 0.69^{abc}	19.55 ± 2.40^{*c}	17.23 ± 1.62^{bc}	12.04 ± 0.89^{abc}	7.86 ± 1.57^{ab}	5.14 ± 0.34^{a}

Adapted from Calderón-Delgado et al. (2019)

Data are shown as mean ± SEM

$^{a, b, c}$Different letters show significant difference among treatments ($p < 0.05$) at each specific time. Asterisks show significant difference with initial time ($p < 0.05$)

13.5 Conclusion

As mentioned initially, the origin of PAHs can be pyrogenic or petrogenic; in the latter the produced waters are an important source of pollution; both sources generate negative impacts on aquatic organisms. The data show evidence of negative effects on the hematological parameters, presence of genotoxicity, alteration in the antioxidant response, and presence of deleterious changes in the architecture of the tissues from fish caught at the site where wastewater is discharged from the oil industry, which could lead to a possible decrease in fish populations exposed to these conditions in the Ocoa River. The results obtained to date, evaluating the impact of oil production waters and polycyclic aromatic hydrocarbons on fish and microalgae, demonstrate the need for more rigorous monitoring in Colombia, including biomarkers of exposure and effect, since limiting the studies only to lethality tests (LC_{50} or IC_{50}), which are the only toxicity tests required by Colombian environmental legislation, evidently neither reflects nor monitors the true impact of pollutants on the health and perpetuation of these aquatic organisms. It is important to highlight that the Ocoa River also receives industrial effluents from other sources which could have a direct impact on the health status of aquatic organism and that mixture could lead to an interaction effect with the compounds present in the oil-produced water.

Acknowledgments The authors are very grateful to the colleagues of the research group BioTox from the University of the Llanos, Villavicencio, Colombia for their support during the experimental work done during the studies, especially to Ivonne Calderón-Delgado and Diego Mora-Solarte.

References

Abdel-Moneim AM, Al-Kahtani MA, Elmenshawy OM (2012) Histopathological biomarkers in gills and liver of *Oreochromis niloticus* from polluted wetland environments, Saudi Arabia. Chemosphere 88:1028–1035

Abdel-Shafy HI, Mansour MSM (2016) A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt J Pet 25(1):107–123

Achuba FI, Osakwe SA (2003) Petroleum – induced free radical toxicity in African catfish (*Clarias gariepinus*). Fish Physiol Biochem 29:97–103

Acuña-González J, Vargas-Zamora JA, Gómez-Ramírez E, García-Céspedes J (2004) Hidrocarburos de petróleo, disueltos y dispersos, en cuatro ambientes costeros de Costa Rica. Rev Biol Trop 52:43–50

Aguilar-León D (2011) Uso de la microalga *Scenedesmus sp*. como potencial especie bioindicadora de calidad de los cuerpos de agua receptores de efluentes petroleros. Tesis de Pregrado, Medicina Veterinaria y Zootecnia, Universidad de los Llanos, 38 p

Ali FK, El-Shehawi AM, Seehy MA (2008) Micronucleus test in fish genome: a sensitive monitor for aquatic pollution. Afr J Biotechnol 7:606–612

Assis HCS, Nicareta L, Salvo LM, Klemz C, Truppel JH, Calegari R (2009) Biochemical biomarkers of exposure to deltamethrin in freshwater fish, *Ancistrus multispinis*. Braz Arch Biol Technol 52:1401–1407

Bakke T, Klungsøyr J, Sanni S (2013) Environmental impacts of produced water and drilling waste discharges from the Norwegian offshore petroleum industry. Mar Environ Res 92:154–169

Barber LB, Lee KE, Swackhamer DL, Schoenfuss HL (2007) Reproductive responses of male fathead minnows exposed to wastewater treatment plant effluent, effluent treated with XAD8 resin, and an environmentally relevant mixture of alkylphenol compounds. Aquat Toxicol 82:36–46

Behera BK, Das A, Sarkar DJ, Weerathunge P, Parida PK, Das BK, Thavamani P, Ramanathan R, Bansal V (2018) Polycyclic Aromatic Hydrocarbons (PAHs) in inland aquatic ecosystems: perils and remedies through biosensors and bioremediation. Environ Pollut 241:212–233

Calderón-Delgado IC, Mora-Solarte DA, Velasco-Santamaría YM (2019) Physiological and enzymatic responses of *Chlorella vulgaris* exposed to produced water and its potential bioremediation. Environ Monit Assess 191:399. https://doi.org/10.1007/s10661-019-7519-8

Calderón-Delgado IC, Mora-Solarte DA, Velasco-Santamaría YM (2020) Respuestas fisiológicas y capacidad antioxidante de *Chlorella vulgaris* (Chlorellaceae) expuesta a fenantreno. Acta Biol Colomb 25(2)

Camargo JA, Alonso A (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ Int 32:831–849

Camargo MMP, Martinez CBR (2006) Biochemical and physiological biomarkers in Prochilodus lineatus submitted to in situ tests in an urban stream in southern Brazil. Environ Toxicol Pharmacol 21:61–69

Carvalho CS, Bernusso VA, Araújo HSS, Evaldo L, Fernandes MN (2012) Biomarker responses as indication of contaminant effects in *Oreochromis niloticus*. Chemosphere 89:60–69

Çavas T, Ergene-Gözükara S (2005) Induction of micronuclei and nuclear abnormalities in Oreochromis niloticus following exposure to petroleum refinery and chromium processing plant effluents. Aquat Toxicol 74:264–271

Çavaş T, Garanko NN, Arkhipchuk VV (2005) Induction of micronuclei and binuclei in blood, gill and liver cells of fishes subchronically exposed to cadmium chloride and copper sulphate. Food Chem Toxicol 43:569–574

Costa DDM, Neto FF, Costa MDM, Morais RN, Garcia JRE, Esquivel BM, Oliveira RCA (2010) Vitellogenesis and other physiological responses induced by 17-β-estradiol in males of freshwater fish *Rhamdia quelen*. Comp Biochem Physiol C Toxicol Pharmacol 151:248–257

Cubillos J, Pug P, Gutiérrez J, Paredes D (2014) Phytoremediation of water and soils contaminated by petroleum hydrocarbons. Ingeniería y competitividad 16(1):131–146

Dazy M, Masfaraud JF, Ferard JF (2009) Induction of oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica* Hedw. Chemosphere 75(3):297–302

Díaz-Martínez E, Alarcón A, Ferrera- Cerrato R, Almaraz- Suarez JJ, García- Barradas O (2013) Crecimiento de *Casuarina equisetifolia* (Casuarinaceae) en suelo con diesel, y aplicación de bioestimulación y bioaumentación. Rev Biol Trop 61(3):1039–1052

Diniz MS, Peres I, Pihan JC (2005) Comparative study of the estrogenic responses of mirror carp (*Cyprinus carpio*) exposed to treated municipal sewage effluent (Lisbon) during two periods in different seasons. Sci Total Environ 349:129–139

EIA (2019) U.S. Energy Information Administration. Country Analysis Executive Summary: Colombia

Elahee KB, Bhagwant S (2007) Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the west coast of Mauritius. Ecotoxicol Environ Saf 68:361–371

García-Medina S, Razo-Estrada C, Galar-Martinez M, Cortéz-Barberena E, Gómez-Oliván LM, Álvarez-González I, Madrigal-Bujaidar E (2011) Genotoxic and cytotoxic effects induced by aluminum in the lymphocytes of the common carp (*Cyprinus carpio*). Comp Biochem Physiol C Toxicol Pharmacol 153:113–118

González N, Simarro R, Molina MC, Bautista LF, Delgado L, Villa JA (2011) Effect of surfactants on PAH biodegradation by a bacterial consortium and on the dynamics of the bacterial community during the process. Bioresour Technol 102:9438–9446

Hansen W, Interwies E, Bär S, Kraemer R.A, Michalke P (2001) Effluent charging systems in the EU member states. Ed. In: Palinkas P(ed) Environment Series, ENVI 104 EN, 09-2001. European Parliament. 140 p

Hellou J, Ross N, Moon T (2012) Glutathione, glutathione S-transferase, and glutathione conjugates, complementary markers of oxidative stress in aquatic biota. Environ Sci Pollut Res 19:2007–2023

Ishikawa NM, Ranzani-Paiva MJT, Lombardi JV, Ferreira CM (2007) Hematological parameters in Nile Tilápia, *Oreochromis niloticus* exposed to sub-letal concentrations of mercury. Braz Arch Biol Technol 50:619–626

Jerônimo GT, Martins ML, Bachmann F, Greinert-Goulart JA, Schmitt-Júnior AA, Ghiraldelli L (2009) Hematological parameters of *Pimelodus maculatus* (Osteichthyes: Pimelodidae) from polluted and non-polluted sites in the Itajaí-Açu river, Santa Catarina State, Brazil. Acta Sci Biol Sci 31:179–283

Katsumiti A, Franca PP, Silva Costa GP, Zandona EM, Benincá C, Silva de Assis HC, Cestari M, Maschio J, Randi M, Silva CA, Roche H, Oliveira Ribeiro CA (2013) Evaluation five years after a Refinery oil spill in freshwater wetland - Paraná State, Southern of Brazil. Ecotoxicol Environ Contam 8:77–87

Kayode SJ, Shamusideen SA (2010) Haematological studies of *Oreochromis niloticus* exposed to diesel and drilling fluid in Lagos, Nigeria. Int J Biodivers Conserv 2:130–133

Kumar G, Nandan B (2014) Copper Toxicity: haematological and histopathological changes and prophylactic role of vitamin C in the fish, *Anabas testudineus* (Bloch, 1792). J Zool Stud 1:4–13

Kumari U, Mittal S, Mittal AK (2012) Surface ultrastructure of the gill filaments and the secondary lamellae of the catfish, *Rita rita*, and the carp, *Cirrhinus mrigala*. Microsc Res Tech 75:433–440

Lawal AT (2017) Polycyclic aromatic hydrocarbons. A review. Cogent Environ Sci 3(1)

Lima ALC, Farrington JW, Reddy CM (2005) Combustion-derived polycyclic aromatic hydrocarbons in the environment—a review. Environ Forensic 6(2):109–131

Liu XJ, Luo Z, Xiong BX, Liu X, Zhao YH, Hu GF, Lv GJ (2010) Effect of waterborne copper exposure on growth, hepatic enzymatic activities and histology in *Synechogobius hasta*. Ecotoxicol Environ Saf 73:1286–1291

Lukin A, Sharova J, Belicheva L, Camus L (2011) Assessment of fish health status in the Pechora River: effects of contamination. Ecotoxicol Environ Saf 74:355–365

Maceda-Veiga A, Monroy M, Viscor G, De Sostoa A (2010) Changes in non-specific biomarkers in the Mediterranean barbel (*Barbus meridionalis*) exposed to sewage effluents in a Mediterranean stream (Catalonia, NE Spain). Aquat Toxicol 100:229–237

Manzetti S (2013) Polycyclic aromatic hydrocarbons in the environment: environmental fate and transformation. Polycycl Aromat Compd 33(4):311–330

Mendelssohn IA, Andersen GL, Baltz D, Caffey R, Carman K, Fleeger J, Joye SB, Lin Q, Maltby E, Overton EB, Rozas L (2012) Oil impacts on coastal wetlands: implications for the Mississippi River Delta ecosystem after the Deepwater horizon oil spill. Bioscience 62:562–574

Miron DDS, Moraes B, Becker AG, Crestani M, Spanevello R, Loro VL, Baldisserotto B (2008) Ammonia and pH effects on some metabolic parameters and gill histology of silver catfish, *Rhamdia quelen* (Heptapteridae). Aquaculture 277:192–196

Moharram SG, Wahbi OM, El-Greisy ZA (2011) Effect of polluted water from the Egyptian Eastern Mediterranean coast on reproductive, toxicological and hematological characteristics of *Siganus rivulatus*. Pak J Biol Sci 14:668–681

Ossana NA, Salibián A (2013) Micronucleus test for monitoring the genotoxic potential of the surface water of Luján River (Argentina) using erythrocytes of *Lithobates catesbeianus* tadpoles. Ecotoxicol Environ Contam 8:67–74

Otero-Paternina AM, Cruz-Casallas PE, Velasco-Santamaría YM (2013) Evaluación del efecto del hidrocarburo fenantreno sobre el crecimiento de *Chlorella vulgaris* (Chlorellaceae). Acta Biol Colombiana 18(1):87–93

Peixoto F, Alves-Fernandes D, Santos D, Fontaínhas-Fernandes A (2006) Toxicological effects of oxyfluorfen on oxidative stress enzymes in tilapia *Oreochromis niloticus*. Pestic Biochem Physiol 85:91–96

Qiu Y-W, Zhang G, Liu G-Q, Guo L-L, Li X-D, Wai O (2009) Polycyclic aromatic hydrocarbons (PAHs) in the water column and sediment core of Deep Bay, South China. Estuar Coast Shelf Sci 83(1):60–66

Saadoun IMK (2015) Impact of oil spills on marine life. Emerging pollutants in the environment - current and further implications

Santos RN, Andrade CC, Santos LN, Santos AFGN, Araújo FG (2006) Testicular maturation of *Oligosarcus hepsetus* (Cuvier) (Actinopterygii, Characidae) in a Brazilian tropical reservoir. Braz J Biol 66:143–150

Schifter I, González-Macías C, Salazar-Coria L, Sánchez-Reyna G, González-Lozano C (2015) Long-term effects of discharges of produced water the marine environment from petroleum-related activities at Sonda de Campeche, Gulf of México. Environ Monit Assess 187(11):723

Serrano MF, Torrado LM, Pérez DD (2013) Impacto de los derrames de crudo en las propiedades mecánicas de suelos arenosos. Ciencia y Tecnología 11:233–244

Silva JPA, Muelbert AE, Oliveira EC, Fávaro LF (2010) Reproductive tactics used by the Lambari Astyanax aff. fasciatus in three water supply reservoirs in the same geographic region of the upper Iguaçu River. Neotrop Ichthyol 8:885–892

Soclo HH, Budzinski H, Garrigues P, Matsuzawa S (2008) Biota accumulation of polycyclic aromatic hydrocarbons in Benin coastal waters. Polycyclic Aromat Compd 28(2):112–127

Srogi K (2007) Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ Chem Lett 5(4):169–195

Ssempebwa JC, Carpenter DO (2009) The generation, use and disposal of waste crankcase oil in developing countries: a case for Kampala district, Uganda. J Hazard Mater 161(2–3):835–841

Talapatra SN, Ganguly P, Mukhopadhyay A, Banerjee SK (2007) Assessment of genetic biomarkers with special reference to micronucleated and binucleated erythrocytes in two fish species grown at industrial vicinity of thermal power plants, Kolkata, India. Asian J Water Environ Pollut 4:139–144

Tavares-Dias M, Ono EA, Pilarski F, Moraes FR (2007) Can thrombocytes participate in the removal of cellular debris in the blood circulation of teleost fish? A cytochemical study and ultrastructural analysis. J Appl Ichthyol 23:709–712

Tavares-Dias M, Moraes FR, Martins ML (2008) Hematological assessment in four Brazilian teleost fish with parasitic infections, collected in feefishing from Franca, São Paulo, Brazil. Boletín do Instituto de Pesca, São Paulo 34:189–196

Van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:57–149

Velasco-Santamaría YM, Calderón-Delgado IC, Corredor-Santamaría W (in press). Gonadal histopathology as biomarker of endocrine disruption in native fish (*Astyanax gr. bimaculatus* and *Aequidens metae*) from a potentially contaminated river in Colombia. Neotrop Icthyol

Velásquez AJA (2017) Contaminación de suelos y cuerpos de agua por hidrocarburos en Colombia. Fitorremediación como estrategia biotecnológica de recuperación. Revista de Investigación Agraria y Ambiental 8(1): 51–167

Vera-Parra NF, Caro LJM, Otero-Paternina AM, Cruz-Casallas PE, Velasco-Santamaría YM (2011) Impacto del agua asociada a la producción de una explotación petrolera sobre la comunidad fitoperifitica del río Acacias (Meta, Colombia) durante la temporada de lluvias. Orinoquia 15(1):31–40

Widdel F, Rabus R (2001) Anaerobic biodegradation of saturated and aromatic hydrocarbons. Curr Opin Biotechnol 12(3):259–276

World Bank Group (2016) The changing wealth of nations: measuring sustainable development in the new millennium

Yu Y, Liu Y, Wu L (2013) Sorption and degradation of pharmaceuticals and personal care products (PPcPs) in soils. Environ Sci Pollut Res Int 20:4261–4267

Zabbey N, Olsson G (2017) Conflicts - oil exploration and water. Global Chall 1(5):1600015

Zaghloul KH, Hasheesh WS, Zahran IA, Marie MA (2007) Ecological and biological studies on the Nile tilapia *Oreochromis niloticus* along different sites of lake Burullus. Egypt J Aquat Biol Fish 11:57–88

Chapter 14
Biomonitoring of Diffuse Contamination in the Subtropical Region of Brazil: Multibiomarker Assessment in Neotropical Freshwater Fishes

Nédia de Castilhos Ghisi and Elton Celton de Oliveira

14.1 Introduction

At the same time Brazil is appointed as an important hotspot of biodiversity, it is affected by environmental catastrophes, principally related to anthropogenic causes, for example, the dams collapse of Brumadinho in 2019 or Mariana in 2015, both in southeastern Brazil. Several other examples can be appointed, as the spill of about 8000 L of the organochlorine endosulfan in November 2008 in Rio de Janeiro State or the explosion of oil ship Vicunha in the coast of Paraná State in 2004, pouring millions of liters of oil and methanol into the sea. Most examples of disasters have two common factors: they are in aquatic environment and in southeastern or south Brazil. In addition to these great and concentrated sources of pollution, there are several daily and continuous nonpoint source pollutions. Certainly, it is not an exclusive problem of Brazil or of the Latin American countries. For this reason, it is necessary to continuously monitor pollution programs and its effects, especially in these areas where human activities are more prevalent. For monitoring programs bioindicator species are used, and the fishes are of great value in this function. In fish, several parameters can be evaluated, such as early-warning signals or biomarkers. Biomarkers are measurements in body fluids, cells, or tissues indicating molecular, biochemical, or other cellular modifications due to the presence and magnitude of xenobiotics (Van der Oost et al. 2003).

Since the 1960s, especially with the publications of the book *Silent Spring* of Rachel Carson (2002), mankind began to worry about the long-term effects of our civilization products (Van der Oost et al. 2003). Since that time, several tests were developed and applied. Now it is important to compile and to have a more integrative response of all obtained information, considering that the aquatic environment presents a mixture of contaminants.

N. d. C. Ghisi (✉) · E. C. de Oliveira
Federal University of Technology – Paraná, Campus Dois Vizinhos (UTFPR-DV), Dois Vizinhos, PR, Brazil

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_14

14.2 Bioindicators: Species of Ecological Interest

A bioindicator is defined as an organism giving information on the environmental conditions of its habitat by its presence or absence or by its behavior (Van der Oost et al. 2003). A bioindicator is defined as an organism that provides response about its environmental conditions through behavioral changes, in the population structure or in the form and intensity of interaction with other species (Van der Oost et al. 2003). A bioindicator can be an organism locally introduced for this purpose or a resident of the studied environment (wild) and selected by specific criteria to perform this function. Generally, a good bioindicator must present most or all of the following characteristics: relevant abundance, broad to moderate geographic distribution, high retention capacity of contaminants in its biomass, genetic stability in its populations, adaptability to the controlled environment (bioassay), well-resolved taxonomy, socioeconomic appeal, and an important associated biological knowledge, among others.

The bioindicators including algae, macrophyte, zooplankton, insect, bivalve mollusks, gastropod, fish, amphibian, and others are numbered and comparable in relation to their advantages and disadvantages in practical biomonitoring of aquatic pollution (Zhou et al. 2008). One of the strong points of fish as good biological models is that they are of immense value to humans. They have been a staple in the diet of many people and are important in the economy of many nations while at the same time presenting incalculable recreational and psychological value. Aspects of several species make themselves ideal to studies of behavior, ecology, evolution, genetics, physiology, and many others. Fish are used as general indicators or associated with other groups to pollution evaluation, principally because of direct benefit and importance to humans (Nelson 2006; CONCEA (Conselho Nacional de Controle de Experimentação Animal) 2017).

The fish of *Astyanax* genus are recommended by the Brazilian Council for Control of Animal Experimentation (CONCEA) and are popularly called "lambaris" or "tetras." The lambaris is the most diversified among the family Characidae, order Characiformes, and presents wide geographic distribution in South America, with hundred species in Neotropical rivers. Moreover, *Astyanax* are frequently used as bioindicators in monitoring studies and toxicological assays because of their abundance, resistance, omnivorous lifestyle, ease of capture, and size that makes them appropriate for laboratory conditions (Bueno-Krawczyk et al. 2015; de Lemos et al. 2008; Ramsdorf et al. 2012; Trujillo-Jiménez et al. 2011). The genus plays an important ecologic role as forage species and is classified as pelagic and active swimmers. The diet of this fish is composed of a wide variety of items, being constituted principally of plants (in some species reaching 91.8%) and small insects (Santos 1981; Hahn et al. 1997). Several researches corroborate that *Astyanax* is a good bioindicator for assessment of the river contamination status, be in wild or lab conditions (Ramsdorf et al. 2012; Ribeiro et al. 2014; Silva et al. 2014), and several ecotoxicology studies were developed with species of the genus, for example, of

A. jacuhiensis (Steffens et al. 2015), *A. bifasciatus* (Bueno-Krawczyk et al. 2015), *A. fasciatus* (Paulino et al. 2014), and *A. aeneus* (Trujillo-Jiménez et al. 2011).

It is also important to investigate the effects of pollution in different trophic levels. In this sense, fish of genera *Hypostomus* (a catfish known as pleco) can be considered good bioindicators of environmental quality. They exhibit nonmigratory benthic behavior, with detritivores feed habits (Bonato et al. 2008). The *Hypostomus* species present nest/brood guard comportment (Sofia et al. 2008) and nocturnal activity (Casatti 2002). They are native from tropical and subtropical South American rivers, with the maximum diversity in number of species occurring in the Paraná-Paraguay system rivers (Weber and Hypostominae 2003). *Hypostomus* are bottom-dwelling loricariid fishes (Siluriformes), occurring in a variety of freshwater ecosystems such as small mountain streams and large lowland river areas (Oyakawa et al. 2005). These species have economic potential and are fished intensely in the region of occurrence. They are highly appreciated for human consumption, because of the absence of Y-shaped spines between the fleshes. These characteristics and its considerable occurrence in urban streams (De Oliveira and Bennemann 2005) make it a good bioindicator species of environmental quality. For this reason, species of genus *Hypostomus* were used in diverse ecotoxicology researches, for example, *H. ancistroides* (Viana et al. 2018; Ghisi et al. 2016), *H. plecostomus* (Normann et al. 2008), *H. punctatus* (Bastos et al. 1992), and others.

14.3 Biomarkers

The biomarkers have an important role in presenting an early warning signal before the species or communities suffer irreversible consequences. Thus, the term biomarker is defined as any biological response caused by environmental disturbance at the subindividual level, being measured within the organisms or of their subproducts (urine, feces, glandular by-products, etc.) (Van der Oost et al. 2003). These responses reflect the health of organisms and can be progressively demonstrated from the molecular and subcellular level, followed by the cellular and tissue level, until reaching the individual level.

For example, isolated dysfunctions from the antioxidant (enzymatic) system of organisms give signal about acute and/or recent contamination. However, associated with cellular abnormalities and tissue pathologies, they present clear signs of chronic pollution, usually related to diffuse contamination. Therefore, when an organism is exposed to a contaminant, typically at the beginning, there is a process of "defense" at the molecular and subcellular level to eliminate the foreign agent or the xenobiotic, affecting the expression of genes and the enzymatic pathways. This response process can be detected and quantified by molecular and biochemical analyses. This first line of response depends on the interaction of the foreign molecule with the cellular environment and usually occurs within minutes or hours after contamination.

In cases where the occurrence of cellular abnormalities and tissue pathologies is already observed, the response is considered slightly later, on a timescale that varies from hours to days. However, these biological dysfunctions present greater relevance and impact on the health of the organism and may have repercussions on its physiology and behavior (Oliveira Ribeiro et al. 2005). Cellular abnormalities can be detected virtually in all systems, from hematopoietic and hepatic to gastrointestinal, with the neuronal changes in enteric system, for example (Pustiglione et al. 2018). Tissue pathologies, in turn, are characterized by the cellular alterations observed in a specific organ, being commonly evaluated the respiratory, hepatic, renal, and tegument tissue. These pathologies can be detected, classified, and quantified according to their importance factor and degree of extension. To evaluate these cellular and tissue biomarkers, the researcher must have a thorough knowledge in the areas of cell biology, histology, and physiology.

Somatic changes in growth rate, nutritional status, or reproductive process of individuals are changes in higher level of biological organization reflecting a later effect, which are related to normal environmental variations and anthropic activities (e.g., pollution). We can consider that this alteration is acting on a timescale ranging from weeks to months (Franzle 2006). In these cases, changes are measured using indexes such as the hepatosomatic and gonadosomatic and also using predictive models, based on biometric data, such as the condition factor. These changes present greater ecological relevance due to the more direct impacts observed in the life cycle of the species, through hatch rate, recruitment, survival, etc. However, these responses are a product of multiple interferences (natural and anthropic), and this fact diminishes the resolution of a study that only involves these metrics.

In this sense, each day, more and more studies have demonstrated the utility of biomarker techniques in the evaluation of pollutants ranging from single compounds to complex mixtures in both the laboratory and the field (Hoffman et al. 2003). In wild environments, once the organisms are exposed to multiple stresses (natural and anthropogenic) over time, the simultaneous evaluation of several biomarkers can better reflect the environmental status. Thus, to facilitate understanding and interpretation of the case studies presented in the next section of this chapter, a brief description of the main biomarkers used in these studies will be presented:

14.3.1 Acetylcholinesterase (AChE)

It is an enzyme used to measure neurotoxic effects in fish and other organisms. AChE has affinity for the neurotransmitter acetylcholine at the nerve endings of the central nervous system and skeletal muscles, hydrolyzing it and allowing the neuron to return to its resting state upon activation. Problems in the functioning of this enzyme indicate abnormal functioning of the sensory and neuromuscular systems, compromising basic functions such as mobility, foraging, and the perception of

predators. To measure the effect of contaminants on AChE, it is necessary to collect a fragment of the brain and/or muscle from the animals and immediately to conserve them in ultrafreezer −86 °C for later biochemical processing of the samples. The determination of AChE activity is better described in the protocol of Ellman et al. (1961).

14.3.2 Micronucleus Test and Erythrocytic Nuclear Abnormalities (ENA)

Micronucleus is a small nuclear fragment separated from the main nucleus formed during the cell division from chromosomal breaks and/or disturbances in mitotic machinery (Hatch et al. 2013). Erythrocytic nuclear abnormalities (ENA) are, in turn, a set of deformities in the structure and shape of the cell's main nucleus. These deformities can be promoted by the disorder of the nuclear lamina, which is formed by a network of intermediate filaments which maintains the stability of the nucleus (Alberts 2002). In fish, micronucleus and ENA are usually evaluated by the simple collection of blood samples from the animals, because the erythrocytes are nucleated cells. In the blood smear, the altered erythrocyte nuclei are evaluated. The technique employed to evaluate these biomarkers in our case studies is better described in Heddle (1973), Schmid (1975) and modified by Ferraro et al. (2004).

14.3.3 Comet Assay

The single-cell gel (SCG) or comet assay is considered as a very useful approach for assessing DNA damage. This technique can detect the DNA damage at the level of the single cell and can be applied to evaluate the blood, liver, kidney, and any other nucleated cell. In the original technique, cells are embedded in agarose onto microscope slide, submitted to detergents to lyse the membranes and high salt, and the liberated DNA electrophoresed under alkaline conditions (pH > 13). Cells with an increased rate of DNA double-strand breaks displayed increased migration of DNA in the direction of the anode. The DNA is stained with ethidium bromide, and the migration of DNA can be evaluated under fluorescence microscope (Tice et al. 2000). As the electrophoresis will separate fragments per size, little DNA fragments will migrate faster, producing the "comet tail." The principle of assay is that cell with more fragmented DNA will produce longer tails. The methodology used in our case studies followed the protocol described by Ferraro et al. (2004).

14.3.4 Histopathology

Histopathology is the study of histological changes found in target tissues of bioindicators and detected by means of histological protocols. Moreover (Bernet et al. 1999), histopathology is also a great biomarker because it lies in an intermediate level of biological organization, i.e., neither so early nor so late in response. In fish, the most commonly used tissues for histopathological evaluation are the gills, liver, kidney, and gonads because of their continuous exposure and/or high sensitivity to contaminants. All of these organs possess vital functions for the bodies, regarding breathing, metabolism, osmoregulation, excretion, and reproduction. The impairment of these functions can lead to a physiological stress of the organism and death. The histological evaluation protocol used in our studies followed the recommendations of Favaro and Oliveira (2012), with modifications. The qualitative and quantitative analysis was performed according to Bernet et al. (1999).

14.3.5 Condition Factor

Condition factor is an estimate that evaluates the physiological state of the fish, assuming that individuals with a larger mass in a specified length are in better condition (Lima-junior et al. 2002). This index reflects the nutritional status of the individuals, being possible to relate it to the natural and anthropic environmental variations. The estimation of the condition factor can be done through Fulton's K condition factor or the allometric condition factor (K). Fulton's K condition factor assumes that the weight-length relationship is always isometric ($b = 3$), while the allometric condition factor considers that variations in the weight-length relationship may occur within a population (da Rocha et al. 2005). The latter is widely used in ecology and toxicology, and it is necessary to a priori establish the animal's weight-to-length ratio to determine the coefficient of the model (b). Subsequently, the slope coefficient is used to estimate the condition factor. This relationship has demonstrated a strong relationship with eutrophic environments, as we will be discussed later.

14.4 Integrative Responses in Multibiomarker Assessment

Nowadays, the aquatic environments, as the ultimate sink for many of the anthropogenic contaminants, are exposed to a great mixture of pollutants that can affect the resident biodiversity of different ways. Thus, the evaluation of the responses to pollutant mixtures needs a set of complementary biomarkers (Flammarion et al. 2002). For this reason, the investigated endpoints must be selected in order to represent several biological processes and responses, and so, several studies are bringing a

multibiomarker approach (Freire et al. 2015; Linde-Arias et al. 2008; Fasulo et al. 2010; Rodrigues et al. 2018). Facing with a great number of possible tests in biomonitoring program, in different levels of response and endpoints, the interpretation of the results becomes complex, making it difficult to have a final conclusion about the environmental situation. Currently, studies have focused on a harder statistical processing of the data, to obtain an integrative response through compilation of the results of the several evaluated biomarkers and bioindicators, as many as possible. Here we will display two examples of researches using this integrative multibiomarker approach in Brazil.

14.4.1 First Case Study (Ghisi et al. 2017)

The first example was a field study evaluating the fish *Astyanax* aff. *paranae*. Field studies comparing impacted and unimpacted environments are advantageous because they allow an assessment of the health conditions of fishes in their own environment. Figure 14.1 shows the sample design of this research. The animals were sampled in three sites, in different environmental conditions of anthropogenic impact:

1. Rebio: The control site (less impacted) in the Biological Reserve of Perobas with 8176 ha of protected area – a Biological Reserve is a Brazilian class of

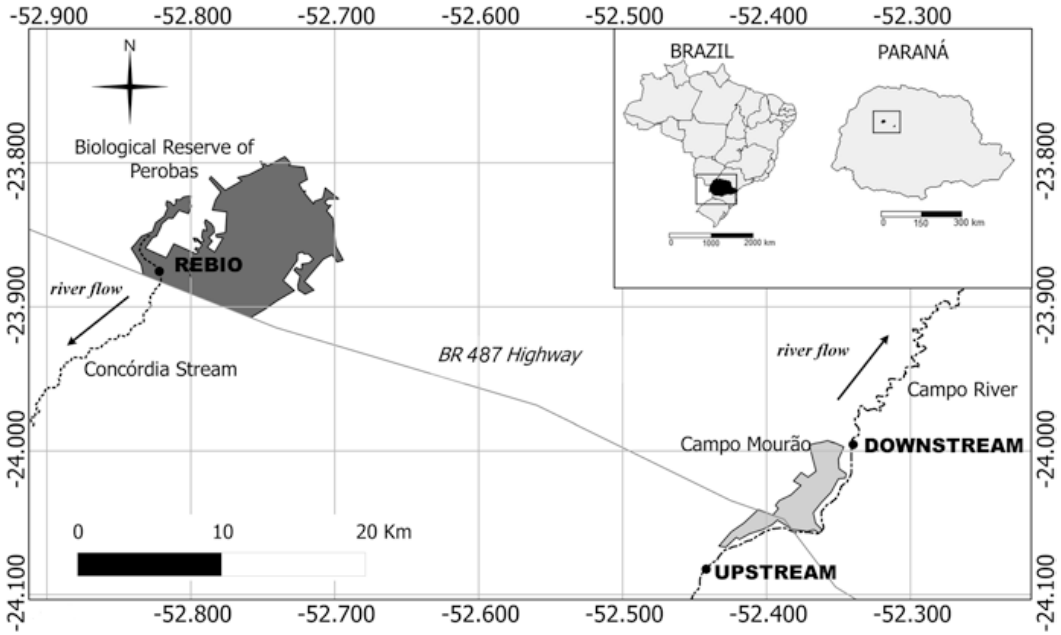

Fig. 14.1 Map displaying sampled sites in the subtropical region of Brazil in Paraná State. (1) REBIO: Concórdia stream in the Biological Reserve of Perobas, Tuneiras do Oeste city. (2) Upstream: site upstream of the Campo Mourão city in the Campo River. (3) Downstream: site downstream of the Campo Mourão city in the Campo River. (Source: Ghisi et al. 2017)

conservation unit created to preserve the biota and other natural characteristics within its boundaries. It is maintained theoretically without human interference, with the exception of the actions required to management and ecosystem recovery (Brasil 2000).
2. Upstream: It is an agricultural site, upstream of an urban zone located in one of the most productive regions of Brazil.
3. Downstream: It is a site downstream from this urban zone. This last site was characterized by the influx of a mixture of effluents, highlighting wastewater from industry, from a sewer treatment plant, and receiving the diffuse influx from agricultural areas. The sites were in Paraná State, in the Campo Mourão city region, and the sites 2 and 3 are in the Campo River.

Campo Mourão is a 90,000 people city and with an economy basically agricultural, where the bigger farming cooperative of South America is located. The Paraná State is notable for its grain productivity and, for years, is considered the country's second largest grain producer, remarkably soy and corn. This high productivity is attributed to technology and external energy input, with a great amount of pesticides applied to avoid losses due plagues. The pesticides were ever a great concern to ecotoxicologists, especially in developing countries as Brazil, that is, appointed as global leader in pesticide use (Rigotto et al. 2014). The Brazilian agrochemical market expanded rapidly in the last decade (190%), at a growth rate that is more than double that of the global market (93%). According to the Department of Agriculture and Supply of Paraná (SEAB/PR), the area of this study recorded the purchase of 10,923 tons during 2011 – the year of the research (Ghisi et al. 2017).

In the same way, the industrial and urban effluents are a great concern too. Previous studies found that this kind of effluents have negative effects on aquatic organisms at the genetic, biochemical, and histological levels. In Brazil, the National Council for the Environment (CONAMA), through the Resolution No. 357/2005, establishes the conditions and criteria in which effluents should be discharged and requires that all effluents dumped into water bodies must not cause toxic effects on aquatic organisms (Brasil 2005).

So, in this scenario, which is probably common in several countries around the world, this first study collected *A.* aff. *paranae* in the three sampled sites aforementioned (Rebio, Upstream, and Downstream), during winter and summer. Normally, in biomonitoring programs, it is recommended to take information in a minimum of two different periods, to capture the seasonal variation. In fish, the following biomarkers were analyzed: comet assay and histopathology with liver tissue; biochemical measures of catalase (CAT), glutathione S-transferase (GST), and the lipid peroxidation levels (LPO); and measurement of acetylcholinesterase activity (AChE) in muscle and brain samples. All the biomarkers were individually analyzed and interpreted, and as expected, we can see that some parameters were more affected than others, providing different responses. More details of these individual analysis can be found in Ghisi et al. (2017).

In order to obtain an integrative response of the parameter, a discriminant analysis was performed to classify the fish's responses in the three sampled sites and to evaluate the percentage of classification in the respective sites. These analyses were performed in IBM SPSS software – version 20. The discriminant analysis selected two variables with discrimination power of the three study sites: the comet assay with liver and the histopathology of liver, evaluated in winter and summer, which presented statistical significance ($p < 0.001$). Two discriminant functions were selected, representing 100% of the explained variance. Only the first discriminant function represents 99.7% of the total variance, being significant ($p < 0.001$), with a high Chi-square value. The comet assay was a central biomarker to ordination in function 1 (Table 14.1). In this sense, considering the importance of first function, we can say that comet assay was a central biomarker in this study. The histopathology in liver was the most important biomarker to function 2. The other variables (AChE in the brain and muscle) were not selected in the analysis, because they have low weights in the two discriminating functions, and are better represented in the first function.

The ordering of the evaluated individuals displays that there are differences among the evaluated areas, despite the occurrence of overlaps. A general overview is shown in Fig. 14.2. We can note a gradient in the data distribution, especially in function 1, where site 1 (Rebio) is on the left, site 2 (upstream) is in the middle, and site 3 (downstream) in the other extremity. Sites upstream and downstream are closer to each other than to site 1.

This discriminant analysis provides also a relation between the response of the individual and the site in what it was sampled. So, 67.5% of the individual were correctly classified in their original sites, but a few of them resemble more with other areas (Table 14.2). This analysis still has the power to identify which individuals were classified outside the original group.

We can see that 75% of individuals from Rebio were correctly classified, 20.8% were classified as Upstream, and only one individual was classified in downstream group. On the other hand, the individuals from upstream and downstream presented more similarity in responses. Of the 23 individuals from Upstream, six were classified in the downstream group. And of the 30 individuals from downstream, 30% were classified in the upstream group. Only two individuals from upstream and two from downstream were classified in Rebio.

Table 14.1 Pooled within-group correlations between discriminating variables and standardized canonical discriminant functions – variables ordered by absolute size of correlation within function (Ghisi et al. 2017)

	Function	
Structure matrix	1	2
Comet assay	**0.824**[a]	−0.567
AChE (brain)[b]	**−0.283**[a]	0.232
AChE (muscle)[b]	**−0.174**[a]	−0.094
Histopathology of the liver	0.436	**0.900**[a]

[a]Largest absolute correlation between each variable and any discriminant function
[b]This variable is not used in the analysis

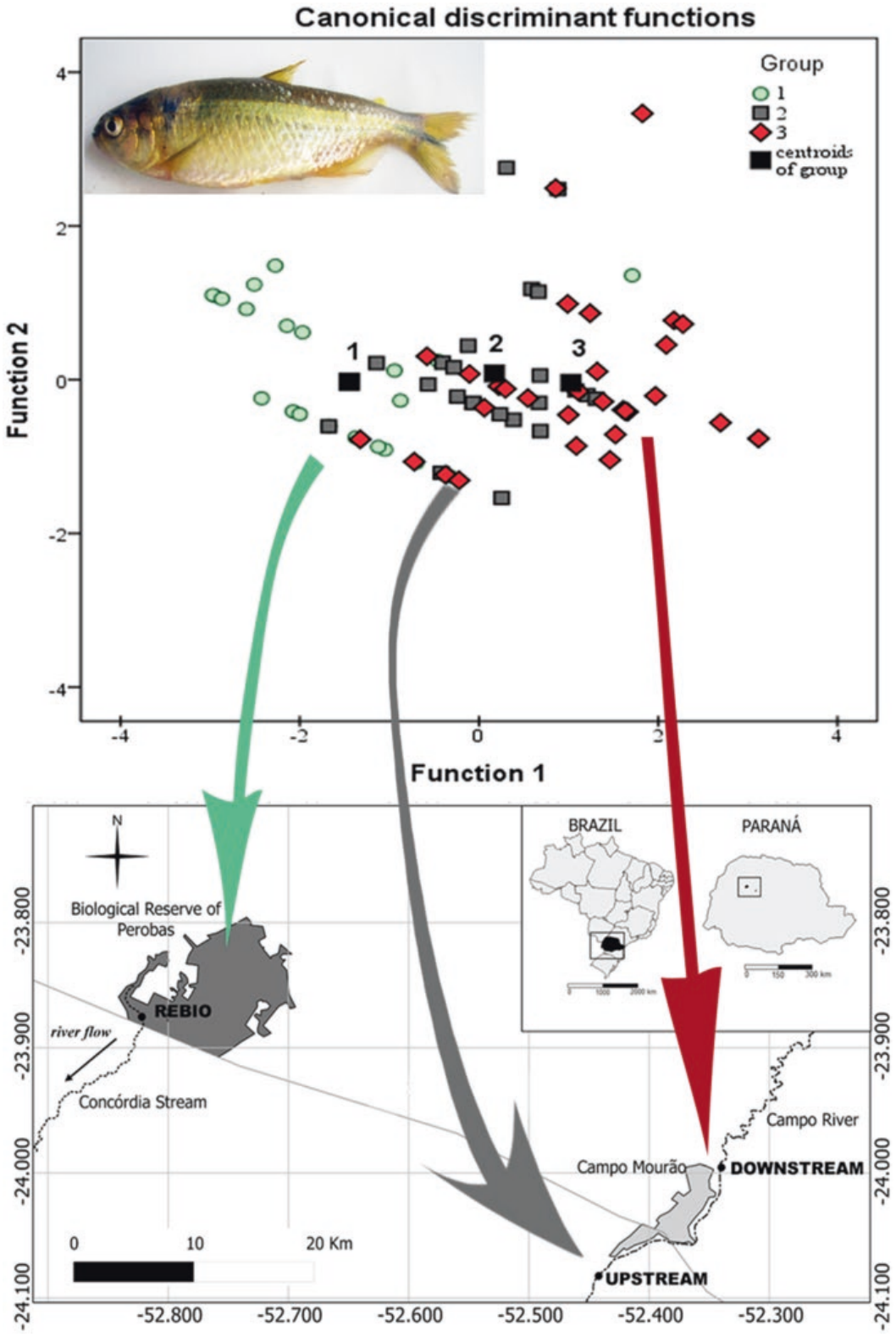

Fig. 14.2 Graph of canonical discriminant functions showing the ordination of the data relative to sampled sites, where 1 Rebio, 2 Upstream, 3 Downstream. Note: the photography of *Astyanax* aff. *paranae* is merely illustrative and out of scale. (Source: Modified from Ghisi et al. 2017)

Table 14.2 Percentage of correct classification of the individuals in the original groups, relative to sites

	Predicted group membership n° (percentage)			
Original site	Rebio	Upstream	Downstream	Total of animals
Rebio	18 (75%)	5 (20.8%)	1 (4.2%)	24
Upstream	2 (8.7%)	15 (65.2%)	6 (26.1%)	23
Downstream	2 (6.7%)	9 (30.0%)	19 (63.3%)	30

[a]67% of the individual were correctly classified in the original locals

We can consider acceptable that some fish were classified as being from another place, especially the overlapping between downstream and upstream sites. It can be justified by the influx of different substance mixtures in these environments and by the different susceptibilities of everyone. The upstream site suffers influence of agricultural pollution, while downstream is affected by urban and agricultural input too. This overlapping can be tolerable, because this is a field study, where several factors can affect the health status of animals, besides the mixture of contaminants.

Moreover, the data were analyzed seasonally by site. In this sense, there was more overlap among individuals of winter than that of summer (Table 14.3). To Rebio individuals, the discriminant analysis also selected the comet assay and histology of liver for the classification of individuals into seasons. There were 85.7% of correct classifications. Of the 14 individuals sampled in the Rebio during winter, 13 were correctly classified, and 1 resembled the individuals sampled in the summer. On the other hand, of the ten individuals sampled in the summer in Rebio, eight were correctly classified and two resembled those of winter.

To upstream site data, no biomarker was selected, and for this reason, it was not possible to elaborate a discriminant function. In other words, there was no differentiation between the individuals (fish) analyzed in winter and summer, as well as their biomarker responses.

Finally, for downstream data, the discriminant analysis selected two variables, namely, comet assay and AChE of the brain. All the individuals of winter were correctly classified in downstream, and only one of summer was classified in the wrong group.

In summary of the first study, we can see that applying a multivariate approach by the discriminant analysis, it is possible to obtain a general overview in comparison to impacted and non-impacted environment. It is possible also to list the more important biomarker or tissue to discriminate different sites and, yet, to perform individual analysis by site and season, isolating spurious factors.

Table 14.3 Percentage of correct classification of the individuals in the original groups, relative to sites per season

		Predicted group membership n° (percentage)		
Original season		Winter	Summer	Total
REBIO[a]	Winter	13 (92.9%)	1 (7.1%)	14
	Summer	2 (20%)	8 (80%)	10
Upstream[b]	–	–	–	–
Downstream[c]	Winter	15 (100%)	0 (0%)	15
	Summer	1 (6.7%)	14 (93.3%)	15

[a]87.5% of the cases correctly classified in the original group (season)
[b]No variable selected, making it impossible to elaborate discriminant functions
[c]96.7% of the cases correctly classified in the original group (season)

14.4.2 Second Study Case (Ghisi et al. 2016)

The second study was developed also in Paraná State, but in a region with greater urban concentration. The fish were collected in Pirapó River, occupying a total area of 5098.10 km^2 and inhabited for a population of around 500,000 people. Besides this, a great part of the basin is occupied by intensive agriculture. For this study, the sample design was conducted with the collection of *Hypostomus ancistroides* in three sites in the same river, from headspring until downstream of the bigger city of the region. It is expected that the headspring is the most preserved locale in a river, with less anthropic influence (Fig. 14.3).

The sites were called Upstream (in the headspring), Middle, and Downstream. In the same way as the first study, the fish were sampled during winter and summer. In this case, measures of length and weight were measured to obtain the inference of condition factor. The tissue was collected to perform the following biomarkers: micronucleus test and other erythrocyte nuclear abnormalities (ENAs), comet assay with blood, AChE in the muscle and brain, and histopathologic evaluation of the gill and liver.

Each biomarker individually was statistically tested, and a multivariate analysis of variance (MANOVA) was performed to all data. The MANOVA is indicated in cases in which there is more than one dependent variable (correlated), and it is advantageous to compare the averages of groups for several variables at the same time. Significant effects of MANOVA can be further explored with a one-way analysis of variance.

In this sense, the MANOVA displayed that the factor's site, season, and interaction were significant ($p = 0.00001**$) (Ghisi et al. 2016). Thus, the data were then tested and interpreted by a two-way analysis, considering the influence of the two factors on the animals present in the environments in the different seasons. More details of individual analysis can be found in (Ghisi et al. 2016).

As in the first study, a discriminant analysis was conducted. As a result, enough dependent variables to elaborate a discriminant function were selected (Table 14.4).

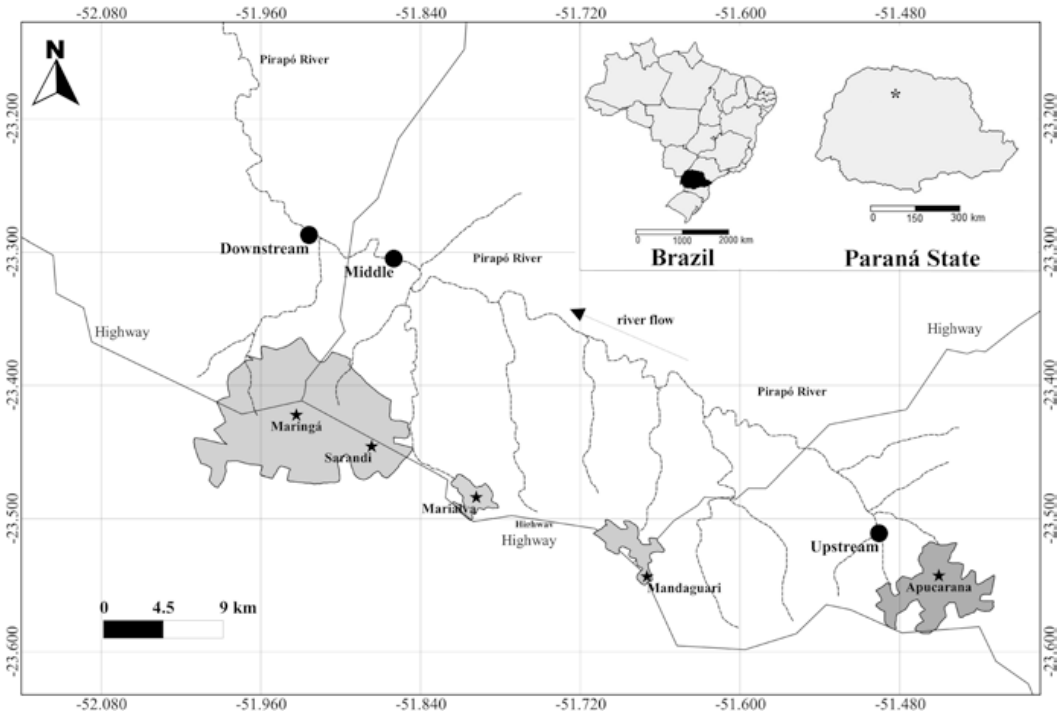

Fig. 14.3 Sample design in subtropical region of Brazil. The collection was performed in Pirapó River Basin, Paraná State. Black dots are the sampled sites. (1) Upstream: headspring of the river upstream of the Maringá city; (2) Middle; (3) Downstream: downstream of the Maringá city. Note: Stars appoint city centers. Shaded areas are the urbanized regions. (Source: Ghisi et al. 2016)

Table 14.4 Pooled within-group correlations between discriminating variables and standardized canonical discriminant functions – variables ordered by absolute size of correlation within function

	Function	
Structure matrix	1	2
Condition factor (k)	**−0.642**[a]	0.060
AChE (brain)	**−0.033**[a]	−0.027
Histopathology (gill)	0.049	**−0.703**[a]
ENA	0.111	**0.471**[a]
Comet assay	0.183	**0.445**[a]
AChE (muscle)	0.018	**−0.239**[a]
Histopathology (liver)[b]	−0.061	**0.087**[a]

[a]Largest absolute correlation between each variable and any discriminant function
[b]This variable is not used in the analysis

We can see the most important biomarkers to discriminate the three sites. The condition factor and the AChE in the brain had the largest absolute correlation with function 1. Function 1 explains 89.6% of variance. Analyzing this information with Fig. 14.4, we can interpret that theses biomarkers were responsible to horizontal segregation of the data in graph. In graph, the upstream site is more distant from the other two sites. Sites middle and downstream presented more similar responses.

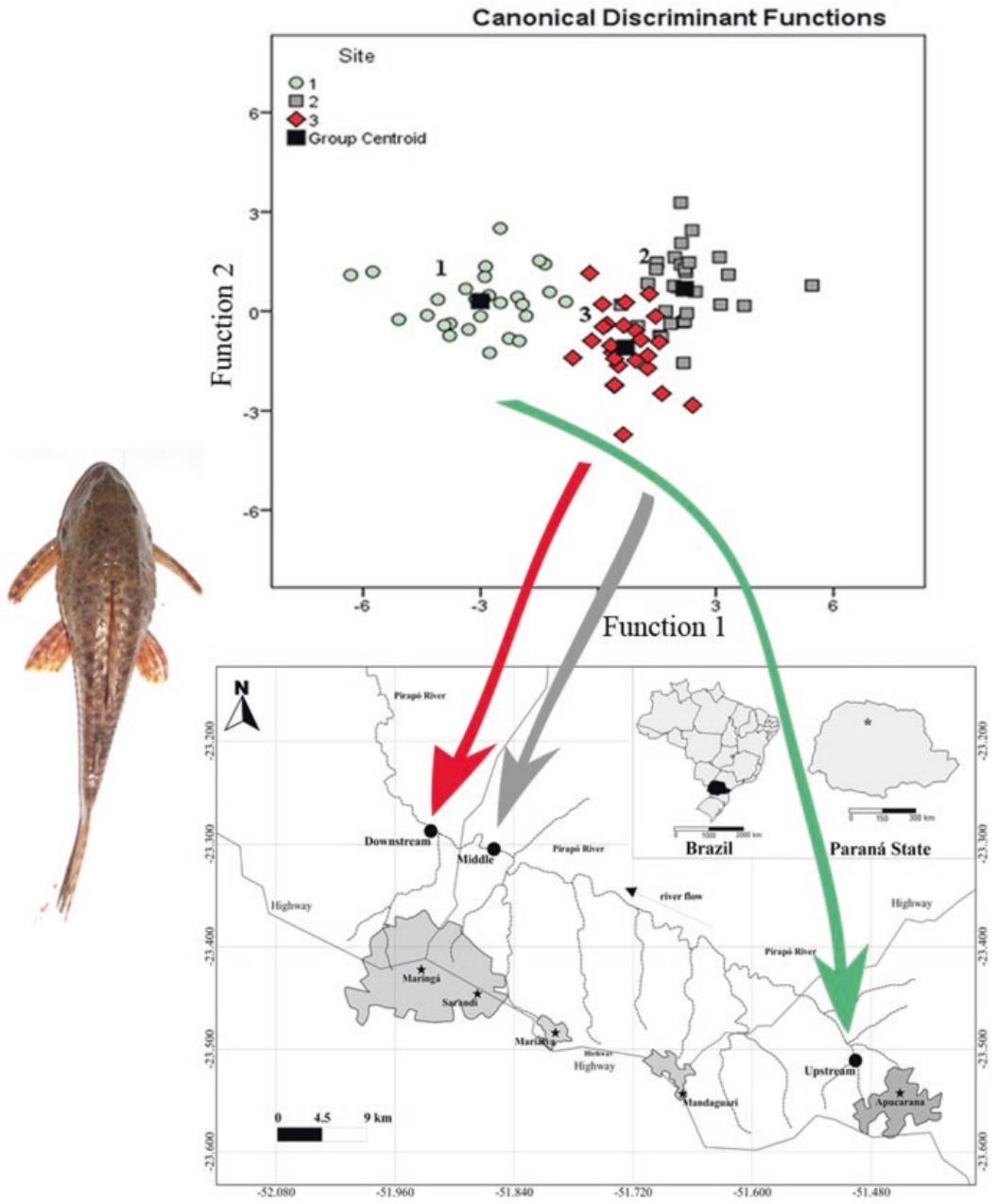

Fig. 14.4 Ordination of data from three sampled sites. Sites 1 (Upstream), 2 (Middle), and 3 (Downstream). Note: The photography of *Hypostomus ancistroides* is merely illustrative and out of scale

This can be attributed to geographical proximity of points (you can return to the map to analyze this) and, consequently, more similar environmental conditions they are subjected to, including the pollution sources.

On the other hand, to constitute function 2, there are several biomarkers important to discriminate the sites. The principal parameters were the histopathology of

Table 14.5 Results of correct classification in the original group[a]

		Predicted group membership			
		Upstream	Middle	Downstream	Total
Original site	Upstream	27 (96.4%)	0 (0%)	1 (3.6%)	28
	Middle	0 (0%)	25 (83.3%)	5 (16.7%)	30
	Downstream	0 (0%)	3 (11.5%)	23 (88.5%)	26

[a] 89.3% of original grouped cases correctly classified

the gill, ENA, and comet assay. In the vertical axes, we can perceive that sites 1 and 2 were closer to zero and site 3 was furthermost in the scale.

In relation to the classification of the responses in the original group, 89.3% of the cases were classified in the original group (Table 14.5). In Upstream, only one case was classified as Middle. Five cases of Middle were classified in Downstream, and three cases of Downstream had more similar response to Middle.

In relation to season, it was possible to obtain only one function, making it impossible to plot a graph. In this function, the variables AChE in the brain and muscle were the fundamental biomarker discriminants. Analyzing the individual results of these biomarkers, we can perceive that this parameter was intensely affected by the season (Fig. 14.5).

In summary of the second study, we can see that it is possible to make a better exploration of the total dataset and obtain an integrative response, with an overview of the responses of all parameter in the sites and the influence of season over specific biomarkers. It was possible to obtain the most important biomarkers to discriminate the sites and the biomarkers more affected by season influence.

14.5 Conclusions

In conclusion, we can see that the refinement and the best elaboration of the information can contribute to improve the understanding of the data collected in biomonitoring programs. It is a fact that not in all situations (and neither with all datasets) will it be possible to apply this same statistic treatment. But, in the cases when it is possible, the multibiomarker approach analyzed by an integrative methodology is a valuable tool to make a better use of the available information.

In this chapter, we presented the situation of two studies in cities of different population size and with different bioindicators. In both case studies, the multibiomarker assessment showed the ordination of less polluted environments differentiated from more polluted sites. Moreover, this approach can appoint the more important biomarkers in each biomonitoring that are not necessarily the same biomarker of another study in different conditions.

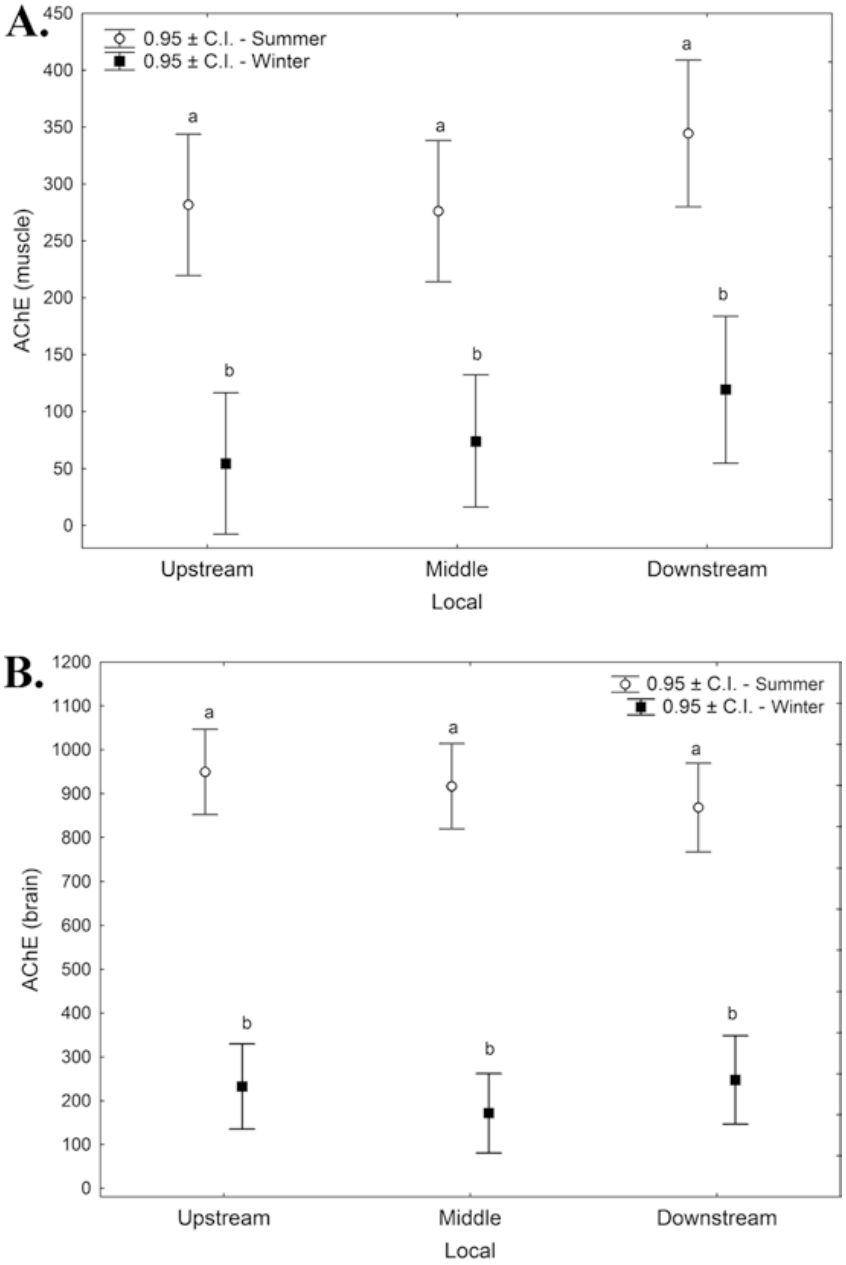

Fig. 14.5 Results obtained from *H. ancistroides* from the three Pirapó River collection sites in the summer and winter as regards (**A**) acetylcholinesterase of the muscle and (**B**) acetylcholinesterase of the brain. Note: Different letters (a, b) represent significant difference in the Fisher LSD post-hoc test ($p < 0.05$). C.I. confidence interval. (Source: Ghisi et al. 2016)

References

Alberts B (2002) Citoesqueleto. In: Biologia molecular da célula. Artmed, Porto Alegre, p 1645

Bastos JC, Bastos VLFC, Rossin A, Fortin H, Faria MVC (1992) Activation of parathion by liver of *Hypostomus punctatus*, a Brazilian benthic fish (Cascudo). Comp Biochem Physiol C Comp Pharmacol 102(3):561–566

Bernet D, Schmidt H, Meier W, Wahli T (1999) Histopathology in fish: proposal for a protocol to assess aquatic pollution. J Fish Dis 22:25–34

Bonato KO, de Araújo MI, Delariva RL (2008) Dieta e morfologia trófica de Hypostomus ancistroides. In: IV Mostra Interna de Trabalhos de Iniciação Cientìfica do Cesumar. Cesumar, Maringá, pp 6–10

Brasil (2000) Sistema Nacional de Unidades de Conservação SNUC, Presidência da República do Brasil. Lei n°. 9985, de 18 de junho de 2000

Brasil (2005) Resolução do Conselho Nacional do Meio Ambiente n° 357, de 17 de março de 2005 [Internet], pp 1–23. Available from: http://www.mma.gov.br/port/conama/res/res05/res35705.pdf

Bueno-Krawczyk AC, Guiloski IC, Piancini LDS, Azevedo JC, Ramsdorf WA, Ide AH et al (2015) Multibiomarker in fish to evaluate a river used to water public supply. Chemosphere [Internet] Elsevier Ltd 135:257–264. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0045653515004038

Carson R (2002) Silent spring, Anniversary edn. Houghton Mifflin Company, New York; 1962. 378 p

Casatti L (2002) Alimentação dos peixes em um riacho do Parque Estadual Morro do Diabo, bacia do Alto Rio Paraná, sudeste do Brasil. Biota Neotrop [Internet] 2(2):1–14. Available from: http://www.biotaneotropica.org.br/v2n2/pt/abstract?article+BN02502022002

CONCEA (Conselho Nacional de Controle de Experimentação Animal) (2017) RN 34. Peixes mantidos em instalações de instituições de ensino ou pesquisa científica para fins de estudo biológico ou biomédico I – Lambari (Astyanax), Tilápia (Tilapia, Sarotherodon e Oreochromis) e Zebrafish (Danio rerio), pp 1–57

da Rocha MA, Ribeiro EL d A, Mizubuti IY, da Silva L d DF, Borosky JC, Rubin KCP (2005) Use of the alometric and the fulton condition factors to compare the carp (Cyprinus carpio) considering sexes and ages. Semin Ciências Agrárias 26(3):429–434

de Lemos CT, Iranço FDA, Oliveira NCD, de Souza GD, Fachel JMG (2008) Biomonitoring of genotoxicity using micronuclei assay in native population of Astyanax jacuhiensis (Characiformes: Characidae) at sites under petrochemical influence. Sci Total Environ 406:3–9

De Oliveira DC, Bennemann ST (2005) Ictiofauna, recursos alimentares e relações com as interferências antrópicas em um riacho urbano no sul do Brasil. Biota Neotrop 5(1):95–107

Ellman GL, Courtney KD, Andres V Jr, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase. Biochem Pharmacol 7:88–95

Fasulo S, Marino S, Mauceri A, Maisano M, Giannetto A, Agata AD et al (2010) A multibiomarker approach in Coris julis living in a natural environment. Ecotoxicol Environ Saf [Internet]. Elsevier 73(7):1565–1573. https://doi.org/10.1016/j.ecoenv.2010.01.008

Favaro LF, Oliveira EC (2012) Ecologia Reprodutiva em Peixes. In: Oliveira-Ribeiro CA, Reis Filho HS, Grotzner SR (eds) Técnicas e métodos para utilização prática de microcopia, 1st edn. Editora Santos, São Paulo, pp 367–389

Ferraro MVM, Fenocchio AS, Mantovani MS, Oliveira Ribeiro CA, Cestari MM (2004) Mutagenic effects of tributyltin and inorganic lead (Pb II) on the fish H. malabaricus as evaluated using the comet assay and the piscine micronucleus and chromosome aberration tests. Genet Mol Biol 27(1):103–107

Flammarion P, Devaux A, Nehls S, Migeon B, Noury P, Garric J (2002) Multibiomarker responses in fish from the Moselle River (France). Ecotoxicol Environ Saf 51:145–153

Franzle O (2006) Complex bioindication and environmental stress assessment. Ecol Indic 6:114–136

Freire CA, Souza-Bastos LR, Chiesse J, Tincani FH, Piancini LDS, Randi MAF et al (2015) A multibiomarker evaluation of urban, industrial, and agricultural exposure of small characins in a large freshwater basin in southern Brazil. Environ Sci Pollut Res 22(17):13263–13277

Ghisi NC, Oliveira EC, Mendonca Mota TF, Vanzetto GV, Roque AA, Godinho JP et al (2016) Integrated biomarker response in catfish Hypostomus ancistroides by multivariate analysis in the Pirapo River, southern Brazil. Chemosphere 161:69–79

Ghisi NC, Oliveira EC, Guiloski IC, Barbosa S, Lima D, Silva HC et al (2017) Multivariate and integrative approach to analyze multiple biomarkers in ecotoxicology: a field study in Neotropical region. Sci Total Environ [Internet]. Elsevier B.V. 609:1208–1218. https://doi.org/10.1016/j.scitotenv.2017.07.266

Hahn NS, Fugi R, Almeida VL, Russo M, Loureiro VE (1997) Dieta e Atividade Alimentar de Peixes do Reservatório de Segredo. In: Agostinho AA, Gomes LC (eds) Reservatório Segredo: Bases ecológicas para o Manejo. EDUEM, Maringá, pp 141–162

Hatch EM, Fischer AH, Deerinck TJ, Hetzer MW (2013) Catastrophic nuclear envelope collapse in cancer cell micronuclei. Cell [Internet]. Elsevier Inc. 154(1):47–60. https://doi.org/10.1016/j.cell.2013.06.007

Heddle JA (1973) A rapid in vivo test for chromosomal damage. Mutat Res [Internet] 18(2):187–190. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10865415

Hoffman DJ, Rattner BA, Burton GA (2003) Handbook of ecotoxicology, 2nd edn. Lewis Publishers, New York. 1290 p

Lima-junior SE, Cardone IB, Goitein R (2002) Determination of a method for calculation of Allometric Condition Factor of fish. Acta Sci 24(2):397–400

Linde-Arias AR, Inácio AF, Novo LA, de Alburquerque C, Moreira JC (2008) Multibiomarker approach in fish to assess the impact of pollution in a large Brazilian river, Paraiba do Sul. Environ Pollut 156(3):974–979

Nelson JS (ed) (2006) Fishes of the world, 4th edn. Wiley, Hoboken; 622 p

Normann CABM, Moreira JCF, Cardoso VV (2008) Micronuclei in red blood cells of armored catfish Hypostomus plecotomus exposed to potassium dichromate. Afr J Biotechnol 7(7):893–896

Oliveira Ribeiro CA, Vollaire Y, Sanchez-Chardi A, Roche H (2005) Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. Aquat Toxicol 74:53–69

Oyakawa OT, Akama A, Zanata AM (2005) Review of the genus *Hypostomus* Lacépède, 1803 from rio Ribeira de Iguape basin, with description of a new species (Pisces, Siluriformes, Loricariidae), vol 27. Magnolia Press, Auckland, pp 1–27

Paulino MG, Benze TP, Sadauskas-Henrique H, Fernandes MN, Margareth FJB et al (2014) The impact of organochlorines and metals on wild fi sh living in a tropical hydroelectric reservoir : bioaccumulation and histopathological biomarkers. Sci Total Environ [Internet] Elsevier B.V. 497–498:293–306. https://doi.org/10.1016/j.scitotenv.2014.07.122

Pustiglione G, Moledo D, Abessa DS, Gusso-choueri PK, Brasil R, Rodrigo A et al (2018) Enteric nervous system analyses: new biomarkers for environmental quality assessment. Mar Pollut Bull [Internet]. Elsevier 137(July):711–722. https://doi.org/10.1016/j.marpolbul.2018.11.015

Ramsdorf WA, Vicari T, Almeida MIM, Artoni RF, Cestari MM (2012) Handling of Astyanax sp. for biomonitoring in Cangüiri Farm within a fountainhead (Iraí River Environment Preservation Area) through the use of genetic biomarkers. Environ Monit Assess 184(10):5841–5849

Ribeiro DL, Barcelos GRM, D'Arce LPG (2014) Genotoxic effects of water from São francisco river, Brazil, in Astyanax paranae. Bull Environ Contam Toxicol 93(3):274–279

Rigotto RM, e Vasconcelos DP, Rocha MM (2014) Pesticide use in Brazil and problems for public health. Cad Saúde Pública 30(7):1360–1362

Rodrigues S, Antunes SC, Correia AT, Nunes B (2018) Ecotoxicological evaluation of gilthead seabream (*Sparus aurata*) exposed to the antibiotic oxytetracycline using a multibiomarker approach. Mar Environ Res 141(June):233–246

Santos E (1981) Peixes de água doce. Belo Horizonte: Ed. Itatiaia; 267p

Schmid W (1975) The micronucleus test. Mutat Res [Internet] 31(1):9–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17174593

Silva MD, Rossi SC, Ghisi NDC, Oliveira Ribeiro CA, Cestari MM, Silva de Assis HC (2014) Using multibiomarker approach as a tool to improve the management plan for a Private Reserve of Natural Heritage (RPPN). Bull Environ Contam Toxicol [Internet]. [cited 2014 July 11]; 92(5):602–608. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24531322

Sofia SH, Galindo BA, Paula FM, Sodré LMK, Martinez CBR (2008) Genetic diversity of Hypostomus ancistroides (Teleostei, Loricariidae) from an urban stream. Genet Mol Biol 31.(Suppl. 1:317–323

Steffens C, Klauck CR, Benvenuti T, Silva LB, Rodrigues MAS (2015) Water quality assessment of the Sinos River – RS, Brazil. Braz J Biol 75(4):S62–S67

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H et al (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 221:206–221

Trujillo-Jiménez P, Sedeño-Díaz JE, Camargo JA, López-López E (2011) Assessing environmental conditions of the Río Champotón (México) using diverse indices and biomarkers in the fish Astyanax aeneus (Günther, 1860). Ecol Indic 11:1636–1646

Van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol [Internet] 13(2):57–149. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21782649

Viana LF, Súarez YR, Cardoso CAL, do Amaral Crispim B, de Carvalho Cavalcante DN, Grisolia AB et al (2018) The Response of Neotropical Fish Species (Brazil) on the Water Pollution: Metal Bioaccumulation and Genotoxicity. Arch Environ Contam Toxicol [Internet]. Springer US 75(3):476–485. https://doi.org/10.1007/s00244-018-0551-9

Weber C, Hypostominae S (2003) In: Reis RE, Kullander SO, Ferraris CF Jr (eds) Check list of the freshwater fishes of South and Central America. EDIPUCRS, Porto Alegre, pp 351–372

Zhou Q, Zhang J, Fu J, Shi J, Jiang G (2008) Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta [Internet]. [cited 2013 Nov 11]; 606(2):135–150. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18082645

Chapter 15
Genotoxicity Biomarkers in Fish Erythrocytes and Water Quality Parameters

Thaís Dalzochio, Natália Bordin Andriguetti, Leonardo Airton Ressel Simões, Günther Gehlen, and Luciano Basso da Silva

15.1 Introduction

Water pollution has become one of the most concerning problems in the past years. Industrial development, intense urbanization, and agricultural practices without the proper planning have a great impact on the water quality. Even low concentrations of metals, pesticides, organic compounds, and "emerging contaminants," e.g., antibiotics, hormones, and nanomaterials, can be found in rivers as a result of anthropogenic activities. These activities may cause harmful effects on biota and consequently to human health. In many countries, the monitoring of water quality is still carried out by means of water physicochemical and microbiological parameters, whereas in more developed countries, ecotoxicological approaches are employed following legal requirements. Physicochemical analyses provide information only on the concentrations of substances present in water, being difficult to estimate the effects of the interaction among these compounds on aquatic biota. On the other hand, the use of ecotoxicological approaches offers great advantages in comparison to the analysis of water parameters, since it is possible to verify the direct and/or indirect effect of contaminants on organisms. Therefore, ecotoxicological studies have been essential in water resources monitoring.

15.2 Water Quality Assessment

In Brazil, water bodies are classified into four classes according to specific characteristics. In a ranking of four classes, class 1 is related to water destined for human consumption after simplified treatment, protection of aquatic communities, and

T. Dalzochio (✉) · N. B. Andriguetti · L. A. R. Simões · G. Gehlen · L. B. da Silva
Programa de Pós-graduação em Qualidade Ambiental, Universidade Feevale, Novo Hamburgo, RS, Brazil

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_15

direct contact recreation, and in class 4, the worst scenario, water can be used only for navigation and landscaping purposes. Surface water quality is monitored only by the means of physicochemical and microbiological (coliforms) parameters and is then classified according to limits established by the Brazilian National Environment Council (CONAMA Resolution 357/2005) (Brasil 2005). Maximum values of some organic and inorganic parameters for freshwaters are listed in Table 15.1.

Considering the parameters aforementioned, class 1 and class 2 waters present similar values for most parameters, except for biochemical oxygen demand, dissolved oxygen, and thermotolerant coliforms. In contrast, most limits are redefined for class 3 waters. There are only a few considerations in the resolution on the conditions and standards for class 4 waters, such as virtually absence of floating materials and sediment substances which contribute to the silting of navigation channels, total phenols <1.0 mg L^{-1} C_6H_5OH, pH between 6 and 9.0, and dissolved oxygen >2.0 mg L^{-1} O_2.

Biochemical oxygen demand, dissolved oxygen, total phosphorous, and thermotolerant coliforms are generally associated with the release of untreated sewage into water resources. The release of high amounts of ammoniacal nitrogen into water can also cause the reduction of dissolved oxygen, negatively affecting aquatic biota (Zoppas et al. 2016). High concentrations of total phosphorus can also indicate the use of fertilizers in agricultural activities and discharge of metallurgical effluents, whereas thermotolerant coliforms can also indicate fecal contamination by swine

Table 15.1 Maximum values of some water quality parameters according to the CONAMA Resolution 357/2005 (Brasil 2005)

Parameter	Class 1	Class 2	Class 3	Class 4
Biochemical oxygen demand (mg O_2 L^{-1})	3.0	5.0	10	n.i.
Dissolved oxygen (mg L^{-1} O_2)	> 6.0	> 5.0	> 4.0	> 2.0
Total phosphorous (mg L^{-1})	0.1	0.1	0.15	n.i.
Ammoniacal nitrogen (mg L^{-1})	0.5–3.7[a]	0.5-3.7[a]	1.0-13.3[b]	n.i.
Thermotolerant coliforms (most probable number – MPN 100 mL^{-1})	200	1000	2500	n.i.
Turbidity (nephelometric turbidity units – NTU)	40	100	100	n.i.
Aluminum (mg L^{-1})	0.1	0.1	0.2	n.i.
Cadmium (mg L^{-1})	0.001	0.001	0.01	n.i.
Lead (mg L^{-1})	0.01	0.01	0.033	n.i.
Copper (mg L^{-1})	0.009	0.009	0.013	n.i.
Total chromium (mg L^{-1})	0.05	0.05	0.05	n.i.
Iron (mg L^{-1})	0.3	0.3	5	n.i.
Manganese (mg L^{-1})	0.1	0.1	0.5	n.i.
Nickel (mg L^{-1})	0.025	0.025	0.025	n.i.
Zinc	0.18	0.18	5	n.i.

n.i. not informed in the resolution

[a]Limit concentration according to the pH: 3.7 mg L^{-1} for pH ≤ 7.5; 2.0 mg L^{-1} for 7.5 < pH ≤ 8.0; 1.0 mg L^{-1} for 8.0 < pH ≤ 8.5; 0.5 mg L^{-1} for pH > 8.5

[b]Limit concentration according to the pH: 13.3 mg L^{-1} for pH ≤ 7.5; 5.6 mg L^{-1} for 7.5 < pH ≤ 8.0; 2.2 mg L^{-1} for 8.0 < pH ≤ 8.5; 1.0 L^{-1} for pH > 8.5

and cattle farms (Blume et al. 2010). Turbidity may be the result of soil leaching and low volume of water causing the suspension of sediments, contributing to circle nutrients as phosphorous (Kieling-Rubio et al. 2015).

The presence of some metals in water, in addition to the deterioration of the physical and chemical equilibrium of water, may also interfere in the food chain causing physiological and morphological alterations on biota (Konzen et al. 2015). Aluminum and iron may be present in water due to the release of domestic sewage without treatment and solid waste disposal near the river (Konzen et al. 2015; Hatje et al. 1998; Alloway 2013; Bergamaschi et al. 2015). In addition, high iron and zinc concentrations in some water resources may be associated with the presence of these metals in the edaphic profile of the region (Streck et al. 2008). The presence of lead in water results from the disposal of urban solid waste that may contain batteries, electronics, material colored with lead paint, package labels and also from the atmosphere given the use of fuel aditives (Oliveira et al. 2008; Bueno-Krawczyk et al. 2015). Chromium is usually associated with leather tanning industries, although air deposition has also been suggested (Terra et al. 2008). Cadmium is a nonessential heavy metal present in the environment resulting from various agricultural, mining, and industrial activities and also from the exhaust gases of automobiles (Foy et al. 1978). It is considered one of the most toxic metals, since it does not play a role in biochemical processes (Kosanovic et al. 2007). Similarly, copper also occurs as a result of agricultural activities related to deforestation and inadequate solid waste management (Sena et al. 2018). Nickel is a heavy metal released into the environment, mainly in electroplating processes and in the manufacture of stainless steel, batteries, and pigments (Marcato et al. 2014). Another metal that can be found in water resources is manganese. Manganese is an essential element involved in the metabolism of living organisms and can be present in water as a result of the natural soil composition and also as a consequence of agricultural practices, due to its presence in some pesticides (Hermes et al. 2013).

It is important to highlight that physicochemical analyses give only information about the nature of the contaminants and their concentrations in the environment, and they cannot predict bioavailability or potential effects on biota (Seriani et al. 2015). On the other hand, ecotoxicological approaches represent a useful indicator of water quality (Gonzalez et al. 1993; Araújo et al. 2014), because they reflect the real conditions of interaction by synergy and/or antagonism among the contaminants and the effects on the organisms (Azevedo et al. 2013; Fuzinatto et al. 2013). Nonetheless, there is no specific information on the use of ecotoxicological tests for assessing water quality in CONAMA Resolution 357/2005.

15.2.1 Biological Indicators

Because chemical analyses can only detect the presence or quantify substances in water, environmental biomonitoring studies with different test organisms have increasingly been required for assessing the real impact of a substance (or mixture of substances) on the health of organisms (Hatje et al. 1998).

Biological indicators are sentinel organisms that respond to changes at various structural levels, from cellular, physiological, biochemical, genetic, and histological factors to variations in patterns of behavior, which may affect the population structure of the species as a response to stressors present in the environment (Rodrigues et al. 2010; Velusamy et al. 2014). Currently, a number of test systems are widely recognized for the environmental monitoring (Mazzeo et al. 2013), e.g., plants and fish.

Plants are excellent biological systems, because they are good bioindicators of toxicity, with high sensitivity to detect cytotoxic and mutagenic agents through different genetic mechanisms, including point mutations and chromosomal aberrations (Matos et al. 2017). The species *Allium cepa* ($2n = 16$) is one of the best systems for evaluating cytotoxicity and mutagenicity of environmental substances (Leme and Marin-Morales 2009) and is widely used in monitoring the effect of pollutants, including heavy metals, cyanotoxins, and hydrophilic and lipophilic chemicals (Bianchi et al. 2011).

Fishes are also often used as biological indicators of water quality and biomonitors for the presence of pollutants (Lemos et al. 2007). The use of fish in monitoring programs is believed to be of importance because of the key position of these organisms in the trophic chain and their high commercial value (Bolognesi and Cirillo 2014). Fishes also provide information of pollutants' bioavailability that contribute to the process of biomagnification (metals) and the risks for human health. Moreover, data from bioassays using fish have shown good correlation with genotoxicity in human cells exposed to mutagens (Marcon et al. 2010).

15.2.2 Biomarkers

Biomarkers are biochemical, histological, physiological, or behavioral variations that can be measured in tissue samples or bodily fluids related to exposure to environmental pollutants (Fasulo et al. 2013; Chavan et al. 2017; Lima et al. 2018). They can be classified in three classes (WHO International Programme on Chemical Safety (IPCS) 1993; Van der Oost et al. 2003; Hook et al. 2014), as shown in Table 15.2.

In general, some biomarkers allow the specific identification of exposure to a class of xenobiotics or alterations of physiological function, but the majority of biomarker applications monitor a general response to disturbance (Trapp et al. 2014). Nevertheless, it is important to note that many non-pollution factors may interfere with biomarker responses. These "confounding" factors include the organisms' health, sex, age, nutritional status, metabolic activity, migratory behavior, reproductive and development status, and population density, as well as factors like season, ambient temperature, heterogeneity of the environmental pollution, and so forth (Van der Oost et al. 2003). Therefore, the use of a set of biomarkers is recommended since there is no single biomarker that can give a complete diagnosis of environmental degradation (Ossana et al. 2016).

Table 15.2 Classes of biomarkers, definition, and examples

Class of biomarker	Definition	Examples of biomarkers
Biomarkers of exposure	They show an early response to contaminants and are typically specific to a particular class of contaminants (Broeg et al. 2005)	Biliary fluorescent aromatic compounds, vitellogenin, cytochrome P4501A mRNA or protein, hepatic ethoxyresorufin-O-deethylase (EROD), and metallothioneins (MT)
Biomarkers of biological effect	Related to measurable biochemical, physiological, or other alterations within tissues or body fluids of an organism that can be recognized as associated with an established or possible health impairment or disease	Heat shock proteins (HSP70 or HSP90), markers of oxidative stress [superoxide dismutase (SOD), glutathione, catalase (CAT), lipid peroxidation (LPO)], condition indices (condition factor, hepatosomatic index, gonadal index), histopathology evaluation, and DNA damage
Biomarkers of exposure and effects	This class is described as "biomarkers that integrate chemical exposure and biological effects"	Acetylcholinesterase activity and also genomic approaches (Hook 2010)

15.2.2.1 Genotoxicity

All living organisms interact with the aquatic environment, and environment degradation by human activities may cause DNA damage in aquatic organisms (Akinboro et al. 2011; Nunes et al. 2011). Changes in the rate of cell division and/or DNA structure are harmful to the cells, which can interfere with vital processes such as DNA replication and gene transcription. In addition, these alterations may also cause gene mutations and chromosomal aberrations that contribute to cancer development and cell death (Ossana et al. 2013).

The complexity of the pollutants in environmental samples demands a multitude of genotoxicity tests, with increasing simplicity, sensitivity, and affordability (Tabrez et al. 2011). In this sense, to evaluate toxicogenetics effects of complex mixtures from river water samples, ecotoxicological tests (cytotoxicity, genotoxicity, and mutagenicity) are carried out in microorganisms, animal cells, and plants, alone or combined (Mazzeo et al. 2013; Zegura et al. 2009).

The DNA damage is assessed mainly by the comet assay (or single-cell gel electrophoresis) and the micronucleus test. These techniques are sensitive, rapid, and extensively used as genotoxic biomarkers (Zapata et al. 2016). The micronucleus test is one of the biomarkers most widely used for in situ monitoring of genotoxic pollution (Al-Sabti and Metcalfe 1995; Bolognesi et al. 2006; Udroiu 2006). Micronuclei are small, extra nuclear chromatin bodies surrounded by a nuclear envelope. They arise in dividing cells from acentric chromosome fragments or whole chromosomes lagging behind in anaphase and are not included into one of the two main daughter nuclei at telophase. They represent chromosome or genome mutations (i.e., chromosomal alterations that have been transmitted to daughter cells) and are frequently used as an endpoint in genotoxicity testing (Al-Sabti and Metcalfe 1995; D'Costa et al. 2017; Hintzsche et al. 2017). Considering the mechanisms underlying the micronuclei induction, clastogenic agents mainly induce chro-

mosomal fragments, and aneugenic agents interfere with the mitotic apparatus and lead to missegregation of whole chromatids or chromosomes during mitosis. In both cases, chromatin is not properly distributed to the daughter nuclei and remains in the cytoplasm as a micronucleus (Fenech 2007; Kirsch-Volders et al. 2011). In addition, during the micronucleus analysis, some authors have observed the occurrence of other nuclear abnormalities in interphase cells, such as lobed, blebbed, and notched nuclei and binuclei (Ayllón and Garcia-Vasquez 2001; Palhares and Grisolia 2002; Çavas and Ergene-Gozukara 2003). The formation of these abnormalities may be connected to cell division failures and cell death processes, as well as to genotoxicity and/or mutagenicity and may complement the micronucleus scoring in routine assays for genotoxicity screening (Souza and Fontanetti 2006).

Micronuclei and other nuclear abnormalities in fish can be visualized in different cell types such as gill, kidney, hepatic cells, and fins (Bolognesi and Hayashi 2011) although the use of peripheral erythrocytes is more widespread because it avoids the complex procedures of cell preparation and the killing of animals (Bolognesi and Cirillo 2014). Some studies related to the use of the micronucleus test in fish erythrocytes to assess water quality are presented in Table 15.3.

Differences found in the mean frequencies of micronucleus and nuclear abnormalities may be attributed to interspecies factors, age, sex, levels of chemicals, and period of exposure, among others. However, it is important to highlight that the presence of cellular and/or cytogenetic effect does not always indicate low environmental quality, since they can be produced by natural stress (basal frequency of micronuclei). Moreover, organisms present efficient repair systems, so that these effects may not affect the organism health at all. Thus, the use of additional methods to confirm if contamination is effectively impairing the health of aquatic biota is recommended (Seriani et al. 2012).

15.3 The Sinos River Case Study

The Sinos River basin is located in the eastern region of the state of Rio Grande do Sul, Southern Brazil, and covers 32 municipalities with different economic activities. Untreated domestic sewage discharges, agricultural runoffs, industrial activities (mainly leather), inadequate waste management, sand removal, and elimination of riparian vegetation are the main impacts observed in this basin (Comitesinos – Comitê de Gerenciamento da Bacia Hidrográfica do Rio dos Sinos 2009; Figueiredo et al. 2010; Benvenuti et al. 2015). Although the basin supplies water for 1.6 million inhabitants, the Sinos River – the main river of the basin – is among the most polluted rivers in Brazil (Instituto Brasileiro de Geografia e Estatística IBGE 2010).

The poor water quality of the Sinos River was evidenced by studies using different approaches, such as water physicochemical analyses (Blume et al. 2010; Konzen et al. 2015), plant assays (Nunes et al. 2011; Costa et al. 2014; Cassanegro and Droste 2017), fish assays (Steffens et al. 2015; Bianchi et al. 2015; Dalzochio et al. 2018a; Scalon et al. 2010), and cell cultures (Terra et al. 2008; Bianchi et al. 2017).

Table 15.3 Mean frequencies of micronuclei and nuclear abnormalities in different fish species used in biomonitoring studies

Fish specie	Type of study	Local	Main impacts	Mean micronuclei frequency[a]	Mean nuclear abnormalities frequency[a]	Reference
Apareiodon affinis	In situ	Paraná River, Argentina	Areas without direct anthropic influence	0	1.1	Furnus et al. (2014)
Astyanax altiparanae[b]	In situ	Água das Araras, Brazil	Intensive agricultural activity	0.5–3.6	1.5–6.1	Vieira et al. (2014)
Astyanax asuncionensis[b]	In situ	Paraná River, Argentina	Areas without direct anthropic influence	0.7	5.7	Furnus et al. (2014)
Astyanax bimaculatus lacustres	In situ	Lago Paranoá, Brazil	Sewage discharge	0.4	12.2	Grisolia et al. (2009)
Astyanax jacuhiensis[b]	In situ	Bom Jardim stream, Brazil	Petrochemical effluents	0.8–3.4	3.4–54.5	Lemos et al. (2008)
Astyanax jacuhiensis[b]	Laboratory	Sinos River basin	Domestic sewage, industrial and agricultural activities	0–0.1	0.2–1.8	Steffens et al. (2015)
Astyanax jacuhiensis[b]	Laboratory	Sinos River basin	Domestic sewage, industrial and agricultural activities	0–0.1	1.8–2.6	Bianchi et al. (2015)
Astyanax jacuhiensis[b]	In situ	Uruguai River, Brazil	Agricultural activity	0–3	3.5–17	Loro et al. (2015)
Astyanax schubarti	In situ	Paraná River, Argentina	Areas without direct anthropic influence	0.44	2.6	Furnus et al. (2014)
Astyanax sp.	Laboratory	Not specified	Hospital waste landfill	3.9–4.5	n.a.	Erbe et al. (2011)
Bryconamericus iheringii	In situ	Feitoria River, Brazil	Areas without direct anthropic influence	0.29	n.a.	Bühler et al. (2012)
Bryconamericus iheringii	In situ	Sinos River, Brazil	Domestic sewage, industrial, and agricultural activities	0–0.5	2.4–4.6	Dalzochio et al. (2018a)

(continued)

Table 15.3 (continued)

Fish specie	Type of study	Local	Main impacts	Mean micronuclei frequency[a]	Mean nuclear abnormalities frequency[a]	Reference
Bryconamericus iheringii	In situ	Sinos River, Brazil	Domestic sewage, industrial, and agricultural activities	0.1–0.3	2.3–3.2	Dalzochio et al. (2018b)
Cnesterodon decemmaculatus	Laboratory	Reconquista River, Argentina	Urban, agricultural, cattle-farming, and industrial effluents	0.1–0.4	3.8–11.2	Ossana et al. (2016)
Cyprinus carpio	Laboratory	Sinos River, Brazil	Domestic sewage, industrial, and agricultural activities	0–0.1	0.3–1	Souza et al. (2016)
Diapoma alburnus	In situ	Sinos River, Brazil	Domestic sewage, industrial, and agricultural activities	0.2	2.2–3.3	Dalzochio et al. (2018b)
Geophagus brasiliensis	Laboratory	Doce River basin, Brazil	Mining activities	0.6–4.5	n.a.	Gomes et al. (2019)
Hyphessobrycon luetkenii	In situ	Reservoir in Canela National Forest, Brazil	Domestic and industrial effluents	0.1–0.5	n.a.	Bühler et al. (2014)
Hyphessobrycon luetkenii	In situ	Sinos River basin	Domestic sewage, industrial, and agricultural activities	0.1	3.9–4.5	Dalzochio et al. (2018b)
Hoplias malabaricus	In situ	Corrente River, Brazil	Urban, automotive mechanical, and agricultural effluents	0.4–1.2	n.a.	Batista et al. (2016)
Hoplias malabaricus	In situ	Contas River basin, Brazil	Domestic and agricultural effluents, mining activities, and sand removal	0.1–0.8	2.3–29.5	Jesus et al. (2016)
Leporinus obtusidens	In situ	Paraná River, Argentina	Areas without direct anthropic influence	0.4	1.8	Furnus et al. (2014)
Leporinus obtusidens	Laboratory	Sinos River basin	Domestic sewage, industrial, and agricultural activities	0.7–3.0	n.a.	Bergamaschi et al. (2015)
Oreochromis niloticus	Laboratory	Cutabão do Sul River	Domestic and industrial effluents, solid waste, and pesticides	0.5–9.8	n.a.	Fuzinatto et al. (2013)

Oreochromis niloticus	In situ	Paraíba do Sul River	Domestic and industrial effluents	0.3–0.6	n.a.	Linde-Arias et al. (2008)
Oreochromis niloticus	Laboratory	Paraíba do Sul River, Brazil	Effluents from oil shale processing plant	0.2–9.4	0–11	Souza and Fontanetti (2006)
Oreochromis niloticus	Laboratory	Poti River, Brazil	Sand and rock extraction, urban effluents, dredging, and horticulture activities	2.1–20.6	1.6–6.9	Matos et al. (2017)
Oreochromis niloticus	Laboratory	Tietê River, Brazil	Domestic sewage and industrial effluents	0–4	9–54	Seriani et al. (2015)
Pimephaes promelas	Laboratory	Caí River, Brazil	Petrochemical effluents	0–8	n.a.	Lemos et al. (2007)
Prochilodus lineatus	In situ (caged)	Tibagi basin, Brazil	Agricultural activities	0.1–0.2	3–5.5	Vieira et al. (2016)
Schizodon nasutus	In situ	Paraná River, Argentina	Areas without direct anthropic influence	0	1.8	Furnus et al. (2014)
Serrasalmus brandtii	In situ	Contas River basin, Brazil	Domestic and agricultural effluents, mining activities, and sand removal	0.1–0.2	1.9–9	Jesus et al. (2016)
Steindachnerina brevipinna	In situ	Paraná River, Argentina	Areas without direct anthropic influence	2.8	20.3	Furnus et al. (2014)
Tilapia rendalli	In situ	Corrente River, Brazil	Urban, automotive mechanical, and agricultural effluents	0.2–1.1	n.a.	Batista et al. (2016)

In situ studies implicate in the collection of fish at sampling sites, whereas laboratory indicated the collection of water samples at sampling sites and then exposure of fish to water samples under controlled laboratory conditions. Caged studies are related to animals caging at the studied sites

n.a. not assessed

[a]Frequencies expressed per 1000 erythrocytes

[b]*Astyanax jacuhiensis*, *Astyanax asuncionensis*, and *Astyanax altiparanae* are currently recognized as new junior synonyms of *Astyanax lacustris* (Lucena and Soares 2016)

In this context, studies using biological organisms should be continuously conducted in order to obtain data on the contamination scenario of the river. Therefore, this case study presents the assessment of the genotoxic potential of the Sinos River basin using the micronucleus test in the fish species *Cyprinus carpio*, as well as the analysis of water physicochemical and microbiological parameters.

Surface water samples were collected at seven sites located in the basin (Fig. 15.1) in January 2014. Water collection, transportation, and analysis were performed according to recommendations of the Standard Methods for the Examination of Water and Wastewater (APHA – American Public Health Association 2005). Physicochemical and microbiological analysis included chemical oxygen demand, biochemical oxygen demand, total phosphorous, ammoniacal nitrogen, aluminum, copper, lead, total chromium, nickel, zinc, and total and thermotolerant coliforms.

The present study was approved by the Ethics Committee for Animal Experimentation of *Universidade Feevale* (n. 01.040.2013), and all procedures were conducted following animal care protocols. Fish (measuring and weighting approximately 5 cm and 4 g, respectively) were provided by a local fish farm, acclimated under laboratory conditions (22 ± 1 °C and natural photoperiod) in dechlorinated tap water for 7 days, fed with commercial fish food every 3 days, and then exposed to the water samples for 72 h (n = 10 per site). A control group was maintained in tap water during the exposure period. Then, fish were killed and blood

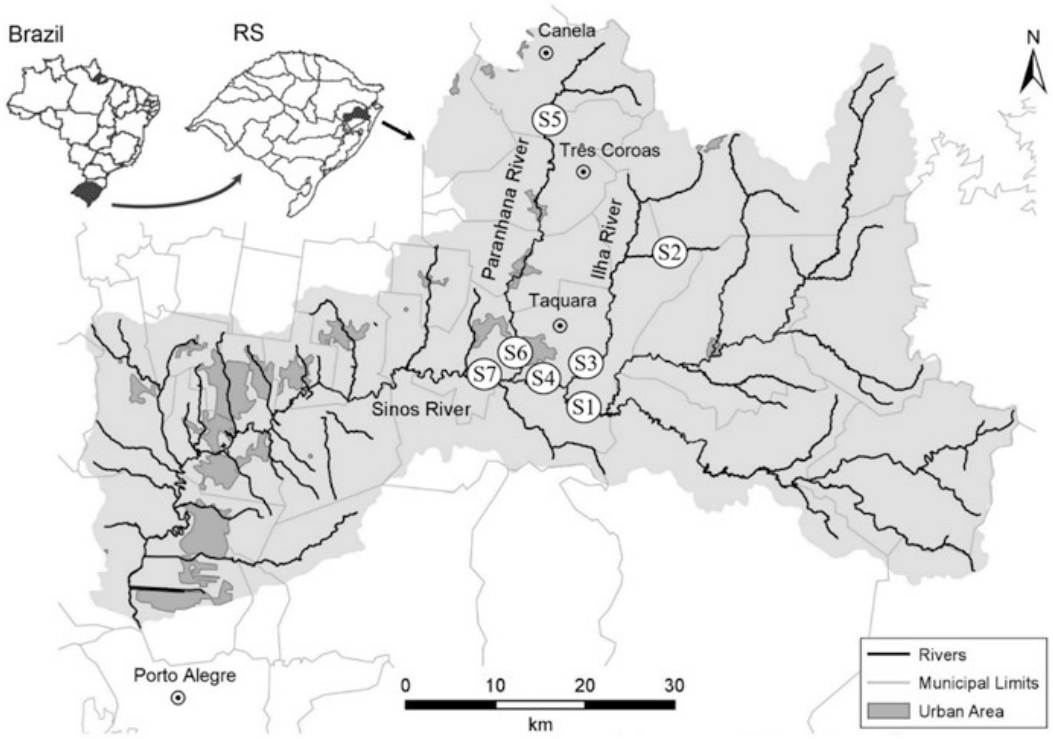

Fig. 15.1 Localization of the samples sites in the Sinos River basin. S1, S4, and S7 are located in the main river – Sinos River; S2 and S3 are located in the source and mouth of the Ilha River, respectively; and S5 and S6 are located in the source and mouth of the Paranhana River, respectively

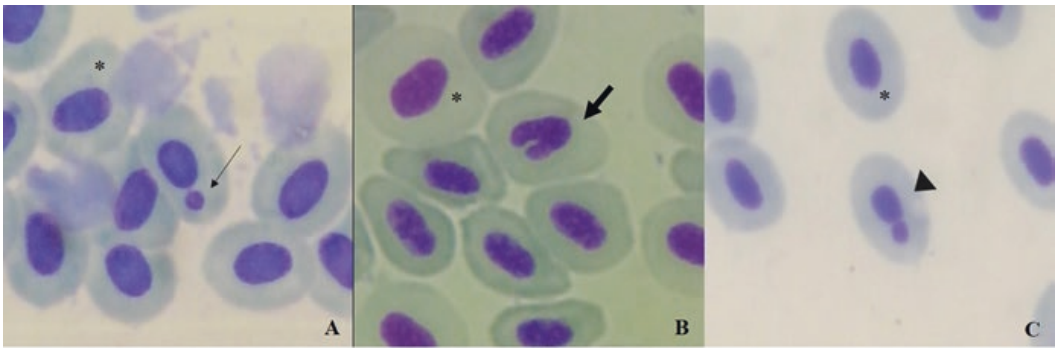

Fig. 15.2 Representative images of the micronucleus test evaluation in *Cyprinus carpio* erythrocytes (1000×). (**a**) Micronucleus (thin arrow), (**b**) invagination (thick arrow), and (**c**) nuclear bud (arrow head). Normal nucleated erythrocytes are indicated (asterisks)

samples were obtained for the blood smears. Slides were fixed in absolute alcohol and stained with Giemsa 5%. A total of 3000 erythrocytes per animal were analyzed for the presence of micronucleated cells and other nuclear anomalies. Micronucleus was considered as rounded non-refractive structures and separated from the main nucleus. Other nuclear anomalies, e.g., invaginations, buds, and binucleated cells, were assessed as described previously (Carrasco et al. 1990) (Fig. 15.2) and were grouped as nuclear abnormalities (Seriani et al. 2015; Vieira et al. 2016). Statistical analysis was performed using the Kruskal-Wallis test in Statistical Package for the Social Sciences (SPSS) 15.0 for Windows. Differences were considered significant when $p < 0.05$.

Levels of aluminum, iron, and lead exceeded the limits established by the Brazilian legislation for class 1 waters at all sampling sites. Thermotolerant coliforms above the limits were found at four sites (S1, S3, S6, and S7), probably as a result of domestic sewage discharges and swine and cattle farms. Copper, total chromium, nickel, and zinc values were within the limits. Micronucleus frequencies ranged from 0 (control, S2, S3, S4, and S5) to 0.2‰ (S6), whereas nuclear abnormality frequencies ranged from 0.2 (S4) to 1.4‰ (S6). No significant differences among sites and the control group were found.

Although no genotoxic effects were observed under the conditions studied, water analyses indicated contamination by metals at all sites, including the sources of the tributaries – areas apparently preserved. Further studies are encouraged in order to better assess the water quality of the Sinos River basin, using long-term exposure periods and samplings during different seasons of the year.

15.4 Conclusions

Water resources may contain a variety of substances that can induce harmful effects on aquatic biota. The analysis of water physicochemical parameters is not sufficient to assess biological effects. Hence, the use of biomarkers in organisms is an

essential approach in monitoring studies. Among others, the micronucleus test in fish erythrocytes is a widely used method to assess the DNA damage induced by water contaminants, given its low cost, sensitivity, and ease to perform.

In southern Brazil, the Sinos River basin is an important water resource used for public supply and is heavily impacted by anthropogenic inputs. A number of studies conducted in the past years have evidenced its poor water quality through the analysis of water physicochemical parameters. More recently, in situ and laboratory studies using biomarkers have also demonstrated the effects of contaminants in organisms. Thus, it is recommended to perform studies using an integrated approach with both water analysis and biological organisms to better estimate the impacts of pollution on aquatic biota. Furthermore, seasonal and temporal variation analysis of such endpoints may also contribute to the comprehension of the real scenario of contamination.

References

Akinboro A, Mohammed K, Rathnasamy S, Muniandy VR (2011) Genotoxicity assessment of water samples from the Sungai Dua river in Pulau Pinang, Malaysia, using the *Allium cepa* test. Trop Life Sci Res 22:23–35

Alloway BJ (2013) Heavy metals in soils. Trace metals and metalloids in soils and their bioavailability.. Environ Poll 22. Springer Netherlands, Netherlands

Al-Sabti K, Metcalfe CD (1995) Fish micronuclei for assessing genotoxicity in water. Mutat Res 343(2–3):121–135

APHA – American Public Health Association (2005) Standard methods for the examination of water and wastewater, twenty-first ed. APHA, Washington, D.C., p 1220

Araújo ALS, Guimarães ET, Seriani R (2014) Mutagenesis in *Tradescantia pallida* as a biomarker of the effects of water polluted with urban effluent. Holos Environ 14(1):97–102

Ayllón F, Garcia-Vasquez E (2001) Micronuclei and other nuclear lesions as in rainbow trout *Oncorhynchus mykiss*. Ecotoxicol Environ Safe 49(3):221–225

Azevedo JS, Braga ES, Assis HCE, Ribeiro CAO (2013) Biochemical changes in the liver and gill of Cathorops spixii collected seasonally in two Brazilian estuaries under varying influences of anthropogenic activities. Ecotoxicol Environ Safe 96:220–230

Batista NJC, Cavalcante AACM, Oliveira MG, Medeiros ECN, Machado JL, Evangelista SR, Dias JF, Santos CEI, Duarte A, Silva FR, Silva J (2016) Genotoxic and mutagenic evaluation of water samples from a river under the influence of diferente anthropogenic activities. Chemosphere 164:134–141

Benvenuti T, Kieling-Rubio MA, Klauck CR, Rodrigues MAS (2015) Evaluation of water quality at the source of streams of the Sinos River Basin, southern Brazil. Braz J Biol 75(S2):S98–S104

Bergamaschi B, Rodrigues MT, Silva JVS, Kluge M, Luz RB, Fleck JD, Bianchi E, Silva LB, Spilki FR (2015) Moving beyond classical markers of water quality: detection of enteric viruses and genotoxicity in water of the Sinos River. Braz J Biol 75(S2):S63–S67

Bianchi J, Espindola ELG, Marin-Morales MA (2011) Genotoxicity and mutagenicity of water samples from the Monjolinho River (Brazil) after receiving untreated effluents. Ecotoxicol Environ Safe 74:826–833

Bianchi E, Goldoni A, Trintinaglia L, Lessing G, Silva CEM, Nascimento CA, Ziulkoski AL, Spilki FR, Silva LB (2015) Evaluation of genotoxicity and cytotoxicity of water samples from the Sinos River Basin, southern Brazil. Braz J Biol 75(S2):S68–S74

Bianchi E, Lessing G, Brina KR, Angeli L, Andriguetti NB, Peruzzo JRS, Nascimento CA, Spilki FR, Ziulkoski AL, Silva LB (2017) Monitoring the genotoxic and cytotoxic potential and the

presence of pesticides and hydrocarbons in water of the Sinos River basin, Southern Brazil. Arch Environ Contam Toxicol 72(3):321–334
Blume KK, Macedo JC, Meneguzzi A, Silva LB, Quevedo DM, Rodrigues MAR (2010) Water quality assessment of the Sinos River, Southern Brazil. Braz J Biol 70(4):1185–1193
Bolognesi C, Cirillo S (2014) Genotoxicity biomarkers in aquatic bioindicators. Curr Zool 60(2):273–284
Bolognesi C, Hayashi M (2011) Micronucleus assay in aquatic animals. Mutagenesis 26:205–2013
Bolognesi C, Perrone E, Roggieri P, Pampanin DM, Sciutto A (2006) Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. Aquat Toxicol 78(Suppl 1):S93–S98
Brasil (2005) National Environment Council—CONAMA Resolução CONAMA 357, de 2005. Available in: http://www.mma.gov.br/port/conama/res/res05/res35705.pdf. Accessed 21 Oct 2018
Broeg K, Westernhagen HV, Zander S, Korting W, Koehler A (2005) The "bioeffect assessment index" (BAI): a concept for the quantification of effects of marine pollution by an integrated biomarker approach. Mar Pollut Bull 50:495–503
Bueno-Krawczyk ACD, Guiloski IC, Piancini LDS, Azevedo JC, Ramsdorf WA, Ide AH, Guimarães ATB, Cestari MM, Silva AHC (2015) Multibiomarker in fish to evaluate a river used to water public supply. Chemosphere 135:257–264
Bühler D, Marinowic R, Rodrigues MAS, Silva L (2012) Evaluation of genotoxicity and cytotoxicity of treated tannery wastewater in Southern Brazil. Int J Environ Technol Manag 15:114–123
Bühler D, Marinowic R, Barros MP, Silva L (2014) Genetic damage induced by water pollutants in the freshwater fish *Hyphessobrycon luetkenii* (Characidae) in a reservoir of the Canela National Forest, Brazil. J Freshw Ecol 29:295–299
Carrasco KR, Tilbury KL, Myers MS (1990) Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. Can J Fish Aquatic Sci 47(11):2123–2136
Cassanegro MBB, Droste A (2017) Assessing the spatial pattern of a river water quality in southern Brazil by multivariate analysis of biological and chemical indicators. Braz J Biol 77(1):118–126
Çavas T, Ergene-Gozukara S (2003) Micronuclei, nuclear lesions and interphase silver-stained regions (AgNORs) as cytogenotoxic indicators in *Oreochromis niloticus* exposed to textile mill effluent. Mutat Res 538(1–2):81
Chavan P, Kumar R, Joshi H, Kirubagaran R, Venugopalan VP (2017) Multimarker study of the effects of antifouling biocide on benthic organisms: results using *Perna viridis* as candidate species. Environ Sci Pollut Res 25(21):20407–20418
Comitesinos – Comitê de Gerenciamento da Bacia Hidrográfica do Rio dos Sinos (2009) Available in: http://www.comitesinos.com.br/planos-estudostecnicos-e-legislacao/1o-plano-de-bacia/. Accessed 29 June 2016
Costa GM, Benvenuti T, Rubio MAK, Rodrigues MAS, Droste A (2014) Monitoramento químico e do potential genotóxico para o diagnóstico da qualidade de corpos hídricos. Rev Bras Ciências Ambient 32:65–74
D'Costa A, Shyama SK, Kumar MKP (2017) Bioaccumulation of trace metals and total petroleum and genotoxicity responses in an edible fish population as indicators of marine pollution. Ecotoxicol Environ Safe 142:22–28
Dalzochio T, Rodrigues GZP, Simões LAR, Souza MS, Andriguetti NB, Silva GJH, Silva LB, Gehlen G (2018a) In situ monitoring of the Sinos River, southern Brazil: water quality parameters, biomarkers, and metal bioaccumulation in fish. Environ Sci Pollut Res Int 25(10):9485–9500
Dalzochio T, Simões LAR, Souza MS, Rodrigues GZP, Schvambach LJ, Lehmann PCA, Gehlen G, Basso LB (2018b) Genotoxic effects on fish species and water quality parameters of two tributaries of the Sinos River, Southern Brazil. Int J Environ Technol Manage 21(3/4):161–173
Erbe MCL, Ramsdorf WA, Vicari T, Cestari MM (2011) Toxicity evaluation of water samples collected near a hospital waste landfill through bioassays of genotoxicity piscine micronucleus test and comet assay in fish *Astyanax* and ecotoxicity *Vibrio fischeri* and *Daphnia magna*. Ecotoxicology 20:320–328

Fasulo S, Mauceri A, Maisano M, Giannetto A, Parrino V, Gennuso F, D'Agata A (2013) Immunohistochemical and molecular biomarkers in *Coris julis* exposed to environmental pollutants. Ecotoxicol Environ Safe 73(5):873–882

Fenech M (2007) Cytokinesis-block micronucleus cytome assay. Nat Protoc 2(5):1084–1104

Figueiredo JAS, Drumm E, Rodrigues MAS, Spilki FR (2010) The Rio dos Sinos watershed: an economic and social space and its interface with environmental status. Braz J Biol 70(S4):1131–1136

Foy CD, Chancy RL, White MC (1978) The physiology of metal toxicity in plants. Ann Rev Plant Phys 29(1):511–566

Furnus GNA, Caffetti JD, García EM, Benítes MF, Pastori MC, Fenocchio AS (2014) Baseline micronuclei and nuclear abnormalities frequencies in native fishes from Paraná River (Argentina). Braz J Biol 74:217–221

Fuzinatto CF, Flohr L, Melegari SP, Matias WG (2013) Induction of micronucleus of *Oreochromis niloticus* exposed to waters from the Cubatão do Sul River, southern Brazil. Ecotoxicol Environ Safe 98:103–109

Gomes LC, Chippari-Gomes AR, Miranda TO, Pereira TM, Merçon J, Davel VC, Barbosa BV, Pereira ACH, Frossard A, Ramos JPL (2019) Genotoxicity effects on *Geophagus brasiliensis* fish exposed to Doce River water after the environmental disaster in the city of Mariana, MG, Brazil. Braz J Biol 79(4):659–664

Gonzalez G, Crespo S, Brusle J (1993) Histo-cytological study of the liver of the cabrilla sea bass, *Serranus cabrilla* (Teleostei, Serranidae), an available model for marine fish experimental studies. J Fish Biol 43(3):363–373

Grisolia CK, Rivero CLG, Starling FLRM, Silva ICR, Barbosa AC, Dorea JG (2009) Profile of micronucleus frequencies and DNA damage in different species of fish in a eutrophic tropical lake. Genet Mol Biol 32:138–143

Hatje V, Bidone ED, Maddock JL (1998) Estimation of the natural and anthropogenic components of heavy metal fluxes in fresh water Sinos River, Rio Grande do Sul State, South Brazil. Environ Technol 19(5):483–487

Hermes N, Schneider RCS, Molin DD, Riegel GZ, Costa AB, Corbellini VA, Torres JPM, Malm O (2013) Environmental pathways and human exposure to manganese in southern Brazil. An Acad Bras Ciênc 85(4):1275–1288

Hintzsche H, Hemmann U, Poth A, Utesch D, Lott J, Stopper H (2017) Fate of micronuclei and micronucleated cells. Mutat Res 771:85–98

Hook SE (2010) Promise and progress in environmental genomics: a status report on the applications of microarray studies in ecologically relevant fish species. J Fish Biol 77:1999–2022

Hook SE, Gallagher EP, Batley GE (2014) The role of biomarkers in the assessment of aquatic ecosystem health. Integr Environ Assess Manag 10(3):327–341

Instituto Brasileiro de Geografia e Estatística e IBGE (2010) Indicadores de Desenvolvimento Sustentável e Brasil. Available in: http://www.ibge.gov.br/home/geociencias/recursosnaturais/ids/default_2010.shtm. Accessed 20 Dec 2016

Jesus IS, Cestari MM, Bezerra MA, Affonso PRAM (2016) Genotoxicity effects in freshwater fish from a Brazilian impacted river. Bull Environ Contam Toxicol 96:490–495

Kieling-Rubio MA, Benvenuti T, Costa GM, Petry CT, Rodrigues MA, Schmitt JL, Droste A (2015) Integrated environmental assessment of streams in the Sinos River basin in the state of Rio Grande do Sul, Brazil. Braz J Biol 75(2):S105–S113

Kirsch-Volders M, Plas G, Elhajouji A, Lukamowicz M, Gonzalez L, Vande Loock K, Decordier I (2011) The in vitro MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. Arch Toxicol 85(8):873–899

Konzen GB, Figueiredo JAS, Quevedo DM (2015) History of water quality parameters - a study on the Sinos River/Brazil. Braz J Biol 75(S2):S1–S10

Kosanovic M, Hasan MY, Subramanian D, Al Ahbabi AAF, Al Kathiri OAA, Aleassa EMAA, Adem A (2007) Influence of urbanization of the western coast of the United Arab Emirates on trace metal content in muscle and liver of wild Red-spot emperor (*Lethrinus lentjan*). Food Chem Toxicol 45(11):2261–2266

Leme DM, Marin-Morales MA (2009) *Allium cepa* test in environmental monitoring: a review on its application. Mutat Res 682:71–81

Lemos CT, Rodel PM, Terra NR, Oliveira CD, Erdtmann B (2007) River water genotoxicity evaluation using micronucleus assay in fish erythrocytes. Ecotoxicol Environ Safe 66:391–401

Lemos CT, Iranço FA, Oliveira NCD, Souza GD, Fachel JMG (2008) Biomonitoring of genotoxicity using micronuclei assay in native population of *Astyanax jacuhiensis* (Characiformes: Characidae) at sites under petrochemical influence. Sci Total Environ 406:337–343

Lima LBD, Morais PB, Andrade RLT, Mattos LV, Moron SE (2018) Use of biomarkers to evaluate the ecological risk of xenobiotics associated with agriculture. Environ Pollut 237:611–624

Linde-Arias AR, Inácio AF, Albuquerque C, Freire MM, Moreira JC (2008) Biomarkers in an invasive fish species, *Oreochromis niloticus,* to assess the effects of pollution in a highly degraded Brazilian river. Sci Total Environ 399(1–3):186–192

Loro VL, Murussi C, Menezes C, Leitemperger J, Severo E, Guerra L, Costa M, Perazzo GX, Zanella R (2015) Spatial and temporal biomarkers responses of *Astyanax jacuhiensis* (Cope, 1894) (Characiformes: Characidae) from the middle Rio Uruguai, Brazil. Neotrop Ichthyol 13:569–578

Lucena CA, Soares HG (2016) Review of species of the *Astyanax bimaculatus* "caudal peduncle spot" subgroup *sensu* Garutti & Langeani (Characiformes, Characidae) from the rio La Plata and rio São Francisco drainages and coastal systems of southern Brazil and Uruguay. Zootaxa 4072:101–125

Marcato ACC, Yabuki AT, Fontanetti CS (2014) Nickel exposure promotes osmoregulatory disturbances in *Oreochromis niloticus* gills: histopathological and energy dispersive spectrometry analysis. Environ Sci Poll Res Int 21(22):13095–13102

Marcon AE, Ferreira DM, Moura MFV, Campos TFC, Amaral VS, Agnez-Lima LF, Medeiros SBR (2010) Genotoxic analysis in aquatic environment under influence of cyanobacteria, metal and radioactivity. Chemosphere 81:773–780

Matos LA, Cunha ACS, Sousa AA, Maranhão JPR, Santos NRS, Gonçalves MMC, Dantas SMMM, Sousa JMC, Peron AP, Silva FCC, Alencar MVOB, Islam T, Aguiar RPS, Melo-Cavalcante BCC, Junior HFJ (2017) The influence of heavy metals on toxicogenic damage in a Brazilian tropical river. Chemosphere 185:852–859

Mazzeo DEC, Matsumoto ST, Levy CE, Angelis DF, Marin-Morales MA (2013) Application of micronucleus test and comet assay to evaluate BTEX biodegradation. Chemosphere 90:1030–1036

Nunes EA, Lemos CL, Gavronski L, Moreira TN, Oliveira NCD, Silva J (2011) Genotoxic assessment on river water using different biological systems. Chemosphere 84:47–53

Oliveira MTG, Rolim SBA, Mello-Farias PC, Meneguzzi A, Lutckmeier C (2008) Industrial pollution of environmental compartments in the Sinos River Valley, RS, Brazil: geochemical-biogeochemical characterization and remote sensing. Water Air Soil Pollut 192:183–198

Ossana NA, Castane PM, Salibian A (2013) Use of lithobates catesbeianus tadpoles in a multiple biomarker approach for the assessment of water quality of the Reconquista river (Argentina). Arch Environ Contam Toxicol 65:486–497

Ossana NA, Eissa BL, Baudou FG, Castañé PM, Soloneski S, Ferrari L (2016) Multibiomarker response in ten spotted live-bearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842) exposed to Reconquista river water. Ecotoxicol Environ Safe 133:73–81

Palhares D, Grisolia CK (2002) Comparison between the micronucleus frequencies of kidney and gill erythrocytes in tilapia fish, following mitomycin C treatment. Gen Mol Biol 25(3):281–284

Rodrigues APC, Maciel PO, Silva LLCP, Albuquerque C, Inácio AF, Freire M, Linde AR, Almosny NRP, Andreata JV, Bidone ED, Castilhos ZC (2010) Biomarkers for mercury exposure in tropical estuarine fish. J Braz Society Ecotoxicol 5(1):9–18

Scalon MCS, Rechenmacher C, Siebel AM, Kayser ML, Rodrigues MT, Maluf SW, Rodrigues MAS, Silva LB (2010) Evaluation of Sinos River water genotoxicity using the comet assay in fish. Braz J Biol 70(4):1217–1222

Sena AR, Machado A, Barbosa SA, Santos LO, Santos MN (2018) Avaliação da qualidade da água do Rio Pitanga, São Cristóvão/SE. Interfaces Científicas-Saúde e Ambiente 6(3):63–74

Seriani R, Abessa DMS, Kirschbaum AA, Pereira CDS, Ranzani-Paiva MJT, Assunção A, Silveira FL, Romano P, Mucci JLN (2012) Water toxicity and cyto-genotoxcity biomarkers in the fish *Oreochromis niloticus* (Cichlidae). J Braz Soc Ecotoxicol 7(2):67–72

Seriani R, Abessa DMS, Moreira LB, Cabrera JPG, Sanches JQ, Silva CLS, Amorim FA, Rivero DHRF, Silva FL, Fitorra LS, Carvalho-Oliveira R, Macchione M, Ranzani-Paiva MJT (2015) In vitro mucus transportability, cytogenotoxicity, and hematological changes as non-destructive physiological biomarkers in fish chronically exposed to metals. Ecotoxicol Environ Safe 112:162–168

Souza TS, Fontanetti CS (2006) Micronucleus test and observations of nuclear alterations in erythrocytes of Nile tilapia to waters affected by refinery effluent. Mutat Res 605:87–93

Souza MS, Rodrigues GZP, Dalzochio T, Goldoni A, Simões LAR, Gehlen G, Silva LB (2016) Avaliação da qualidade da água do Rio dos Sinos (Brasil) por meio do teste de micronúcleos em *Cyprinus carpio* e de análises físico-químicas e microbiológicas. Acta Toxicol Argent 24(3):193–199

Steffens C, Klauck CR, Benvenuti T, Silva LB, Rodrigues MAS (2015) Water quality assessment of the Sinos River, RS, Brazil. Braz J Biol 75(4):62–67

Streck EV, Kämpf N, Dalmolin RSD, Klamt E, Nascimento PC, Schneider P, Giasson E, Pinto LFS (2008) Solos do Rio Grande do Sul, 2nd edn. EMATER/RS, Porto Alegre, 222 p

Tabrez S, Shakil S, Urooj M, Damanhouri GA, Abuzenadah AM, Ahmad M (2011) Genotoxicity testing and biomarker studies on surface waters: an overview of the techniques and their efficacies. J Environ Sci Health C 29:250–275

Terra NR, Feiden IR, Fachel JMG, Lemos CT, Nunes EA (2008) Ecotoxicological evaluation of sediment and water samples from Sinos River, Rio Grande do Sul, Brazil, using *Daphnia magna* and V79 cells. Acta Limnol Bras 20(1):63–72

Trapp J, Armengaud J, Salvador A, Chaumot A, Geffard O (2014) Next-generation proteomics: toward customized biomarkers for environmental biomonitoring. Environ Sci Technol 48:13560–13572

Udroiu I (2006) The micronucleus test in piscine erythrocytes. Aquat Toxicol 79(2):201–204

Van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13:149–157

Velusamy A, Kumar PS, Ram A, Chinnadurai S (2014) Bioaccumulation of heavy metals in commercially important marine fishes from Mumbai Harbor, India. Mar Pollut Bull 81(1):218–224

Vieira CED, Almeida MS, Galindo BA, Pereira L, Martinez CBR (2014) Integrated biomarker response index using a Neotropical fish to assess the water quality in agricultural areas. Neotrop Ichthyol 12:153–164

Vieira CED, Costa PG, Lunardelli B, Oliveira LF, Cabrera LC, Risso WE, Primel EG, Meletti PC, Fillmann G, Martinez CBR (2016) Multiple biomarker responses in *Prochilodus lineatus* subjected to short-term in situ exposure to streams from agricultural areas in Southern Brazil. Sci Total Environ 542:44–56

WHO International Programme on Chemical Safety (IPCS) (1993) Biomarkers and risk assessment: concepts and principles, Environmental Health Criteria 155. World Health Organization, Geneva

Zapata LM, Bock BC, Orozco LY, Palacio JA (2016) Application of the micronucleus test and comet assay in *Trachemys callirostris* erythrocytes as a model for in situ genotoxic monitoring. Ecotoxicol Environ Safe 127:108–116

Zegura B, Heath E, Cernosa A, Filipic M (2009) Combination of in vitro bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. Chemosphere 75:1453–1460

Zoppas FM, Bernardes AM, Meneguzzi Á (2016) Parâmetros operacionais na remoção biológica de nitrogênio de águas por nitrificação e desnitrificação simultânea. Bio Rev Engenharia Sanitária 21(1):29–42

Chapter 16
Health Diagnosis of the Fish *Scomberomorus cavalla* from Tecolutla, Ver. México

Verónica Ramírez-Trejo, Pamela Becerra-Amezcua, Irma Hernández-Calderas, Patricia Ramírez Romero, Alfonso Vázquez-Botello, and Xochitl Guzmán-García

16.1 Introduction

The Gulf of Mexico has been seriously contaminated with pollutants such as pesticides, detergents, bleaches, cosmetics, drugs, wastewater discharges, hydrocarbons, and heavy metals, among others (Caso et al. 2004), which can have implications on the physiology of those organisms that live in this aquatic ecosystems.

Some metals such as Cr, Cu, Fe, Mo, Se, and Zn are essential but can be toxic if their concentrations are high in the environment (Espina and Vanegas 2005). Heavy metals represent an environmental risk because they are highly stable in the face of degradation process, so they do not disappear from the environment; they are only transferred among compartments where they can be combined with organic substances through microbial processes, frequently leading to more toxic forms (Svobodová et al. 1993; Mancera-RodrÍguez and Álvarez-León 2006).

V. Ramírez-Trejo · I. Hernández-Calderas · X. Guzmán-García (✉)
Universidad Autónoma Metropolitana, Unidad Iztapalapa, Mexico City, Mexico
e-mail: xgg@xanum.uam.mx

P. Becerra-Amezcua
Laboratorio de Biotecnología y Toxicología Molecular, Departamento Recursos del Mar, Centro de Investigación y de Estudios Avanzados, Unidad Mérida, Mexico City, Mexico

P. R. Romero
Laboratorio de Ecotoxicología, Departamento de Hidrobiología, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Unidad Iztapalapa, Mexico City, Mexico

A. Vázquez-Botello
Universidad Nacional Autónoma de México, Instituto de Ciencias del Mar y Limnología, Ciudad Universitaria, Ciudad de México, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_16

16.2 Effects of Metals in Aquatic Organisms

Among the metals, mercury, lead, and chromium have toxicological relevance because they can have an impact on the physiology of aquatic organisms (Páez-Osuna 2005). Mercury (Hg) is released to the environment by geological phenomena such as volcanism, degassing of the earth's crust, and soil erosion (Nuñez et al. 1998). However, as a result of industrial growth and pollution, concentrations of this metal have increased worldwide and now represent a growing threat to wildlife and human health (Stafford and Haines 1997; Fitzgerald et al. 1998). The bioaccumulation of mercury occurs when living organisms absorb this metal faster than their bodies can eliminate it, so the metal bioaccumulates in their tissues or organs (Rand et al. 1995; Gray 2002). Bioaccumulated mercury is transferred from one trophic level to another increasing its concentration through the trophic chain, what is known as biomagnification (Gray 2002; Campos 1987; Regnell et al. 1997). In the case of methylmercury, the EPA established a limit concentration for edible fish of 0.3 mg/Kg of wet weight, a concentration that should not be exceeded assuming a total fish consumption of 0.175 Kg/day. In Mexico, the official Mexican standard NOM-SSA1–027-1993 establishes that fish containing concentrations greater than or equal to 1.0 mg/Kg wet weight of total mercury should not be consumed.

Lead (Pb) is an electropositive metal with high affinity for sulfhydryl groups. The enzymes that depend on those groups are inhibited in the presence of Pb. Also, as a divalent metal, it is similar to calcium and can compete in cellular processes such as mitochondrial respiration and in some neurological functions (Landis and Yu 1999). It is well known that Pb interacts with nucleic acids, causing an increase or decrease in protein synthesis. In humans, chronic effects lead to intoxication of gastrointestinal, neuromuscular, hematological, renal, reproductive, and the central nervous system (Moreno 2003). In fish, severe lead toxicity is initially characterized by damage to the gill epithelium, causing death by asphyxia. The characteristic symptoms of chronic lead toxicity include changes in blood parameters with severe damage to erythrocytes and leukocytes and degenerative changes in the parenchymal organs as well as damage to the nervous system (Svobodová et al. 1993).

In the case of chromium (Cr), the trivalent form and its salts are the most stable forms. Trivalent chromium is essential for humans and is required to have a normal balance in cholesterol metabolism, insulin, and glucose homeostasis (Páez-Osuna 2005). The hexavalent form is the less stable but it is the most biologically reactive (Hellawell 1989). In the marine environment, the dominant species is the hexavalent form Cr^{+6} (Bruland 1983) which represents an ecological risk, produced through industrial processes, becoming a risk to the health of organisms; however, the high concentrations of this metal in the environment are due to industrial activities like the processing of fossil fuels and the incineration of municipal solids, but also by drilling mud (Páez-Osuna 2005). It is recognized that all foods contain chromium at levels of 0.5 ppm wet weight; however, the daily intake of chromium in humans has been estimated in a range of 0.03 to 0.1 mg/person/day (Páez-Osuna 2005).

16.3 Bioindicator and Biomarkers in Ecotoxicological Studies

Fish are considered good indicators of the quality of the environment, so a great diversity and abundance of fish in rivers, lakes, and seas indicate a healthy environment for them and other forms of life (Fossion et al. 2018; Helfman et al. 1997). The species *Scomberomorus cavalla* (Cuvier, 1829) plays an important role as a top predator, and its absence can cause drastic increases in prey populations, significantly altering the equilibrium of marine and coastal ecosystems (Díaz-Sánchez and Aguilar III 2008). In addition, its dietary habits, rapid growth, and high metabolic demand for the pelagic lifestyle can lead to the incorporation of high concentrations of metals in their tissues, including the muscle, which constitutes a risk for the human health (Kuklyte 2012; Lacerda et al. 2000; Monteiro et al. 1996). It is known that the consumption of contaminated marine fish is the main known source of human exposure for some metals such as mercury, lead, or chromium (Fitzgerald and Clarkson 1991).

The toxic effects of metals in indicator organisms can be measured through biomarkers. A biomarker is defined as a biological response: biochemical, physiological, morphological, or histopathological that is associated with the exposure to xenobiotic agents and provides early warnings of lesions caused by contaminants (Huggett et al. 1992; Martínez-Jerónimo 1991). Biomarkers can be classified into different categories depending on the level of biological organization: (1) biotransformation enzymes (involved in the detoxification of xenobiotics and their metabolites), (2) biotransformation products, (3) amino acids and proteins, (4) hematological, (5) immunological, (6) reproductive and endocrine, (7) neuromuscular, (8) genotoxic, and (9) physiological-morphological (Farris and Van Hassel 2007).

Physiological and morphological responses can be evaluated through histopathology, since this allows detection at the tissue level of the early responses generated by the contaminants (Guzmán-García 2007). Histopathology has been extensively used in aquatic organisms, such as fish, to evaluate the effect of pollutants from environmental exposures (Bernet et al. 1999), and it has been proposed as a biomarker because it is at an intermediate position with respect to the levels of biological organization (between the atom and the organism) and represents a rapid method to detect effects, especially chronic ones, in various tissues and organs. Some authors consider that histological changes appear as a medium-term response to sublethal stressors (Adams et al. 1989; Johnson et al. 1993). The relevance of an injury depends on its pathological importance, that is, how it affects organ function and the ability of organisms like fish to survive. This is taken into account through an importance or impact factor (FI) assigned to each alteration observed in the histological description (Bernet et al. 1999).

At a finer level of biological organization, the evaluation of protein and enzymatic biomarkers has also been proposed, as well as the examination of tissues and cells by histochemical and cytochemical techniques, which provide information on the cellular chemical composition, as well as the structural elements and their loca-

tion. The demand and great popularity for the identification of specific substances through immunohistochemical methods have opened a new era for histopathology and histotechnology. Likewise, some proteins that can be evaluated by means of immunohistochemistry such as Hsp70 and metallothioneins (MTs) have been used to evaluate environmental stress since they are overexpressed in different organs or tissues such as gills, gonads, the liver, and muscle, as a result of exposure to various environmental stressors (Clark and Kaufman 1997).

Heat shock proteins (HSPs) are overexpressed when cells are subjected to different stimuli such as heat stress, radiation, various drugs, viral infections, heavy metals, among others (Coronato et al. 1999). HSPs are biomarkers of exposure to environmental contamination in a large variety of biota; they show the effect of a contaminant in a time interval and integrate various aspects of protein damage such as the increase of unfolded proteins, together with an increase in the unfolding and formation of protein aggregates, all of which cause a response of the cell mediated by a molecular synthesis of proteases and molecular chaperones, which will try to repair the damaged proteins; the HSP adds value to environmental biomonitoring (Bierkens 2000; Cruz-Rodríguez et al. 2000; Nollen and Morimoto 2002; Radlowska and Pempkowiak 2002).

On the other hand, metallothioneins (MTs) are thermostable and water-soluble proteins that lack aromatic amino acids. They are characterized by a low molecular weight (6–10 kDa) and a sequence of 61 or 62 amino acids, with a high proportion of cysteine, a sulfur-containing amino acid, which represents between 20 and 30% of the total amino acid residues (a total of 20 cysteines) (Ureña-Robles 2007). Although they are found in a large number of different tissues, they are found predominantly in the liver and kidney of vertebrates and in the digestive gland and intestine of invertebrates (Roesijadi 1992). The synthesis of MTs can be induced by a wide variety of stimuli, including metals, hormones, and toxins (Klaassen et al. 1999), with metals being the most potent inductors, which is why MT is considered as a good biomarker of exposure to metals (Roesijadi 1992).

16.4 Tissue and Protein Biomarkers for the Evaluation of Physiological Condition

Biomarkers used in ecotoxicological studies have been evaluated in different organs or tissues for the effects induced by pollutants. One of the organs of greater interest has been the liver, because from a morphological as well as physiological point of view, it is a glandular component of the digestive system, and it is in charge of several functions that include assimilation of nutrients, production of bile, detoxification, and maintenance of the body's metabolic homeostasis (processing of carbohydrates, proteins, lipids, and vitamins). It also plays an important role in the plasma protein synthesis, such as albumin and fibrinogen, and complement factors (Genten et al. 2009; Di Giulio and Hinton 2008). The

normal structure and function of the liver in fish make it a target organ for contaminants; therefore, morphological, histological, immunohistochemical, and molecular tests have been proposed in this organ to detect alterations caused by contaminants (Di Giulio and Hinton 2008).

Another organ of interest in ecotoxicological studies is the fish muscle. Muscle is divided in smooth and striated, and striated is divided into skeletal and cardiac. Skeletal muscle of fish is responsible for providing movement and is the most edible part of the body (Takashima and Hibiya 1995). In this tissue, there are inducible markers of environmental contamination, which include the alteration of locomotor activity, as well as tissue lesions that are generated by exposure to contaminants like pesticides and metals (Koca et al. 2005; Mughal et al. 2004; Wang et al. 2004). The accumulation of pollutants in the muscle represents a risk for the organism itself when it affects its locomotor activity, and it can have an effect on human health by consumption, since it represents the edible part of the fish.

In Mexico, there are just a few studies in fish communities of degraded sites, and there is not any follow-up in terms of space and time of the environmental changes. In the absence of biomonitoring programs in our country, it is pertinent to use tools that allow evaluating the effect of environmental stress on organisms. The use of analytical techniques to determine the concentration of contaminants in the tissues of organisms, combined with the application of biomarkers, has been widely recommended for the evaluation of environmental impact. This type of study also provides information that can help prevent human health from fish extracted. Recent years have seen an increased interest in the variability and complexity analysis of physiological responses through pathophysiology. However, a general understanding is lacking of which variables and variability are indicators of good health and when, on the contrary, variability implies a risk factor (Fossion et al. 2018). Consequently, the present work used the *S. cavalla* specimens from the Tecolutla, Ver., Mexico to determine the concentration of critical metal and evaluate their physiological state by applying tissue and protein biomarkers as an alternative for the evaluation of environmental stress.

16.5 *Case Study:* Health Diagnosis of the Fish *Scomberomorus cavalla* of the Tecolutla, Ver., Mexico

Ten specimens of *Scomberomorus cavalla* organisms were obtained in May and December of 2013 and March of 2014 in the municipality of Tecolutla, Ver., México (Fig. 16.1), according to the recommendation by Padrós and Zarza (2005), who indicated that five specimens of sizes between 100 and 150 g are statistically significant in a health analysis. The average sizes and weight obtained were 82.6 cm and 3400 g; these measures are within the range reported for adult organisms and commercial sizes of this species (length of 70 cm and a weight of 3300 g) (Collette

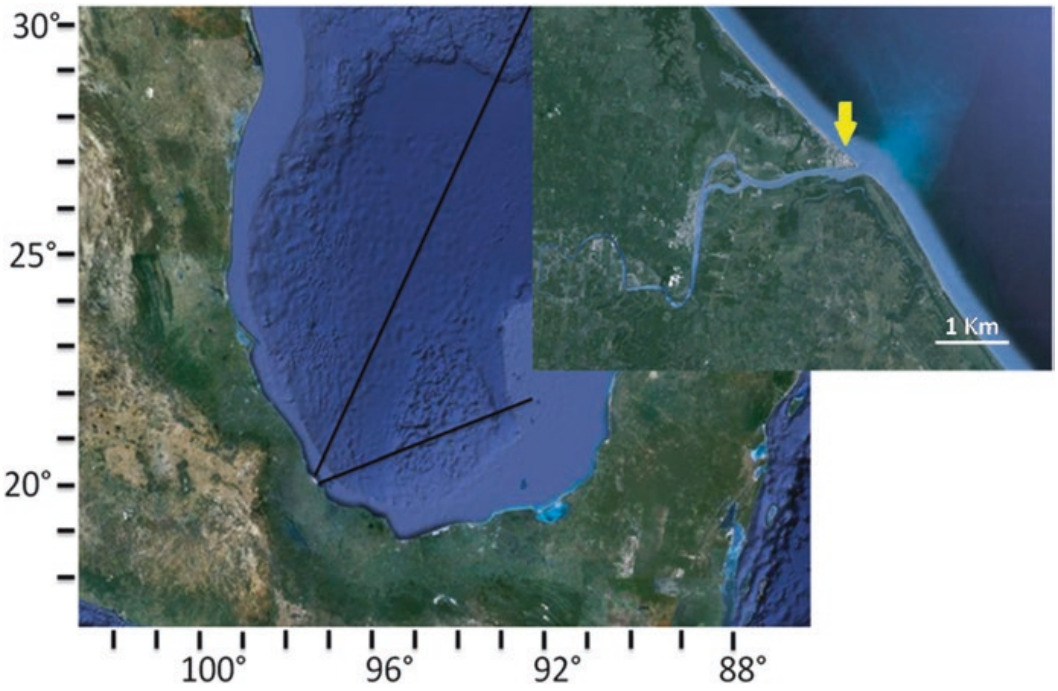

Fig. 16.1 Study area, Tecolutla, Veracruz, Mexico

and Nauen 1983). Length-weight relationships are often used to model biomass trends based on size and are commonly used in the management of economically important populations (Treer et al. 2008; Agboola and Anetekhai 2008).

The analysis of the physiological condition requires the monitoring of parameters that allow us to estimate organisms' macroscopic responses. The recognition of the species in question is important, as well as the condition index, morphometric and biological parameters of reference, and in some cases for tissues analytical records and histology/histhopatology analysis.

The taxonomic determination of the species was carried out using FAO's Species Identification Guide. To calculate the condition index (CI), the relationship between weight and total length was used, using the formula proposed by Maddock and Burton (1999) (Maddock and Burton 1998):

$$\mathrm{CI} = \frac{\left(\text{Total weight}\right)}{\left(\text{Total length}\right)^3} \times 100$$

The reference values for the CI were established according to the intervals proposed by Barnabé and Martínez (1996), considering <1 = thin and elongated fish; > 1 = well-nourished specimens; and 1.4 = females prepared to spawn.

Fishes were evaluated macroscopically through biological parameters such as the presence of ecto- and endoparasites, fungal or bacterial infections, soft tissue deformities, apparent lesions, as well as bad odors.

Tissue Analysis Liver samples and muscle samples from different organisms were used for the metal analysis; these were dried in an oven (BLUE M® brand model SW-11TA) at 60 °C for 48 hours. Subsequently, the tissue was macerated and placed in plastic bags for further analysis quantification of Hg, Pb, and Cr carried out by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) AGILENT® Series 7500. Finally, statistical analysis was performed using one-way ANOVA and student's T test (NCSS & PAS Trial 2001 program). An adjustment of the registered concentrations was made since they were determined in dry weight, considering that the relative humidity of the fish muscle is 65–70% (Primo, 1997). The values are presented in wet weight to make the comparison with the corresponding standards.

Histology/Histopathology The liver and muscle samples were fixed in 10% buffered formalin, dehydrated, and infiltrated in paraffin with an automatic tissue processor (LEICA® model TP1020) and an inclusion center (LEICA® model EG1140H). Each subsample was cut into 5 μm in duplicate with a microtome (MICROM® model HM315). The histological sections were stained with hematoxylin and eosin. Each histological preparation was observed under optical microscope (CARL ZEISS® Primo Star), and photomicrographs of the morphophysiological unit of the liver and muscle were taken by means of a camera (CANON® model PowerShot G10) attached to the microscope. Histopathological analysis of the liver and muscle was performed by constructing a matrix of prevalence of lesions associated with circulatory disturbances and regressive and progressive changes in the organs, accounting for the number of lesions present for each organism. Subsequently, we proceeded to obtain the percentage of prevalence of each one in the tissues analyzed. Finally, tissue injuries were associated with an impact factor proposed by Barnabé and Martínez (1996), who points out that a "0" value corresponds to the absence of tissue lesions, "1" easily reversible lesions (minimal pathological importance), "2" reversible lesions (moderate pathological importance), and "3" generally irreversible lesions (severe pathological importance).

Protein Analysis The analysis of protein biomarkers to assess the presence of Hsp70 was carried out on slides that presented lesions with impact factor 1, 2, and 3 in the liver and in muscle with impact factor 0, since this was the only factor found. Metallothionein analysis was carried out in organisms that presented lesions in the liver with impact factor 1, 2, and 3 and in organisms that had lesions in muscle with impact factor 1 and 0. The blocks were cut to 5 microns and placed on electrostatically charged slides (SFH1103, BIOCARE MEDICAL®) to deparaffinate and hydrate them with distilled water. Recovery of antigen sites was performed with DIVA solution (pH 6 DAKO® recovery: K0679), the primary antibodies Hsp70 (heat shock 70KDa Protein 1A, Genetex), and MTs (Mmp14a + b antibody, Genetex).

16.5.1 Condition Index

The result of the condition index (CI) showed an average of 0.6, which corresponds to thin and elongated organisms according to Barnabé and Martínez (1996) and represents a low condition, despite having values close to those indicated for commercial size. According to these authors, organisms with values greater than 1.2 correspond to well-nourished specimens, and values of 1.4 are recorded in females prepared to spawn. The values obtained in the present study indicate that the organisms analyzed were not in optimal conditions. Costopoulos and Fonds (1989) (Costopoulos and Fonds 1989) point out that the CI also constitutes a measure of the energy reserves related to environmental conditions, maturity states, food, or parasitic effects. This would indicate that the studied specimens had been subject to one or more of the previously mentioned characteristics.

16.5.2 Biological Parameters

Most organisms (90%) showed no apparent lesions, fungal or bacterial infections, soft tissue deformities, bad smells, or ectoparasites, while 10% recorded the presence of endoparasites in the liver and gonad. The presence of parasites, fungi, or bacteria is a macroscopic indicator that allows evaluating the health state of the fish, since their presence can trigger diseases that cause reversible lesions or even affect vital functions that cause death (Feist and Longshaw 2008).

16.5.3 Hg, Pb, and Cr Concentrations

The metal analysis in the liver and muscle indicated the presence of mercury and chromium, but lead was found below the detection level of the equipment (0.01 mg/Kg). In the liver, the average Hg concentration was 1.18 mg/Kg (wet weight) and in the muscle 0.92 mg/Kg (wet weight).

The average concentration of Cr in the liver and muscle was 0.46 mg/kg (wet weight) and 0.47 mg/Kg (wet weight), respectively.

The average concentration of Hg in muscle tissue exceeded the reference value for the consumption of fish meat according to the European Union (EU), which establishes a limit of 0.5 mg/Kg of total mercury, but not the FDA (Food and Drug Administration) and the Official Mexican Standard NOM-SSA1–027-1993 of 1.0 mg/Kg. Regarding the liver, there are no published norms specifically for this organ; the registered concentrations for both types of tissue were found within the same order of magnitude (liver 1.19 and muscle 0.92 mg/kg Hg humid weight), without significant difference between the Hg contents ($t = 0.8098$, $P = 0.46$).

In the case of Cr, FAO (Food and Agriculture Organization) and FDA set 1.0 mg/kg as the reference value. The results obtained in this work indicate that this limit was not exceeded. The average concentration registered in the liver was 0.46 mg/Kg (wet weight) and in muscle 0.45 mg/Kg (wet weight); these concentrations did not show statistically significant differences between the tissues analyzed ($t = -0.2850$; $P = 0.79$). Figure 16.2 shows the comparative analysis of the metals analyzed in the liver and muscle.

The concentrations of Hg, Pb, and Cr in the liver and muscle recorded in this study provide valuable information because so far there is not any report on the concentrations of the aforementioned metals for the species (*Scomberomorus cavalla*) collected in Tecolutla, Veracruz. In this way, the present study is relieved that the concentrations of registered metals exceed the acceptable values for their consumption. This report will provide useful elements to avoid risks to the health of consumers. The recorded concentrations of Hg in the liver and muscle in this study exceeded or were close to the reference values established in the environmental legislation for the consumption of fish can.

Cai (2005) and Adams and McMichael (1999) conducted studies on the concentration of Hg in *S. cavalla* in the northwest area of the Gulf of Mexico. They found that the average concentrations of Hg in the liver and muscle were 0.78 and 0.94 mg/Kg, respectively. Although these concentrations did not exceed the limits, they were found close to it, as observed in the present study. On the other hand, higher concentrations of this element have been identified in the same species in

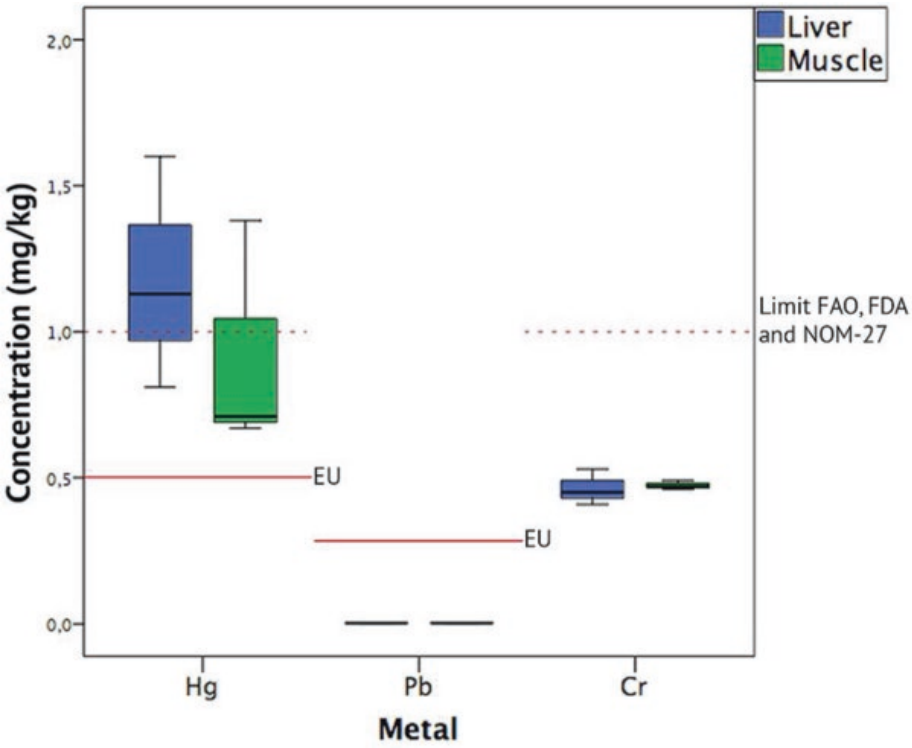

Fig. 16.2 Concentrations of metals in the liver and muscle of *S. cavalla* from Tecolutla, Ver

the southeastern United States, where Kuklyte (2012) recorded an average concentration of 1.04 mg/kg of Hg in muscle. The value exceeds the maximum limit permitted by the Mexican regulation and is comparable to the average recorded in the present study.

In the same work, it was found that the concentration of lead in the liver and muscle of *S. cavalla* was lower than the limit of detection of the device used, which means that it does not represent a risk to human health, since the concentrations were found below the reference value established by the FAO of 0.20 mg Pb/Kg of wet weight in fish muscle. This result differs from those obtained by some authors in marine species collected in the north of the Gulf of Mexico (Feldhausen and Johnson 1983; Hanson 1997; Vázquez et al. 2001), which indicates that the levels of Pb in the muscle of fish were below 10 mg/kg. In contrast, Ploetz et al. (2007) reported lower levels of Pb and other metals in the liver and muscle of *Scomberomorus cavalla* collected in Alabama, finding a lower concentration of Pb in the liver (1 mg/kg) than in muscle (1.8 mg/kg).

The average concentration of Cr in the liver and muscle of *S. cavalla* (0.46 and 0.45 mg/kg of wet weight, respectively) was below what is established by FDA and FAO (1 mg/kg). Cr can be eliminated in feces and urine, but it can also be bioaccumulated and cause some damage in humans, such as acute gastrointestinal symptoms (Galvão and Corey 1987); however, the most important acute effects of high levels of hexavalent Cr ingestion are renal lesions in glomeruli and tubules (Moreno 2003). The mortal dose of Cr for human being is 6–8 g/Kg/d. It is important to notice that in Mexico there is no law that regulates the permissible levels of this metal in the biota despite the damage it can generate, which is worrying because of the concentrations observed in human consumption species.

16.5.4 Tissue Analysis

Histologically, hepatic parenchyma and stroma were found in the morphological unit of the liver. The parenchyma was formed mainly by polyhedral-shaped hepatocytes with a central round nucleus and its characteristic nucleolus, while the stroma presented loose connective tissue, which supports the parenchyma; blood vessels formed by an inner wall of endothelial cells with nucleated erythrocytes were also observed (Fig. 16.3). This composition is associated with the function of biotransformation, detoxification, and blood irrigation of the organ.

The tissue section of the lateral line of the muscle includes the most exposed part, the skin, which is composed of two principal layers: epidermis and dermis. In the histological sections with skin, it proved possible to differentiate the layers that make up the epidermis, the dermis, and the hypodermis (Fig. 16.4). Within the dermis, uniformly aligned dark-colored pigment cells called melanophores (Fig. 16.4) were observed. The diameter of the pigment cells varied between 20.5 and 29.4 μm. In the muscle, the presence of muscle fibers with crossed striation and several ovoid nuclei located in the periphery of the muscle fiber (Fig. 16.5) is characteristic, while muscle bundles of a triangular, hexagonal, or muscle shape

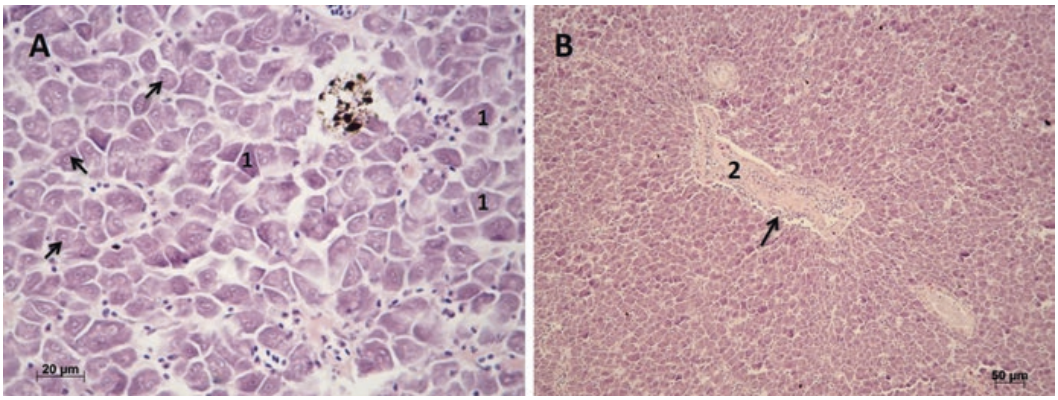

Fig. 16.3 Tissue appearance of the liver. In **a**, hepatocytes are observed in a polyhedral form (1) with characteristic central nucleus (arrows), and the disposition of the hepatocytes toward the vein was evident; in **b**, a blood vessel in the center (2) with erythrocytes inside (arrow). H&E stain

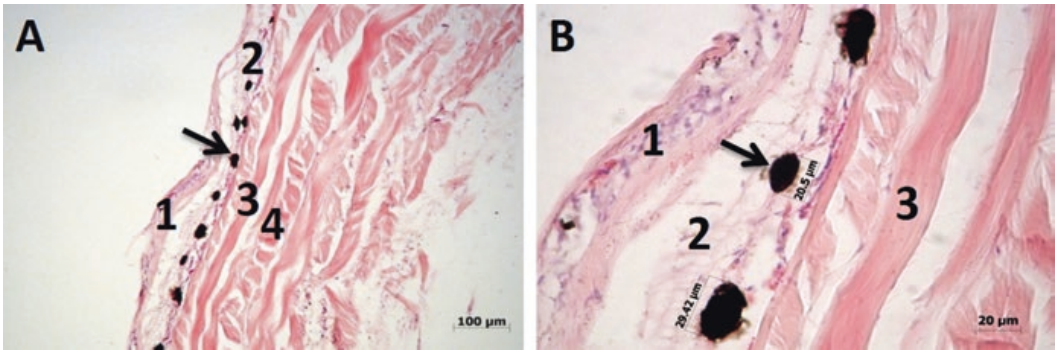

Fig. 16.4 Tissue composition of the muscle section of the *S. cavalla* fish. In **a**, the epidermis (1), dermis (2) with pigment cells (arrow), fibers and muscle bundles in section longitudinal and transversal, respectively (3 and 4); in **b**, approaching the layers of the skin, epidermis (1), dermis (2) with pigment cells (arrow), and longitudinal muscle fibers (3). H&E staining

were observed in a transversal section. Irregular polygons with their peripheral ovoid nuclei are surrounded by connective tissue (Fig. 16.5). Vessels were present within the connective tissue, indicating enough supply of blood to the organ. It was possible to observe the intercellular spaces of the connective tissue. The typical structure of muscle (striated muscle fibers) is linked to locomotor activity in fish.

The lesions found in the liver were of different types: the hepatic parenchyma showed focal inflammations, erythrocyte infiltration in the hepatic stroma, and granulomas from connective tissue (Fig. 16.6). Brown melanomacrophage centers associated with the blood conduits, congestive vessels, eosinophilic secretions, and vacuolated hepatocytes (Fig. 16.7) were also noted. These tissue responses are associated with protection mechanisms (inflammation, eosinophilic secretions), disturbances of the circulatory system (congestive vessels and infiltration), and immunological mechanisms (granulomas and melanomacrophage centers). Analogously, histopathological analysis of the muscle showed circulatory lesions as congestive blood vessels, where nucleated and oval erythrocytes were observed (Fig. 16.8).

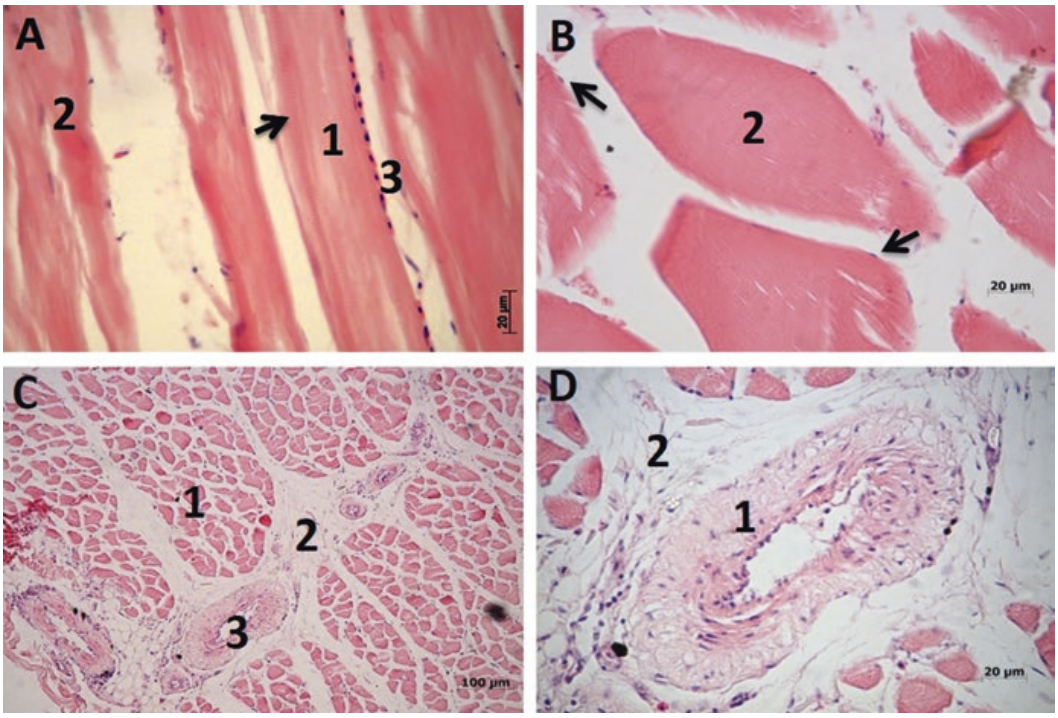

Fig. 16.5 Disposition of fibers and muscle bundles in *S. cavalla*. In **a**, muscle fibers (1) are observed in longitudinal section with peripheral ovoid nuclei (2) as well as characteristic striations (arrow) and a blood channel with nucleated erythrocytes (3); in **b**, in a cross section muscle bundles (2) with peripheral ovoid nuclei (arrows) are observed. They are observed in **c**, muscle bundles (1) and the presence of blood vessels (3) immersed in connective tissue (2); in **d**, approach of a blood vessel (1) and connective tissue (2). H&E staining

16.5.5 Prevalence Matrix or Tissue Evidence

Most of the analized organisms (80%) showed circulatory alterations such as congestive areas and erythrocyte infiltration, whereas 20% presented reversible lesions such as vacuolated hepatocytes and atrophy. Progressive-type lesions were recorded in more than 40% of organisms (eosinophilic secretions, inflammations, melano-macrophage centers, and granulomas).

In the muscle, only congestive vessels were observed with a prevalence of 20%, and not reversible or progressive lesions were observed, which indicates a better condition in this tissue.

The different biological responses were associated with the impact factor, and in general terms, it can be observed that the organisms presented tissue injury whose impact factor fluctuated between 1 and 3 (minimal, moderate, and severe pathological importance, respectively). The muscle presented lesions associated with circulatory disturbances with an impact factor of 1 (minimal importance). However, all the specimens analyzed showed congestion, infiltration, eosinophilic secretions, and melanomacrophage centers.

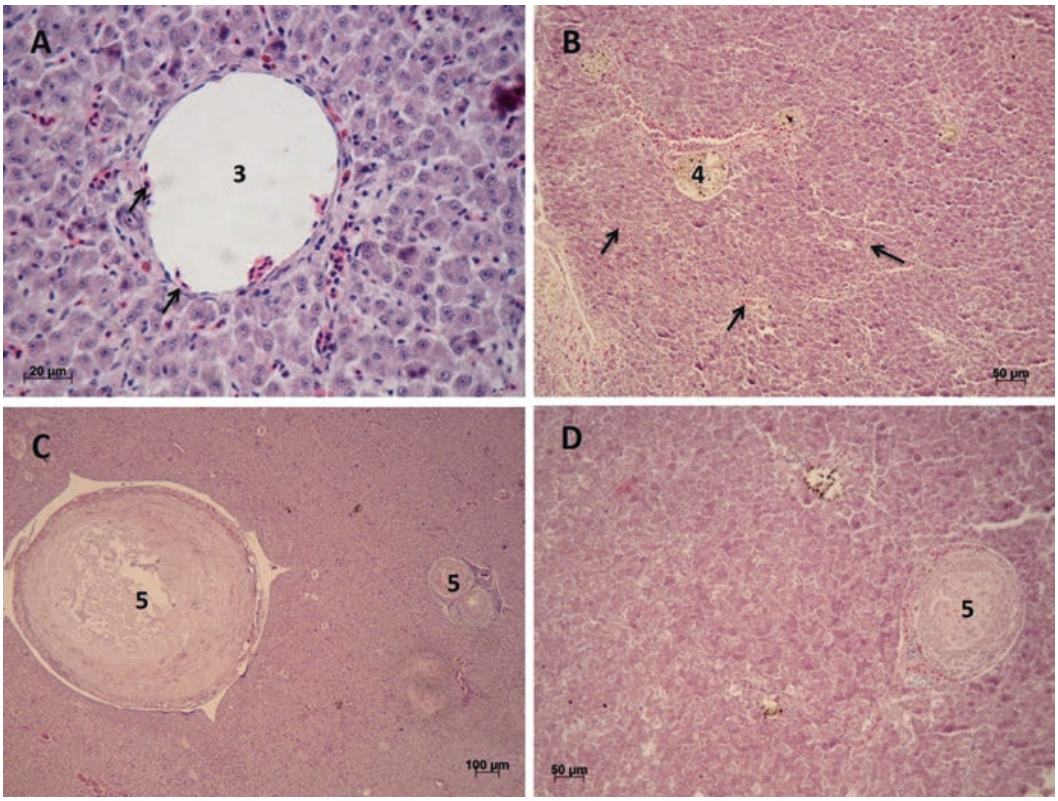

Fig. 16.6 Hepatic lesions related to the circulatory system, inflammations, melanomacrophage centers, and granulomas. In **a**, a focal inflammation (3) is observed as well as some nucleated erythrocytes (arrows); in **b**, it is possible to observe a melanomacrophage center (4) and infiltration in the stroma (arrows); in **c** and **d**, granulomas are observed (5). H&E stain

The main tissue alterations that were observed in the analyzed organs were infiltrations, congestion in the blood conduits, focal inflammations in the hepatic parenchyma, melanomacrophage centers, granulomas, eosinophilic secretions, and, to a lesser extent, some vacuolated hepatocytes, as well as focal atrophy of hepatocytes, which have been described by various authors as pathological lesions in tissues (Takashima and Hibiya 1995; Mumford et al. 2007). Some histological alterations observed in the liver have also been reported in fish species developed in wastewater-impacted ecosystems. Reported lesions include vascular degeneration in hepatocytes, focal areas of necrosis and fibrosis, aggregation of inflammatory cells among hepatocytes, dilation and congestion in blood sinusoids, and the formation of central vein thrombosis (Mohamed 2009).

Another important pathological process in the liver of fish is inflammation, which is observed in 50% of the organisms analyzed. The purpose of the inflammatory response is to dilute, isolate, and destroy the harmful agent to facilitate healing and repair (Mumford et al. 2007). Some protozoa, metazoans, and bacterial and mycotic agents have been reported as agents responsible for inflammatory processes (Humphrey 2007); however, for this study the corresponding analysis was not performed for the identification of agents that provoke this response. The

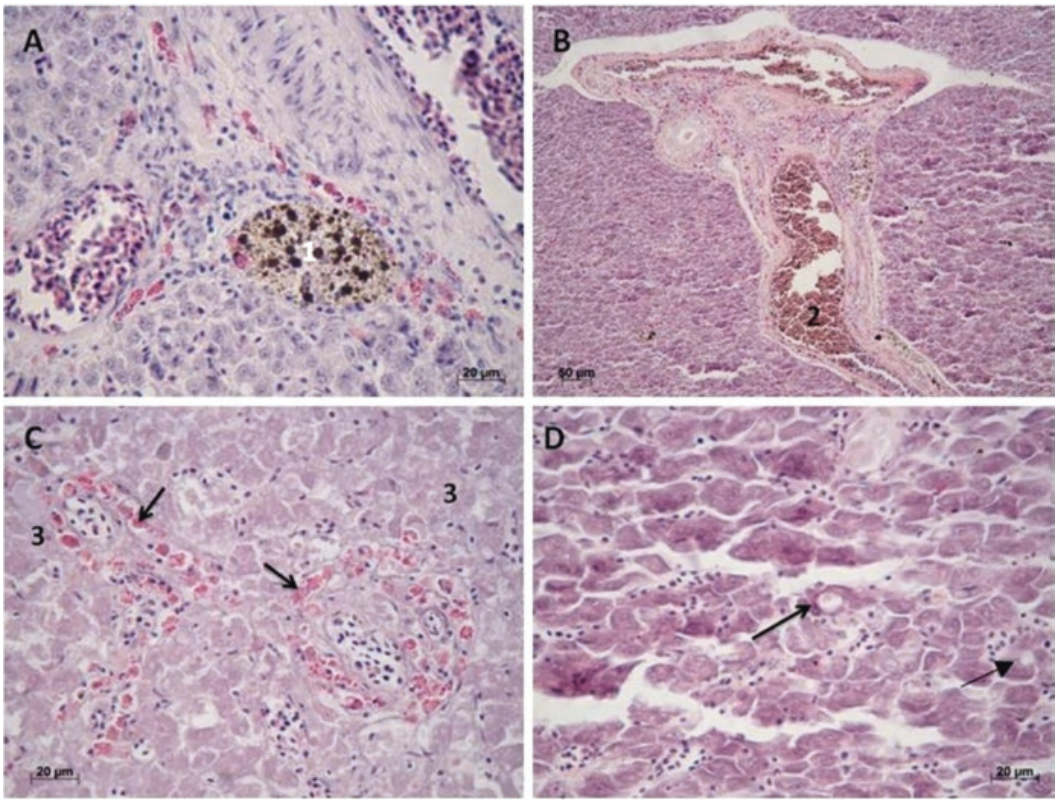

Fig. 16.7 Histopathological lesions in the morphological unit of the liver. In **a**, a melanomacrophage center can be seen in the stroma (1); in **b**, a considerable congestive area (2) is observed; in **c**, it is possible to observe eosinophilic secretions (arrows) as well as atrophy of some hepatocytes (3); in **d**, vacuolated hepatocytes (arrow). H&E stain

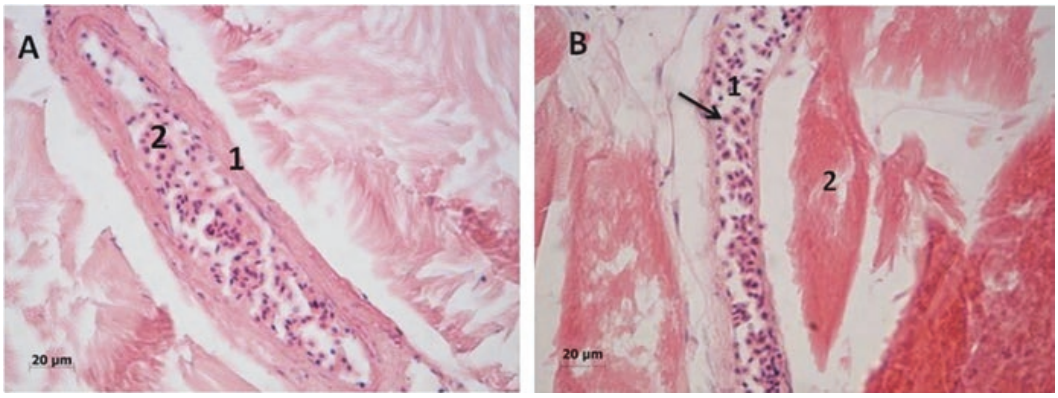

Fig. 16.8 Appearance of congested areas in the muscle of *S. cavalla*. In **a** and **b**, the presence of a congestive blood vessel (1 and 2) with nucleated erythrocytes (arrow) is observed. Staining: H&E stain

melanomacrophagous centers (CMMs) observed in the liver of fish had an incidence of 80%. These have been reported mainly in organs such as the spleen, kidney, liver, gonads, thyroid, and even in the thymus (Humphrey 2007). CMMs are constituted by macrophages, reticular cells, lymphocytes, and plasma cells; they receive that name because pigments such as lipofuscin, melanin, hemosiderin, etc. are produced and stored in the macrophages, among others (Humphrey 2007; Dalmo et al. 1997; Agius and Roberts 2003). CMMs are the product of an immune response to environmental stressors such as heavy metals or in response to infections by intracellular bacteria resistant to phagocytosis, such as *Streptococcus*, *Mycobacterium*, *Renibacterium*, and *Myxobolus* sp., or by nodaviruses, where CMMs increase in number and their macrophages trap large amounts of antigen or whole bacteria and inside it also increases the amount of pigments; however, the role of CMMs in response to infection is still speculative (Humphrey 2007; Agius and Roberts 2003; Hernández et al. 2009). In this work, the high prevalence of CMMs was associated with the presence of Hg and Cr registered in the liver.

The inflammatory response is associated with granulomas, which consist essentially of macrophages and are characterized by circular nodular formations (focal accumulation of macrophages) that occur when chronic inflammatory processes occur (Klatt 2006). Therefore, the presence of granulomas in the liver of *S. cavalla* despite having an incidence of 40% is an important response to consider due to the chronic effect it represents, because it indirectly implies the response of the organisms to conditions of stress in the environment in which they develop.

At the same time, it should be emphasized that eosinophilic secretions were also recorded in 80% of organisms; although its function is not clear, the eosinophilic secretions report a segregation of chemicals that have an affinity to eosin. These responses have been considered as indicative that the organism generates protection measures against a stressor (Sander 1990), as is the case of exposure to pesticides in juvenile tilapia males, where they observed vacuolar changes, eosinophilic inclusions in the cytoplasm of hepatocytes, sinusoidal congestion, and infiltration of eosinophilic granular cells associated mainly with large portal vessels and nuclear alterations (Chaparro et al. 2013).

The morphophysiological structure of the muscle analyzed in the present work indicates that despite the lesions found in the liver as well as the concentrations of metals registered in both organs, no outstanding effects have been recorded in muscle tissue, which forms the edible part of these animals.

16.5.6 Protein Biomarkers by Immunohistochemistry

Hsp70 proteins were detected in the cytoplasm of hepatocytes of organisms with reversible and progressive alterations. These alterations had an impact factor between 1 and 3. Hsp70 proteins in the muscle were not immunodetected (Fig. 16.9).

Besides, as we had mentioned before, the impact factor considers many kinds of quantifications among the alterations. As the alterations presented in the liver

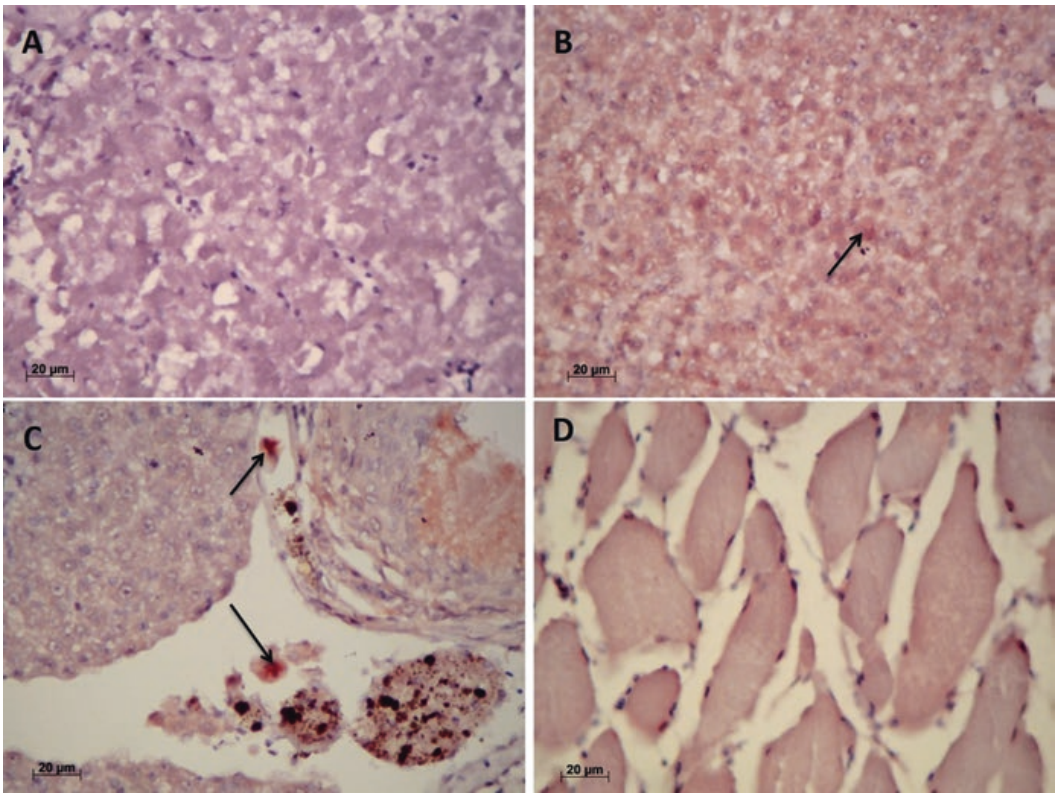

Fig. 16.9 Location of Hsp70 proteins in the liver and muscle sections of *S. cavalla*. In **a**, the negative control is observed; in **b** and **c**, the presence of Hsp70 proteins is evident by the reaction that is visualized in red in the cytoplasm of the hepatocytes (arrows); in **d**, the reaction in muscle tissue was not observed

had an impact factor between 1 and 3, we believed this organ should show presence of metallothionein among the tissue. The antibody we used was first designed for mollusks, so we cannot discard the possible lack of specificity for fish metalloproteins.

Hsp70 is part of an immune response that triggers in the presence of stress factors such as heat or chemical substances (Hoekstra et al. 1999). The immunolocalization of Hsp70 in the cytoplasm of hepatocytes has been reported by several authors, in the presence of heavy metals (Rajeshkumar and Munuswamy 2011; Liu et al. 2012); therefore, there is a possibility that these proteins could be associated with the presence of Hg and Cr in the liver of the *S. cavalla*. Negative results with the MTs antibody in both tissues, and even the absence of Hsp70 in the muscle, should be investigated further.

16.6 Conclusion

It is important that tissue diagnostics have the normalization and systematization to let us establish models to estimate variability through mathematical relations. The responses of the biomarkers analyzed in this study, which are the low index of condition, the presence of mercury in the tissues which exceeded the reference limits, the prevalence of tissue lesions in the liver both reversible and progressive, and the presence of Hsp70 stress proteins in the liver, suggest that fish *S. cavalla* present in Tecolutla, Veracruz, are subject to environmental stress, which directly impacts fish health.

Acknowledgments We thank Meztli Matadamas Guzman for her comments on the manuscript.

References

Adams D, McMichael RH (1999) Mercury levels in four species of sharks from the Atlantic coast of Florida. Fish Bull 97(2):372–379

Adams SM, Shepard KL, Greeley MS et al (1989) The use of bioindicators for assessing the effects of pollutant stress on fish. Mar Environ Res 28(1):459–464

Agboola JI, Anetekhai MA (2008) Length–weight relationships of some fresh and brackish water fishes in Badagry creek, Nigeria. J Appl Ichthyol 24(5):623–625

Agius C, Roberts RJ (2003) Melano-macrophage centres and their role in fish pathology. J Fish Dis 26(9):499–509

Barnabé G, Martínez EC (1996) Bases biológicas y ecológicas de la acuicultura. Acribia, España, p 451

Bernet D, Schmidt H, Meier W et al (1999) Histopathology in fish: proposal for a protocol to assess aquatic pollution. J Fish Dis 22(1):25–34

Bierkens JGEA (2000) Applications and pitfalls of stress-proteins in biomonitoring. Toxicology 153(1):61–72

Bruland GW (1983) Trace elements in seawater. In: Riley JP, Chester R (eds) Chemical oceanography. Academic Press, New York, pp 159–221

Cai Y (2005) Bioaccumulation of mercury in pelagic fishes in NW Gulf of Mexico. A&M University, Texas, p 64

Campos H (1987) Los metales pesados: su contaminación y sus efectos tóxicos. Contaminación ambiental 9(17):63–70

Caso M, Pisanty I, Ezcurra E (2004) Diagnóstico ambiental del Golfo de México, vol 1. Secretaría de Medio Ambiente y Recursos Naturales. Instituto Nacional de Ecología. Instituto de Ecología A.C. Harte Reaserch Institute for Gulf of Mexico Studies 626, México

Chaparro GJ, Aranzazu Taborda DA, Urrea L et al (2013) Immunolocalización hepática de CYP P450 en un experimento de toxicidad subaguda por Clorpirifos 0,0-dietil 0-(3, 5, 6-tricloro-2-piridil fosforotioato) en machos juveniles de tilapia *Oreochromis* spp. CES Medicina Veterinaria y Zootecnia 8:61–72

Clark SL, Kaufman WI, et al (1997) Histopathology, metallothionein, stress and enzyme and inmunohistochemistry as biomarker for cadmium exposure in Asia clam. *Potamocurbula amurensis*. SETAC, USA

Collette BB, Nauen CE (1983) An annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. In: FAO (ed) Scombrids of the world. Food and Agriculture Organization of the United Nations, Rome

Coronato S, Di Girolamo W, Salas M et al (1999) Biología de las proteínas del shock térmico. Medicina 59:477–486

Costopoulos CG, Fonds M (1989) Proximate body composition and energy content of plaice (Pleuronectes platessa) in relation to the condition factor. Neth J Sea Res 24(1):45–55

Cruz-Rodríguez LA, Baucum AJ, Soudant P et al (2000) Effects of PCBs sorbed to algal paste and sediments on the stress protein response (HSP70 family) in the eastern oyster, Crassostrea virginica. Mar Environ Res 50(1):341–345

Dalmo RA, Ingebrigtsen K, Bøgwald J (1997) Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). J Fish Dis 20(4):241–273

Di Giulio RT, Hinton DE (2008) The toxicology of fishes. CRC Press, Boca Raton, p 1071

Díaz-Sánchez A, Aguilar C III (2008) Simposium Nacional de Tiburones y Raya, SOMEPEC U, Alianza WWF-Telcel, editor. México, p 252

Espina S, Vanegas C (2005) Ecotoxicología y Contaminación. In: Botello AV, Rendón von Osten J, Gold Bouchot G, Agraz Hernández C (eds) Golfo de México, contaminación e impacto ambiental: diagnóstico y tendencias (2nd ed., p. 79–120). Campeche, Mexico: Centro EPOMEX. Universidad Autónoma de Campeche

Farris JL, Van Hassel JH (Eds) (2007) Freshwater bivalve ecotoxicology. Florida, USA: SETAC-CRC Press

Feist SW, Longshaw M (2008) Histopathology of fish parasite infections – importance for populations. J Fish Biol 73(9):2143–2160

Feldhausen PH, Johnson D (1983) Ordination of trace metals in Syacium papillosum (dusky flounder) from the eastern Gulf of Mexico. Northeast Gulf Sci 6:9–21

Fitzgerald WF, Clarkson TW (1991) Mercury and monomethylmercury: present and future concerns. Environ Health Perspect 96:159–166

Fitzgerald WF, Engstrom DR, Mason RP et al (1998) The case for atmospheric mercury contamination in remote areas. Environ Sci Technol 32(1):1–7

Fossion R, Fossion J, Rivera AL et al (2018) Homeostasis from a time-series perspective: an intuitive interpretation of the variability of physiological variables. In: Olivares-Quiroz L, Resendis-Antonio O (eds) Quantitative models for microscopic to macroscopic biological macromolecules and tissues. Springer International Publishing, Cham, pp 87–109

Galvão LAC, Corey G (1987) Arsenico. Serie Vigilancia 3. Centro Panamericano de Ecología Humana y Salud, Organización Panamericana de la Salud, Organización Mundial de la Salud, México, p 70

Genten F, Terwinghe E, Danguy A (2009) Atlas of fish histology. J Fish Biol 75. . USA: Science Publishers:757–758

Gray JS (2002) Biomagnification in marine systems: the perspective of an ecologist. Mar Pollut Bull 45(1):46–52

Guzmán-García X (2007) Empleo de biomarcadores para evaluar el proceso de daño en ostión Crassostrea virginica y su respuesta ambiental. Universidad Autónoma Metropolitana, México, p 100

Hanson PJ (1997) Response of hepatic trace element concentrations in fish exposed to elemental and organic contaminants. Estuaries 20(4):659

Helfman G, Collette B, Facey D (1997) The diversity of fishes. Wiley, Malden

Hellawell JM (1989) Biological indicators of freshwater pollution and environmental management. Elsevier Applied Science, London, p 546

Hernández E, Figueroa J, Iregui C (2009) Streptococcosis on a red tilapia, *Oreochromis* sp., farm: a case study. J Fish Dis 32(3):247–252

Hoekstra KA, Iwama G, Nichols CR et al (1999) Increased heat shock protein expression after stress in Japanese Quail. Stress 2:265–272

Huggett RJ, Kimerle RA, Mehrle PM et al (1992) Biomarkers: biochemical, physiological, and histological markers of anthropogenic stress. Lewis Publishers, Boca Raton, p 347

Humphrey JD (2007) Systemic pathology of fish: a text and atlas of normal tissues in teleosts and their response in disease. J Fish Dis 30(6):381–382

Johnson LL, Stehr CM, Olson OP et al (1993) Chemical contaminants and hepatic lesions in winter flounder (*Pleuronectes americanus*) from the northeast coast of the United States. Environ Sci Technol 27(13):2759–2771

Klaassen CD, Liu J, Choudhuri S (1999) Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu Rev Pharmacol Toxicol 39(1):267–294

Klatt EC (2006) Robbins and Cotran atlas of pathology, 3rd edn. Elsevier Saunder, Savannah, p 600

Koca YB, Koca S, Yıldız Ş et al (2005) Investigation of histopathological and cytogenetic effects on Lepomis gibbosus (Pisces: Perciformes) in the Çine stream (Aydın/Turkey) with determination of water pollution. Environ Toxicol 20(6):560–571

Kuklyte L (2012) Mercury contamination in pelagic fishes of the Gulf of Mexico. Texas A&M University, College Station

Lacerda LD, Paraquetti HHM, Marins RV et al (2000) Mercury content in shark species from the south-eastern Brazilian coast. Rev Bras Biol 60:571–576

Landis WG, Yu MH (1999) Introduction to environmental toxicology. Lewis Publishers, New York, p 390

Liu D, Chen Z, Zhou X (2012) Detection of heat shock proteins 70 in the gill, liver, and cardiac muscle of Carassius auratus with confocal microscopy. Microsc Res Tech 75(4):531–536

Maddock DM, Burton MPM (1998) Gross and histological observations of ovarian development and related condition changes in American plaice. J Fish Biol 53(5):928–944

Mancera-RodrÍguez NJ, Álvarez-León R (2006) Estado del conocimiento de las concentraciones de mercurio y otros metales pesados en peces dulceacuícolas de Colombia. Acta Biológica Colombiana 11(1):3–23

Martínez-Jerónimo F (1991) El papel del bioensayo en la evaluación de la toxicidad acuática. In: Figueroa MC et al (eds) Fisicoquímica y Biología de las lagunas Costeras Mexicanas. "Grandes temas de la Hidrobiología". UAMI, México, pp 57–65

Mohamed FAS (2009) Histopathological Studies on Tilapia zillii and Solea vulgaris from Lake Qarun, Egypt. World J Fish and Marine Sci 1(1):29–39

Monteiro LR, Costa V, Furnes RW et al (1996) Mercury concentrations in prey fish indicate enhanced bioaccumulation in mesopelagic environments. Mar Ecol Prog Ser 141:21–25

Moreno Grau MD (2003) Toxícología ambiental. Evaluación de riesgo para la salud humana. Madrid, España: McGraw-Hill

Mughal M, Malkani N, Jahan N (2004) Histopathological changes in kidney and muscle of *Labeo rohita* due to cadmium intoxication. Biologia 50:39–45

Mumford S, Heidel J, Smith C, et al (2007) Fish histology and histopathology manual. U. S. Fish & Wildlife Service: National Conservation Training Center, p 356. USA

Nollen EA, Morimoto RI (2002) A heat shock response: cellular and molecular responses to stress, misfolded proteins, and diseases associated with protein aggregation. In: Wiley J (ed) Encyclopedia of molecular medicine. Wiley, New York, pp 1553–1556

Nuñez G, Bautista-Ordoñez J, Rosiles-Martinez R (1998) Concentración y distribución de mercurio en tejidos del cazón (*Rhizoprionodon terraenovae*) del Golfo de México. Vet Mex 29:15–21

Padrós F, Zarza C (2005) Manual de Técnicas Básicas de diagnóstico patológico en peces. In: V curso de Ictiopatología Práctica para Piscicultores. Situación Sanitaria Actual del cultivo de la Dorada y Lubina

Páez-Osuna F (2005) Efectos de los metales, in Golfo de México. Contaminación e Impacto ambiental: Diagnostico y Tendencias, Botello A, et al, Editors., Instituto de Ecología, Pesquerías y Oceanografía del Golfo de México: Universidad Nacional Autónoma de México, Instituto de Ciencias del Mar y Limnología: México. p. 696

Ploetz DM, Fitts BE, Rice TM (2007) Differential accumulation of heavy metals in muscle and liver of a marine fish, (king mackerel, *Scomberomorus cavalla* Cuvier) from the northern Gulf of Mexico, USA. Bull Environ Contam Toxicol 78(2):134–137

Radlowska M, Pempkowiak J (2002) Stress-70 as indicator of heavy metals accumulation in blue mussel *Mytilus edulis*. Environ Int 27(8):605–608

Rajeshkumar S, Munuswamy N (2011) Impact of metals on histopathology and expression of HSP 70 in different tissues of Milk fish (*Chanos chanos*) of Kaattuppalli Island, South East Coast, India. Chemosphere 83(4):415–421

Rand GM, Wella PG, McCarthy JF (1995) Introduction to aquatic ecology. In: Rand GM (ed) Fundamentals of aquatic toxicology. Taylor and Francis, London, pp 3–53

Regnell O, Ewald G, Lord E (1997) Factors controlling temporal variation in methyl mercury levels in sediment and water in a seasonally stratified lake. Limnol Oceanogr 42(8):1784–1795

Roesijadi G (1992) Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat Toxicol 22(2):81–113

Sander B (1990) In: JF MC, Shugart LR (eds) Stress proteins: potential as multitiered biomarkers, in biomarkers of environmental contamination. Lewis Publishers, Boca Raton, pp 165–192

Stafford CP, Haines TA (1997) Mercury concentrations in Maine sport fishes. Trans Am Fish Soc 126(1):144–152

Svobodová Z, Lloyd R, Máchová J et al (1993) Water quality and fish health. FAO, Roma, p 67

Takashima F, Hibiya T (1995) An atlas of fish histology: normal and pathological features, 2nd edn. Kodansha Limited, Tokyo

Treer T, Šprem N, Torcu-Koc H et al (2008) Length–weight relationships of freshwater fishes of Croatia. J Appl Ichthyol 24(5):626–628

Ureña-Robles R (2007) Metalotioneínas en peces y gasterópodos: su aplicación en la evaluación de la contaminación. In: Departamento de Biología Funcional. Universidad de Valencia, Valencia, p 218

Vázquez FG, Sharma V, Mendoza QA et al (2001) Metals in fish and shrimp of the Campeche Sound, Gulf of Mexico. Bull Environ Contam Toxicol 67:756–762

Wang D, Ueng JP, Huang BQ (2004) Structural changes in the muscular tissue of thornfish (Terapon jarbua, Forsskal) under TBT (tributyltin) exposure. J Fish Soc Taiwán 31:225–234

Chapter 17
Histopathological Assessment of Organisms in Ecotoxicological Studies from Mexico

Meztli Matadamas-Guzman, Irma Hernández-Calderas, Juan Carlos Segoviano Ramírez, and Xochitl Guzmán-García

17.1 Introduction

Environmental monitoring offers tools to detect toxic components and evidence their effects on living organisms over time, through a process of sampling and analyzing specific environmental media (soil, water, plants or animals). Organisms, which have been exposed to toxic agents, show changes at different biological levels. Tissues are at a middle level, so histopathology is a useful tool to detect early changes produced in organs before irreversible damage reaches higher biological levels (Bernet et al. 1999). Histological characterization of specific organs can reflect the physiological condition and integrate different time scales and also endogenous and exogenous impact coming from lower biological levels (Van Dyk et al. 2009). Currently, environmental monitoring programs are in a development phase, so histopathology is a fast and low-cost tool to evaluate the contaminants exposure level. Although the interpretation can be subjective, consequently, it is necessary to standardize protocols to evaluate tissue alterations and set scales to establish a health index. This health index might be used as a reference in aquatic environmental monitoring programs.

In different studies, fish alterations have been trusted biomarkers of the presence of contaminants. Fish are often exposed to diverse contaminants; these contaminants induce changes in target organs, especially on the liver and gills. The liver and gills are the most typical organs studied in environmental monitoring, as they are the

M. Matadamas-Guzman
Doctorado en Ciencias Biomédicas, UNAM, México City, Mexico

I. Hernández-Calderas · X. Guzmán-García (✉)
Universidad Autónoma Metropolitana, Unidad Iztapalapa, México City, Mexico
e-mail: xgg@xanum.uam.mx

J. C. Segoviano Ramírez
Unidad de Bioimagen del Centro de Investigación y Desarrollo en Ciencias de la Salud (CIDICS), Universidad Autónoma de Nuevo León (UANL), Monterrey, Nuevo León, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_17

major targets of contaminants. In fish, the liver is a key organ for digestion, nutrient absorption, sexual hormone metabolism, and xenobiotic metabolism. As well, gills are susceptible organs, in charge of respiration, osmoregulation, acid-base balance, and nitrogen excretion (Liebel et al. 2013; Reddy and Rawat 2013). Thus, the study of tissue from the liver and gills reflects directly the effects of toxic components in the organisms before their death.

17.2 Assessment Methods to Value Tissue Alterations in Fish

Currently, different histopathological studies are trying to integrate environmental effects through health indexes. However, evaluation protocols have not been standardized, and there are many differences that make difficult to establish fish condition and, indirectly, environment health status. Otherwise, each study assesses lesions on organisms in different ways, so the results of these analyses are not comparable between them. Here we present two established methods to score tissue alterations in different organs, also to assess their impact in fish health, and finally to predict environment health status (Bernet et al. 1999).

The first method was developed by Bernet et al. (1999); it is divided into two stages, histological description and histopathological evaluation. During histological description, pathological alterations on each organ are evaluated and classified in five categories, called reaction patterns:

1. Circulatory disturbances.
2. Reversible changes.
3. Progressive changes.
4. Inflammation.
5. Tumors.

These reaction patterns include different alterations which involve either functional units of an organ (Fig. 17.1). Subsequently, on histological evaluation, each alteration listed in the histological description is rated depending on the importance factor and score value. The importance factor (w) depends on how the lesion affects organ function and fish survival. Three importance factors are used: (1) minimal pathological importance, (2) moderate, and (3) high importance, depending if it is reversible or not. Moreover, the score value (a) rank every lesion from 0 to 6, depending on the occurrence. Table 17.1 shows fish data analyzed from the gills, liver, and skin ranking different lesions.

Once this analysis is finished, we can estimate diverse health indexes. Organ index (I_{org}) and reaction index of an organ ($I_{org\,rp}$) are applicable when only one organ is examined. Organ index represents the degree of damage to an organ, while reaction index of an organ represents the quality of the lesions in an organ. On the other hand, total index (Tot-I) and total reaction index (I_{rp}) are applicable when several organs are examined. Total index represents a measure of the overall health status based on histological lesions, while total reaction index represents the quality

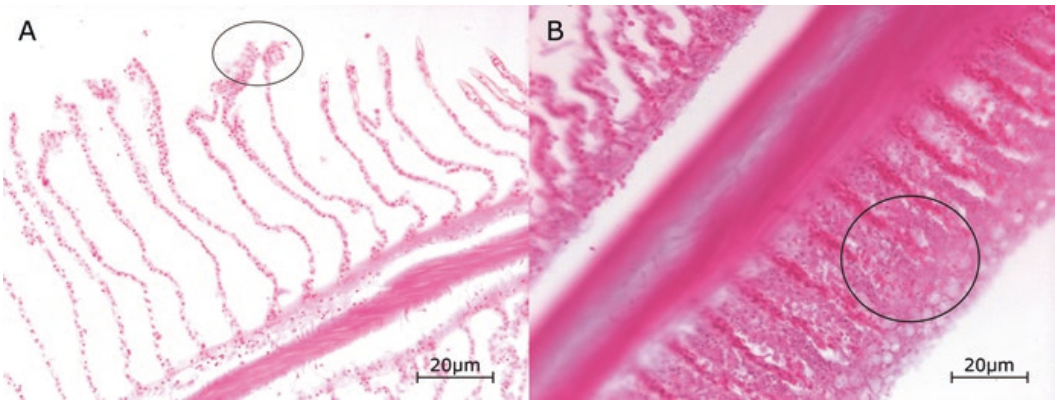

Fig. 17.1 Examples of alterations seen on the gills. (**a**) Epithelial tissue with edema in second gill lamellae (importance factor = 1 and score value = 2). (**b**) Hyperplasia and fusion of secondary lamellae (importance factor = 2 and score value = 2)

of the histological lesions in all examined organs of an individual fish. As all these indexes are calculated with the same algorithm each time for each organ or each complete organism, we can compare between the same organ of various fish or between different fish.

Further to complement this method, the second method, developed by Zimmerli et al. (2007), propose to classify lesions in four categories, according to values of organ indexes:

- Class 1: $I_{org} < 10$ (normal/healthy structure) Tissue architecture and histology are well developed and show no impairments or pathological changes.
- Class 2: I_{org} 11–20 Slight modifications of normal tissue architecture and morphology (e.g., change in cell size) are present.
- Class 3: I_{org} 21–30 Moderate modifications of normal tissue architecture and morphology are present.
- Class 4: I_{org} 31–40 Pronounced modifications of normal tissue architecture and morphology are present.
- Class 5: $I_{org} > 40$ Serious alterations of normal tissue architecture and morphology are present.

These categories allow us to differentiate between fish with pathologically altered organs from those with non-altered organs. Using this method we can easily correlate the health status of organisms with the health status of a specific place, allowing us to compare different places or the same place over time. Therefore nowadays, the combination of different methods enables the standardization of histological analysis, making histology a semiquantitative method to score the health status of the environment.

Table 17.1 Importance factor assigned to each alteration found in different tissues. We added histopathological alterations found on fish collected at Tecolutla. The importance factor is used later to calculate organ and total indexes

Reaction pattern	Functional unit	Alteration	Tecolutla	Importance factor
Gills				
Circulatory disturbances		Hemorrhage/hyperemia/ aneurysm	Yes	1
		Intercellular edema	No	1
		Telangiectasia∗	Yes	2
Regressive changes	Epithelium/supporting tissue	Structural alterations	No	1
		Plasma alterations	No	1
		Atrophy	Yes	2
		Necrosis	No	3
		Rupture of the pillar cells	Yes	2
Progressive changes	Epithelium/supporting tissue	Hypertrophy	No	1
		Hyperplasia	Yes	2
		Lamellar fusion∗	Yes	3
Inflammation		Exudate	No	1
		Activation of RES	No	1
Tumor		Benign tumor	No	2
		Malignant tumor	No	3
Liver				
Circulatory disturbances		Hemorrhage/hyperemia/ aneurysm	No	1
		Intercellular edema	No	
		Structural alterations	No	1
		Plasma alterations	No	1
Regressive changes	Liver tissue/interstitial tissue/bile duct	Atrophy	Yes	2
		Necrosis	No	3
		Vacuolar degeneration	Yes	3
Progressive changes	Liver tissue/interstitial tissue/bile duct	Hypertrophy	Yes	1
		Hyperplasia	No	2
		Melanomacrophage aggregates∗	Yes	2
Inflammation		Exudate	No	1
		Activation of RES	No	1
Tumor		Benign tumor	No	2
		Malignant tumor	No	3
Skin				
Circulatory disturbances		Hemorrhage/hyperemia/ aneurysm	Yes	1
		Intercellular edema	No	1
		Structural alterations	No	1
		Plasma alterations	No	1

(continued)

Table 17.1 (continued)

Reaction pattern	Functional unit	Alteration	Tecolutla	Importance factor
Regressive changes	Epidermis/dermis	Atrophy	No	2
		Necrosis	No	3
Progressive changes	Epidermis/dermis	Hypertrophy	No	1
		Hyperplasia	No	2
		Hyperplasia of mucous cells	No	2
Inflammation		Exudate	No	1
		Activation of RES	No	1
Tumor		Benign tumor	No	2
		Malignant tumor	No	3

17.3 Semiquantitative Analysis of Organisms from Tecolutla, Veracruz, Mexico

Tecolutla, Veracruz, has been monitored for many years in the Gulf of Mexico, since this place is an estuary. An estuary is the part of a river where it joins the sea and where freshwater and saltwater are mixed. Organisms that live in this place are affected by different pollutants, coming from industry and people around the place. As fishing is one of the main economic activities of Tecolutla, looking for the health status of the organisms is very important. Here we applied the methods above to fish collected in Tecolutla, to further score the health status of fish and the environment.

The gills, liver, and skin were the target organs of this study. These organs were fixed in buffered 10% formalin, embedded in paraffin wax, and sectioned according to routine histological protocols. The 5 μm sections were stained with hematoxylin and eosin (HE stain). A total of 378 fields were observed in order to get tissue alterations of these organs (Fig. 17.1). We identify the alterations and classified them in the five reaction patterns described above (Table 17.1).

In average, total index of these organisms was between 21 and 30. This average indicates organisms from Tecolutla have lesions of class 3 (moderate modifications). Thus, pollutants are starting to affect fish in Tecolutla. Despite this, we cannot discard the possibility that regional pollutants are affecting other organisms or that fish have been adapting enough to not have evident lesions on the target organs. It is important to make this kind of studies over time to compare the effects of pollutants on fish. Also, it is relevant to develop these types of semiquantitative analysis in other organisms used as biomarkers, like oysters and plants, among others.

Many labs have used and modify health indexes with the aim to unify criteria about how tissues of diverse organs are altered by environmental pollutants (Table 17.2). However it is important that coming studies use the same methods to make data comparable and assess correctly the environmental health status. Also, the histopathological diagnosis should consider the basal state of the organisms; these organisms normally have moderate presence of alterations, like minimal

Table 17.2 Histopathological evaluations that use indexes to assess health status on diverse organs and species

Organs	Species	Index	Reference
Gill, liver, and muscle	*Scomberomorus cavalla*	I_{org}, $I_{org\ rp}$, *Tot-I*, I_{rp}	Reyes-Márquez (2018)
Liver and gill	*Notothenia coriiceps* and *Notothenia rossii*	I_{org}	Donatti et al. (2012)
Gill and digestive glandule	*Ruditapes decussatus*	I_{org} (modified)	Costa et al. (2013)
Liver and gill	*Astyanax aff. Fasciatus* *Oreochromis niloticus*	I_{org}	Liebel et al. (2013)
Gill, kidney, and liver	*Odontesthes argentinensis*	I_{org}	Pereira et al. (et al. 2012)
Liver	*Clarias gariepinus*	I_{org}	van Dyk et al. (2012)
Gill	*Salmo salar*	I_{org}	Mitchell et al. (2012)
Kidney, liver, and gills	Demersal fish	I_{org}	Lukin et al. (2011)
Liver	*Clarias gariepinus*	I_{org}	van Dyk et al. (2009)
Liver	*Clarias gariepinus*	I_{org}	Marchand et al. (2009)
Liver and kidney	*Salmo trutta*	I_{org}	Zimmerli et al. (2007)

structural changes and inflammatory reactions (Marchand et al. 2009). This assessment is crucial over time to have records of how environment is changing and take better decisions on future environmental legislation.

17.4 Conclusions

Semiquantitative evaluation of tissue alterations allows objective validation of the diagnosis. However, it involves strict procedures for sample handling, storage, and microscopic analysis. Histopathological protocols, for the evaluation of environmental stress, have proven to be very useful to evaluate the physiological or health status of the organisms and the system where they develop. It is possible to improve routine diagnosis through the use of computational programs and digital systems to allow more robust statistics, such as the application of serological analysis. Nevertheless, all studies that use semiquantitative methods help to determine the health status of the environment and also to keep a record of the environmental changes over time. This study of Tecolutla is the first one that scores the health status of this place. Being the closest beach to Mexico City, it is important to track the environmental status of Tecolutla, Veracruz.

Histopathology is a useful and low-cost tool that allows to establish health indexes. These indexes could be reference values which can be used to establish strategies for the use, care, and exploitation of aquatic resources. Finally, we suggest that these health indexes should be considered within Mexican legislation as a parameter of environmental health.

Acknowledgments We thank Dr. Jaime García Juárez for assistance with image edition and analysis, at Unidad de Bioimagen del Centro de Investigación y Desarrollo en Ciencias de la Salud (CIDICS), Universidad Autónoma de Nuevo León (UANL), and Dr. Mayra Pamela Becerra Amezcua for comments that greatly improved the manuscript.

References

Bernet D, Schmidt H, Meier W et al (1999) Histopathology in fish: proposal for a protocol to assess aquatic pollution. J Fish Dis 22(1):25–34

Costa PM, Carreira S, Costa MH et al (2013) Development of histopathological indices in a commercial marine bivalve (Ruditapes decussatus) to determine environmental quality. Aquat Toxicol 126:442–454

Donatti L, Rios F, Machado C et al (2012) Histopathological Alterations on Antarctic Fish *Notothenia Coriiceps and Notothenia Rossii* as Biomarkers of Aquatic Contamination. INCT-APA Annual Activity Report, 139–143

Liebel S, Tomotake MEM, Ribeiro C (2013) Fish histopathology as biomarker to evaluate water quality. Ecotoxicol Environ Contamin 8:9–15

Lukin A, Sharova J, Belicheva L et al (2011) Assessment of fish health status in the Pechora River: effects of contamination. Ecotoxicol Environ Saf 74(3):355–365

Marchand MJ, Van Dyk JC, Pieterse GM et al (2009) Histopathological alterations in the liver of the sharptooth catfish *Clarias gariepinus* from polluted aquatic systems in South Africa. Environ Toxicol 24(2):133–147

Mitchell SO, Baxter EJ, Holland C et al (2012) Development of a novel histopathological gill scoring protocol for assessment of gill health during a longitudinal study in marine-farmed Atlantic salmon (*Salmo salar*). Aquac Int 20(5):813–825

Pereira Gusmão E, Vieira Rodrigues R, Bonucci Moreira C et al (2012) Growth and histopathological effects of chronic exposition of marine Pejerrey Odontesthes argentinensis larvae to petroleum water-soluble fraction (WSF). Ambio 41(5):456–466

Reddy P, Rawat SS (2013) Assessment of aquatic pollution using histopathology in fish as a protocol. Vol 2:79–82

Reyes-Márquez A (2018) Histopatología de Scomberomorus cavalla Cuvier 1829 (Perciformes: Scombridae) como biomarcador de la contaminación acuática. In: *Posgrado en Biología*. Universidad Autónoma Metropolitana, Mexico, p 74

Van Dyk JC, Marchand MJ, Smit NJ et al (2009) A histology-based fish health assessment of four commercially and ecologically important species from the Okavango Delta panhandle, Botswana. Afr J Aquat Sci 34(3):273–282

Van Dyk JC, Cochrane MJ, Wagenaar GM (2012) Liver histopathology of the sharptooth catfish *Clarias gariepinus* as a biomarker of aquatic pollution. Chemosphere 87(4):301–311

Zimmerli S, Bernet D, Burkhardt-Holm P et al (2007) Assessment of fish health status in four Swiss rivers showing a decline of brown trout catches. Aquat Sci Res Across Bound 69:11–25

Chapter 18
Histopathological Analysis of the Intestine from *Mugil cephalus* on Environment Reference Sites

Gabriela Huerta-Aguirre, Kartagena María Paredes-Ramos, Mayra Pamela Becerra-Amezcua, Irma Hernández-Calderas, Meztli Matadamas-Guzman, and Xochitl Guzmán-García

18.1 Introduction

Pollution problems, caused by the increase in the incorporation of chemical substances in the environment, have received great attention during the last two decades, because of their ecological impact in the biota and potential damage to the health of consumers (Sarasquete Reidiz et al. 1999).

Since pollution is a global issue, environmental monitoring programs have been established. Actually, these programs are no longer limited to the evaluation of physicochemical parameters of the environmental sites of interest but also include ecotoxicological analysis. Currently, long-term environmental monitoring programs are considered indispensable tools to take decisions on environmental management. In Mexico, the National Environmental Monitoring and Assessment Program-Programa Nacional de monitoreo y evaluación ambiental (PRONAME) was implemented in the period 2010–2014. To achieve this purpose, different campaigns are carried out in different parts of the country, where samples of water, sediment, soil and biota are collected for the detection of chemical substances that determine the toxicity, and finally, histopathological studies are carried out in fish to determine their health. This is the case of Guasimas, Sonora, and Coatzacoalcos, Ver., in Mexico, called sites of reference in the PRONAME. Histology is a tool that allows us to detect changes induced by contaminants in organism's tissues as an intermediate response to sublethal stressors, especially the chronic ones in the

G. Huerta-Aguirre · K. M. Paredes-Ramos · I. Hernández-Calderas · X. Guzmán-García (✉)
Universidad Autónoma Metropolitana, Unidad Iztapalapa, Mexico City, Mexico
e-mail: xgg@xanum.uam.mx

M. P. Becerra-Amezcua
Laboratorio de Biotecnología y Toxicología Molecular, Departamento Recursos del Mar, Centro de Investigación y de Estudios Avanzados, Unidad Mérida, Mérida, Yucatan, Mexico

M. Matadamas-Guzman
Doctorado en Ciencias Biomédicas, UNAM, Mexico City, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_18

tissues of organisms (Johnson et al. 1993). Tissues are a group of cells that have the same embryonic origin and that are differentiated and grouped to fulfill different functions (Welsch and Sobotta 2008).

In the Gulf of Mexico, there are several pollution problems; Botello and collaborators (2005) report studies on fish, mollusks, and crustaceans at Coatzacoalcos, Veracruz, where they found high concentrations of polycyclic aromatic hydrocarbons (PAHs) of high toxicity and potentially carcinogenic, according to the surrounding refineries, among other anthropogenic activities. On the Pacific side of the state of Sonora, at Guasimas, Arreola-Lizarraga (1995) reports that there are high levels of pollution due to untreated wastewater deposition, generated by human settlements and agricultural activities. The results presented by Villegas and collaborators (1985) showed concentrations of pesticides in sediment, among the most frequent were DDT and heptachlor, which are extremely toxic and persistent compounds.

The fishes have developed certain adaptations that allow them to avoid damage to external pathogens, for example, the scales. However, other pollution sources can easily enter your body through your mouth. This route is the entry of numerous bacteria, viruses, protozoans, helminths, and toxic components, among others, reflecting several alterations on the digestive system induced by the environment (Reichenbach 1977).

18.2 The Digestive Systems in *Mugil cephalus*

The digestive system is formed by a modified conductor (digestive tube) that extends from the mouth to the anus. In the different groups of vertebrates, the digestive system presents the same structural plan: an anterior intestine formed by the mouth, pharynx, esophagus, and stomach, a medium or small intestine, and a posterior large intestine that ends in the rectum and anus (Estrada-Flores and Uribe-Aranzábal 2002). During millions of years of evolution, the digestive system of fish has been widely modified showing marked differences in function and morphology as an adaptation to the abuse on the environment (Bullock and Bunton 2000). The differences observed at specific sites of the digestive tract are related to the type of food, the feeding habits, body size, shape, and sex of the fish (ÇInar and Şenol 2006).

The smooth fish or *Mugil cephalus* (Fig. 18.1) is representative of the Mugilidae family. They constitute a group within commercial fishing (Castelló Orvay 1993); according to CONAPESCA (2013), it is reported that the mullet for its volume is positioned in the 17th place of the fishing production in Mexico with an annual catch of 9898 tons.

Some important biological features that distinguish this species from other fish is that it has a cylindrical and robust body; its head is wide; the labial teeth of the upper jaw are small, straight, dense, and usually in several lines; the buccal commissure ends below the posterior nasal; its blue/greenish color on the back, flanks and

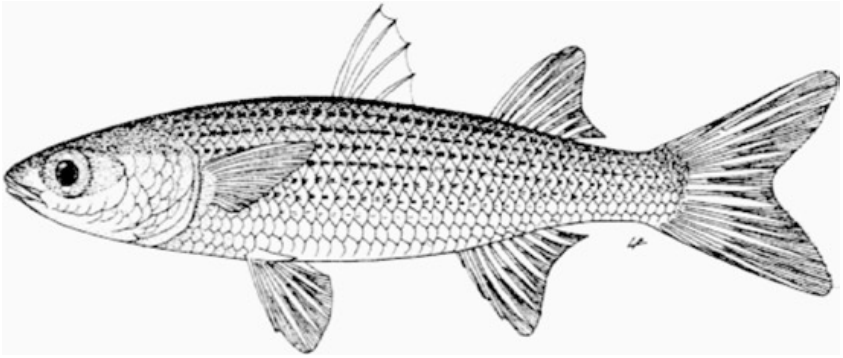

Fig. 18.1 External anatomy of *M. cephalus*. (Picture of FAO 2016)

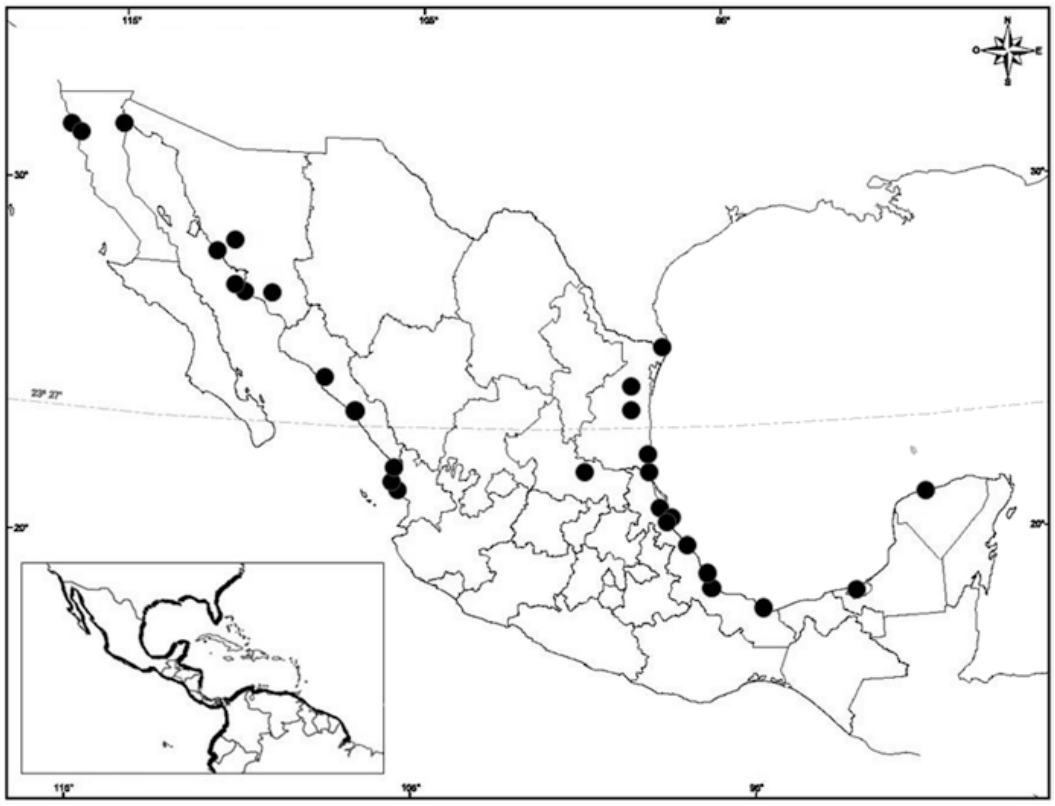

Fig. 18.2 Distribution of *M. cephalus* in Mexico and North and Sur America. (Picture of Rush et al. 2009)

abdomen pale or silver; scales on the back and flanks aligned to form longitudinal stripes; and dark pectoral axillary stain (Saleh 2006). The maximum observed length is 120 cm, and the maximum weight is 8 Kg; the duration of life is between 4 and 16 years (Fischer et al. 1995). This fish has diurnal eating habits, consuming mainly zooplankton, decaying plant material and detritus (Saleh 2006). They inhabit subtropical and tropical seas in Mexico (Fig. 18.2) (Robins et al. 1991).

Since the digestive system is one of the organs where the alterations induced by the environment can be seen, the aim of this study was to evaluate the health on the fish *Mugil cephalus* of Guasimas, Sonora, and Coatzacoalcos, Veracruz, in Mexico, through the characterization and analysis of the tissue and histopathological structure of the midgut.

18.3 Methods

Study Area Fishes *M. cephalus* ($n = 3$) were collected in two zones from México (Fig. 18.3).

Macroscopic Description and Histological Analysis The morphometric parameters (total length, width, high, and weight) were analyzed with an ichthyometer and Vernier. The intestine was delimited, dissected, and measured. The middle part of the intestine was fixed in 10% formaldehyde for its subsequent histological process that includes dehydration and paraffin embedding. For this procedure, a LEICA® brand tissue processor (TP1020), a LEICA® brand inclusion system (EG1140-H), and a LEICA® brand chiller plate model EG1140-C were used. For light microscopy, a MICROM® rotary microtome (HM3156) was used to cut 5-micrometer-thick tissue sections which were mounted on a glass microscope slide, making three replicates of each one. The obtained sections were stained with H&E stain. The tissue structures were analyzed using a CARL ZEISS® Primo Star optical microscope coupled with a CANON® digital camera model PowerShot G10.

18.4 Results and Discussion

Macroscopic Description The fish of the species *M. cephalus* presented on average a length of 32 cm, a height of 22.25 cm, a width of 3.15 cm, and a weight of 237 g (Fig. 18.4).

The digestive tract included the oral cavity, esophagus, stomach, and intestine. The oral cavity was small and was located in the middle part of the head; the tongue and teeth were absent. The stomach had a firm consistency, and it was followed by the liver, the intestine, and the anus. The intestine was found toward the ventral part of the abdominal cavity, and it was found completely wrapped, with a total length of 1.74 m (Figs. 18.5 and 18.6).

According to the description of the digestive tract, we determined that *Mugil cephalus* is a fish with detritivore feeding habits; this means they feed from the superficial layer of the sediment which contains organic matter (Yáñez-Arancibia and Nuget 1975). The intestine of *M. cephalus* has a sandy consistency, due to the fact that during its feeding this fish eats large quantities of fine sand that helps the

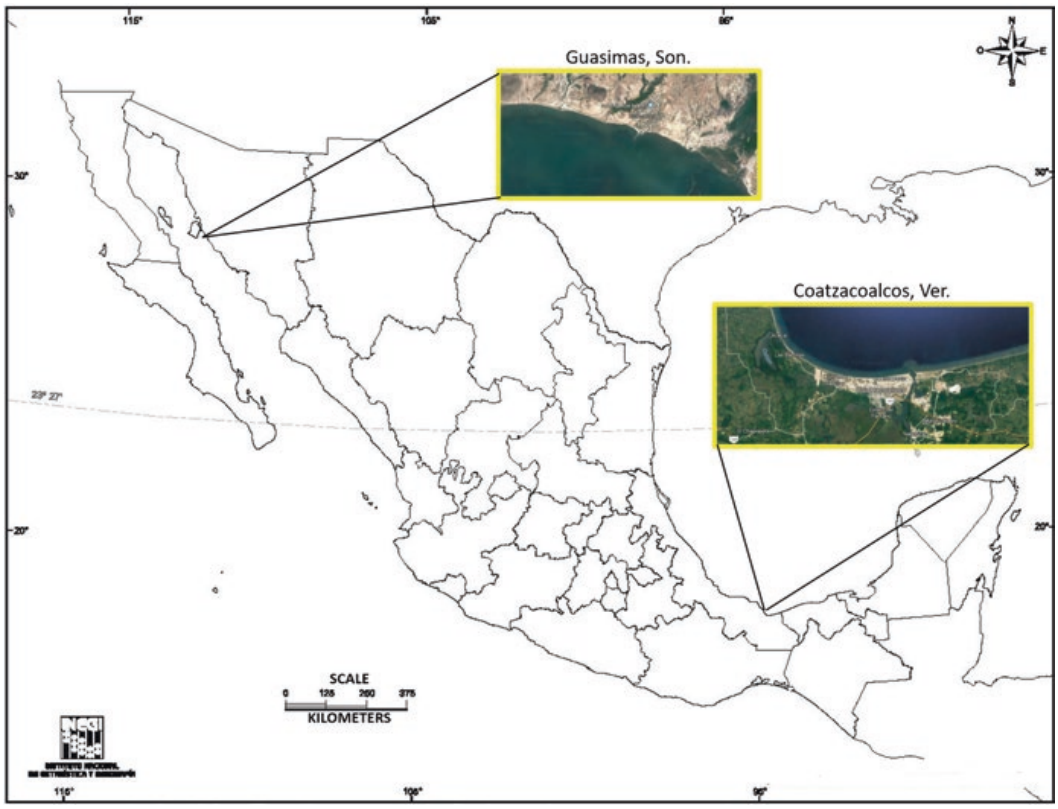

Fig. 18.3 Geographic location of study areas

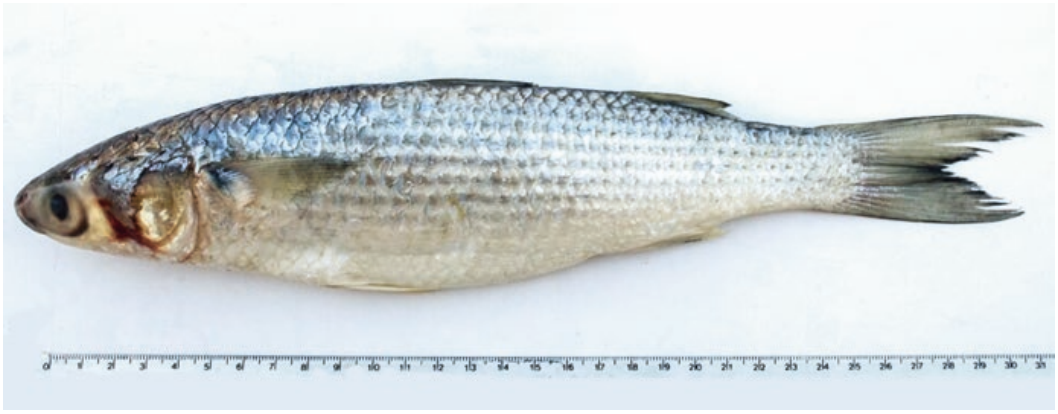

Fig. 18.4 Appearance external of *M. cephalus*

digestive processes (Sánchez 1995). All these characteristics allow these organisms to optimize the swallowing and absorption of sediments and detritus (Sánchez 1995).

Histological Analysis Fifty histological sections were analyzed, characterizing the four constitutive layers of the intestine middle part: mucosa, submucosa, muscular layer, and serosa (Fig. 18.7).

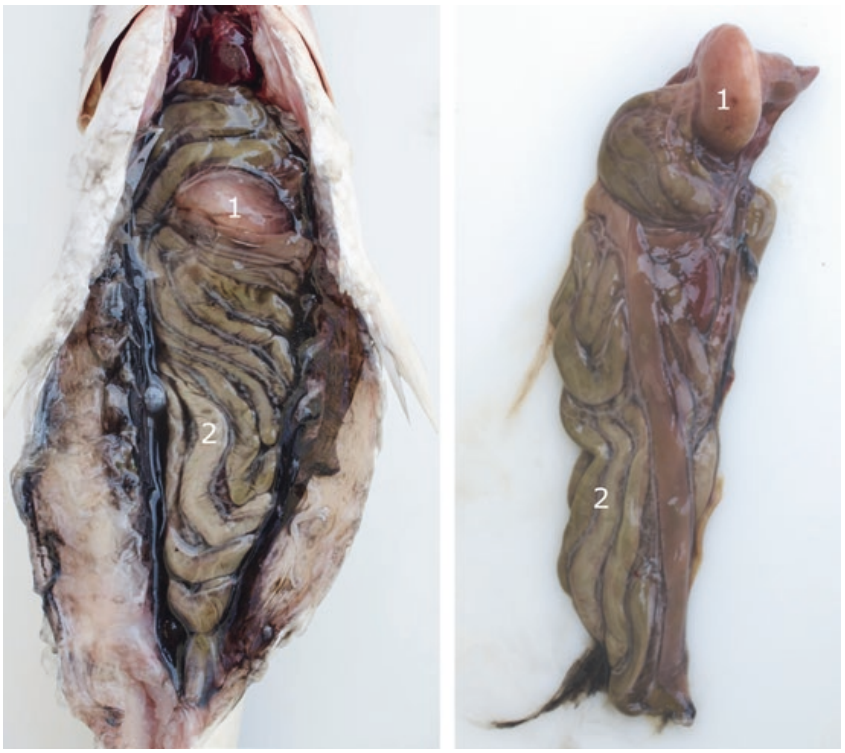

Fig. 18.5 Internal anatomy of *M. cephalus* where the digestive tract is observed. (1) Stomach and (2) intestine

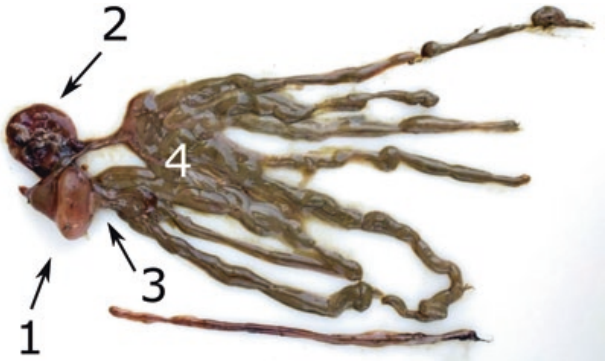

Fig. 18.6 *M. cephalus* digestive tract. (1) Stomach, (2) liver, (3) anterior intestine, (4) medium intestine, and (5) posterior intestine

The innermost layer of the gut is the mucosa, which presents finger-like projections called villi. The villi are formed by cylindrical epithelium that consists of three cell types: coating cells, goblet cells, and enterocytes. The coating cells are cylindrical cells with a striated edge and have participation in the absorption process. The goblet cells produce a kind of mucus that protects the intestinal wall. Finally the enterocytes secrete substances that go directly to the lamina propria and are distributed by the blood flow (Estrada-Flores and Uribe-Aranzábal 2002). In this study, we observed the cylindrical epithelium with elongated cells and vacuolated epithelium (Fig. 18.8). This vacuolated epithelial tissue has not been reported for detritivore fish, as *M. cephalus*, but according to Serrano et al.

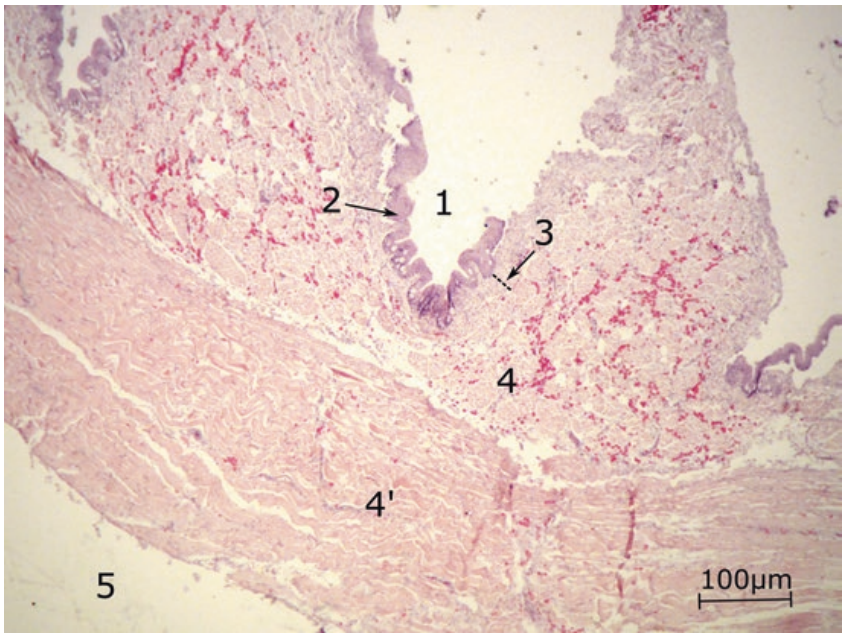

Fig. 18.7 Medium intestine of *M. cephalus*. (1) Lumen, (2) mucosa layer, (3) submucosa layer, (4) longitudinal muscle layer (4) circular muscle layer, and (5) serosa. H&E staining

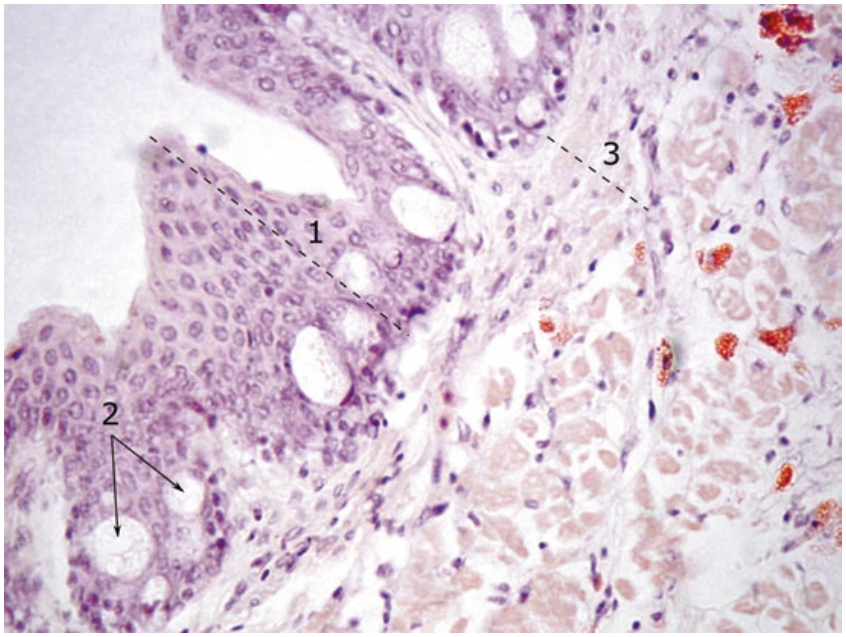

Fig. 18.8 Medium intestine of *M. cephalus*. (1) Mucosa layer, (2) vacuolated epithelial tissue, and (3) submucosa. H&E staining

(2014), there is the presence of vacuolated epithelial tissue in a carnivorous fish (*Merluccius australis*). This presence was related to a greater activity of acidic and alkaline phosphatases, demonstrating that this region of the digestive tract is where the main processes of absorption of nutrients occur.

The submucosa is composed of dense connective tissue with blood vessels, erythrocytes, and fibroblasts (Fig. 18.8). The submucosa layer is characterized by the abundance of blood vessels, lymphatic vessels, and fibroblasts (Welsch and Sobotta 2008; Groman 1982).

The muscular layer consists of two layers of muscle, the inner and outer layer. The inner layer is arranged in circular rings of muscle around the tract, whereas the outer layer is arranged longitudinally. In the outer layer, we could see muscle fibers and elongated cells with peripheral and elongated nuclei (Fig. 18.8). The outer layer is thinner than the inner one (Welsch and Sobotta 2008). The coordinated movements of both layers that move the intestinal contents are called peristalsis (Ross and Wojciech 2007).

Finally, we observed the serosa layer formed by loose connective tissue and mesothelium (Fig. 18.8).

Histopathological Analysis In general, three alterations were observed: eosinophilic secretions, congested vessels, and inflammation. These alterations differ between Coatzacoalcos and Guasimas organisms. In Coatzacoalcos, muscular layer of intestine showed eosinophilic secretions and congested vessels, while submucosa layer showed eosinophilic secretions and inflammation. Unlike in Guasimas, mus-

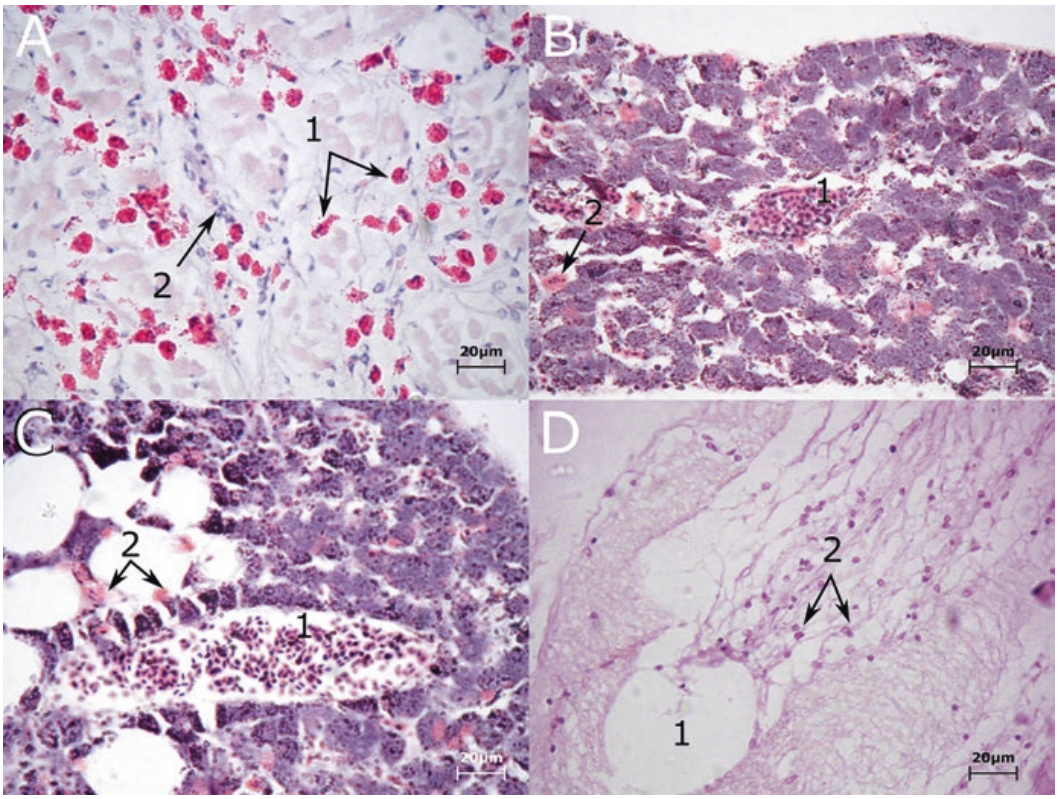

Fig. 18.9 Medium intestine of *M. cephalus*. (**a**) Muscle layer with (1) eosinophilic secretions and (2) muscle fibers, (**b**) eosinophilic secretion (2) and congestive vessel (1), (**c**) congestive vessel (1) and eosinophilic secretions (2), and (**d**) connective tissue with (1) inflammation and (2) fibroblasts. H&E staining

cular layer presented only inflammation, while submucosa layer presented eosinophilic secretions and inflammation (Fig. 18.9).

Eosinophilic secretions are blood cells that belong to the family of polymorphonuclear leukocytes. These secretions are characterized by having cytoplasmic granules that are associated with acid stains such as eosin. The main function of the secretions is to defend the organism against helminth parasites. When the tissue has already suffered infection or inflammation, these secretions are highly exhibited (Megías et al. 2015). Congested vessels are indicators of an excess of blood inside the duct in a certain region, as a result of too much blood delivered by the arteries (active) or very little evacuation through the veins (passive) (Eiras et al. 2008). Inflammations are processes of connective tissue, where an extracellular fluid that looks like clear spaces in the stroma separates the cells from the parenchyma (Fernández Cossío 2007). Edema is frequently found with inflammation in tissues that have suffered injuries (Eiras et al. 2008).

18.5 Conclusion

The results indicate that the long digestive tract of *Mugil cephalus* is largely due to its nutrition. The constitutive layers of the intestine found in this fish agree with the typical conformation of this tissue, except for the presence of vacuolated epithelial tissue which is reported usually in carnivorous fish. The histopathological analysis showed three main injuries: eosinophilic secretions, congested vessels, and inflammation. The prevalence of injuries was higher in Coatzacoalcos, indicating the contamination of this place has more impact on the organism's health. Finally, this study recommends the histopathological analysis of the digestive tract to evaluate the health status of fish and the relation with the environment. However it is important to standardize and establish new semiquantitative methods to assess the impact of pollutants on the living organisms.

Acknowledgments We thank our colleagues from ecotoxicology lab from Universidad Autónoma Metropolitana, Iztapalapa Unit, who provided insight and expertise that greatly assisted the research.

We thank Dalia Itzkopilli Luis Hernández for assistance in taking and editing the macroscopic photographs and José Roberto Jerónimo-Juárez for comments that greatly improved the manuscript.

References

Arreola-Lizarraga JA (1995) Diagnósis Ecológica de la Bahía de Lobos, Sonora. In: Centro Interdisciplinario de Ciencias Marinas. Instituto Politécnico Nacional, Mexico

Botello AV, Rendón von Osten J, Gold Bouchot G, Agraz Hernández C (Eds) (2005) Golfo de México: contaminación e impacto ambiental: diagnóstico y tendencias (2nd ed.). Campeche, México: Centro EPOMEX. Universidad Autónoma de Campeche

Bullock G, Bunton TE (2000) Foreword. In: Ostrander GK (ed) The laboratory fish. Academic Press, London, p xvii

Castelló Orvay F (1993) Acuicultura marina: fundamentos biológicos y tecnología de la producción, 2nd edn. Universitat de Barcelona, Barcelona, España, p 739

ÇInar K, Şenol N (2006) Histological and histochemical characterization of the mucosa of the digestive tract in flower fish (*Pseudophoxınus antalyae*). Anat Histol Embryol 35(3):147–151

CONAPESCA (2013) Anuario estadístico de acuicultura y pesca 2013. Available from: https://www.gob.mx/conapesca/documentos/anuario-estadistico-de-acuacultura-y-pesca

Eiras JC, Segner H, Kapoor BG (Eds) (2008) Fish Diseases. Enfield, NH, USA: Science Publishers

Estrada-Flores E, Uribe-Aranzábal MC (2002) Atlas de histología de vertebrados. Universidad Nacional Autónoma de México, México, p 222

Fernández Cossío S (2007) Respuesta tisular a materiales de relleno : estudio experimental. Universidad de Santiago de Compostela, España, p 29

Fischer W, Krupp F, Schneider W et al (1995) Pacífico centro-oriental. Vertebrados – Parte 2. Guía FAO para la Identificación de Especies para los Fines de la Pesca, vol III. FAO Organización De Las Naciones Unidas Para La Agricultura Y La Alimentación, Rome, p 1298

Groman DB (1982) Histology of the striped bass, vol 116. American Fisheries Society, Bethesda

Johnson LL, Stehr CM, Olson OP et al (1993) Chemical contaminants and hepatic lesions in winter flounder (*Pleuronectes americanus*) from the northeast coast of the United States. Environ Sci Technol 27(13):2759–2771

Megías M, Molist P, Pombal MA (2015) Atlas de Histología vegetal y animal: Tipos celulares Eosinófil*o*. In: Biología funcional y Ciencias de la Salud. Universidad de Vigo, España

Reichenbach L (1977) Trabajos sobre Histopatología de los peces, vol 100. Acribia, España

Robins R, Bailey RM, Bond CE, et al (1991) Common and scientific names of fishes from the United States and Canada. In: AFS ComMittee on Names of Fishes (ed) American Fisheries Society Special Publication 20 (5th ed.)

Ross MH, Wojciech P (2007) Histología: texto y atlas de color con biología celular y molecular, 5th edn. Médica Panamericana, Buenos Aires, p 994

Saleh MA (2006) Cultured aquatic species information programme *Mugil cephalus*. In: Cultured aquiatic species information programme. FAO Fisheries and Aquaculture Deparment, Rome

Sánchez RMP (1995) Análisis de los hábitos alimenticios de Mugil cephalus (Linnaeus, 1758) y Mugil curema con base en la presencia de diatomeas. Universidad Nacional Autónoma de México, Mexico

Sarasquete Reidiz C, González de Canales ML, Muñoz Cueto JA (1999) Patología, fisiología y biotoxicología en especies acuáticas. Consejo Superior de Investigaciones Científicas-CSI Press, España, p 290

Serrano E, Leiva C, Lonza C et al (2014) Caracterización Histológica y Morfológica del Tracto Digestivo de la Merluza Austral (*Merluccius australis*). Int J Morphol 32:1079–1084

Villegas RE, Gracia BD, Barcelo MP (1985) Estudio de la contaminación por residuos de plaguicidas en las Bahías de Yavaros y Lobos, Sonora. In: Ciencias Químicas. Universidad de Sonora, Mexico, p 89

Welsch U, Sobotta J (2008) Histología. Médica Panamericana, Buenos Aires, p 85

Yáñez-Arancibia LA, Nuget RS (1975) Síntesis de antecedentes bióticos y abióticos de los ecosistemas estuarinos de Nayarit, México. Vol. In Memoriam Dr. E. Caballero Mexico: Ciencias Biológicas. Universidad Autónoma de Nuevo León

Chapter 19
Impacts Caused by Manganese in the Aquatic Environments of Brazil

Gabriela Zimmermann Prado Rodrigues, Mateus Santos de Souza, and Günther Gehlen

19.1 Introduction

Water pollution resulting from erroneous anthropic actions favors the presence of metals and other contaminants in water (Kassim et al. 2011). Among the sources responsible for the occurrence of metals in water bodies, we highlight mining (Li et al. 2015), release of industrial and domestic effluents without previous treatment (Chen et al. 2015), and the excessive use of chemical fertilizers, which is the subject of several studies at the global level (Bhowmik et al. 2015; Wu et al. 2016; Xiao et al. 2017; Silva et al. 2018; Benson et al. 2018). In addition to anthropogenic sources, natural processes also contribute to the occurrence of these elements in groundwater and surface water (Winkel et al. 2008; Martin et al. 2015).

After being released into the water bodies, metals may either be deposited in the sediments or stay in the water column (Simpson and Spadaro 2016), which causes concern due to their persistence, toxicity, bioaccumulation capacity in aquatic organisms (Islam et al. 2015), and the consequent impact on the quality of the ecosystem. The enrichment of heavy metals in organisms through the food chain is a threat to humans (Lei et al. 2016), which is aggravated by the fact that the majority of the current systems of sewage treatment and water purification are inefficient for the removal of these substances.

Manganese is one of the metals whose uncontrolled release into the environment raises concern due to its toxic potential. Although it is an essential element to various physiological processes of plants and animals, when in excess, it is harmful. Therefore, it is important to monitor the levels of manganese, as well as other metals, in different environmental compartments – water, air, soil, and biota.

G. Z. P. Rodrigues (✉) · M. S. de Souza
Laboratório de Histologia comparada, Universidade Feevale, Novo Hamburgo, RS, Brazil

G. Gehlen
Programa de Pós-graduação em Qualidade Ambiental, Universidade Feevale, Novo Hamburgo, RS, Brazil

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_19

19.2 Manganese as a Worldwide Aquatic Pollutant

Manganese is a metal that is widely distributed in the earth's crust and is also present in the atmosphere and water (Mena 1980). It is the most abundant transition metal after iron (Fe) and titanium (Ti), and due to its electronic configuration, it can present several states of oxidation (0 to +7).

It is a widely used element for the manufacture of iron and steel alloys, batteries, glassware, fireworks, fertilizers, fungicides, varnishes, and animal supplementation (Patil et al. 2016; HSDB 2001). In addition, methylcyclopentadienyl manganese tricarbonyl (MMT) is used in Canada, Europe, Asia, and South America as an additive in unleaded gasoline to increase the octane number and reduce the effects of engine knocking (Lynam et al. 1999; Smith et al. 2018).

Historically, high levels of manganese in surface water may be associated with industrial pollution and mining effluents (WHO, 1981). However, the same structure involved in the water potability process, such as wells, pumps, and storage tanks, may contribute to manganese contamination (Farrag et al. 2016). Many countries have regulations that define acceptable levels of manganese in the waters. In Brazil, the limit of manganese in rivers ranges from 0.1 mg L^{-1} to 0.5 mg L^{-1} (National Council of the Environment of Brazil, CONAMA, 2005) and should not exceed 0.4 mg L^{-1} in drinking water (BRASIL, 2011). In countries such as Canada, the United States, France, and Spain, the manganese limit in drinking water is 0.05 mg L^{-1} (Marsidi et al. 2018).

Until 2011, the World Health Organization (WHO) recommended in its guidelines for drinking water quality the concentration limit of 0.4 mg L^{-1} for manganese. However, this limit was removed by the justification that it was well above the values normally found in drinking water, which would make the formal definition of a limit unnecessary (WHO 2011). However, Frisbie et al. (2012) reviewed the WHO guidelines over time and presented data from studies conducted in several countries that had manganese concentrations in drinking water above 0.4 mg L^{-1}, concluding that WHO should reassess the withdrawal of the manganese limit from its guidelines, as well as assessing the reduction of that value.

Recently, Marsidi et al. (2018) have described that manganese can be found in water in combination with carbonates, sulfates, chlorides, and phosphates, which hinders its detection. A considerable number of reports of high concentrations of manganese in rivers around the globe have been made. Some examples are the concentrations of 4.32 mg L^{-1} found in Kenya (Kakoi et al. 2016) and 0.34 mg L^{-1} in Myanmar (Bacquart et al. 2015). In Ecuador, in areas close to small-scale gold mining, values were found from 2.66 mg L^{-1} to 3.99 mg L^{-1} (González-Merizalde et al. 2016).

In Bolivia, concentrations of up to 0.457 mg L^{-1} were recorded in rivers and streams in a region of oil fields (Alonso et al. 2010). In Nigeria, mean concentrations of 0.430 mg L^{-1} and 0.510 mg L^{-1} were recorded in a bitumen exploration area (Ayandiran et al. 2018), and in India, mean values of up to 1.309 mg L^{-1} were recorded in a tributary of the Ganges River (Khan et al. 2017). Such occurrences

cause concern, since overexposure or prolonged exposure to manganese has long been reported as a cause of neurodegenerative diseases and deficits in humans, including a syndrome called manganism, with symptoms similar to Parkinson's (Couper 1837; Mena et al. 1967), in addition to the toxicity already described in animal models (Wang et al. 2015; Altenhofen et al. 2017; Liu et al. 2017).

19.3 Occurrence of Manganese in Brazilian Aquatic Environments

In Brazil, reports of high concentrations of manganese vary from 0.5 mg L^{-1} in stretches of the Sinos River (one of the most polluted in the country) (Nascimento et al. 2015) to 19.3 mg L^{-1} in the Pampulha basin in Minas Gerais (Rietzler et al. 2001). There are also reports of high concentrations in drinking water (Khan et al. 2017; Alvarez-Bastida et al. 2018).

Machado et al. (2017) detected concentrations up to 84% higher than those recommended by the Brazilian legislation in the Pardo River, which flows through the states of São Paulo and Minas Gerais. These values were found especially in regions with a predominance of sugar cane cultivation, due to the application of corrective micronutrients in the soil. Previously, Alves et al. (2014) had also reported concentrations of manganese up to 2.5 times higher than allowed by legislation in the Pardo River waters, as well as high concentrations in sediment.

Brito et al. (2018) reported concentrations of manganese more than 15 times greater than allowed by Brazilian legislation in all sample points evaluated in the Upper Iguaçu River (Paraná state). The region is classified as highly impacted by urban and industrial activities on the river. The sample point with the highest concentration of manganese is impacted by the largest sewage treatment plant in the region, which releases about 1.120 L s^{-1} of treated effluent in the river. The water from this sample point caused the highest mortality rate in fish larvae of the species *Rhamdia quelen* after 96 hours of exposure, even at the lowest dilution (33%). This fact demonstrates the toxic potential of these waters to the aquatic biota.

Quadra et al. (2019) quantified the main trace metals in samples collected along the Doce River 10 days after the disaster at the Fundão dam in Mariana city, state of Minas Gerais, in 2015. Among the metals detected, manganese was highlighted because it has exceeded the limits allowed by the legislation and may have influenced the reduction of mitotic index and the occurrence of genotoxicity in *Allium cepa* cells reported by the authors. Previously, other authors had also reported high concentrations of Mn in suspended particles after this same disaster (Hatje et al. 2017).

In southern Brazil, Hermes et al. (2013) evaluated a region known to be impacted by manganese in the Pardinho River Basin; the authors report high concentrations in water, soil, food produced in the region, and hair samples provided by study participants. In this case, the contamination occurs especially by the soil that is used for agronomic purposes, and the risk to the population is great.

The studies described above demonstrate that manganese is a frequent aquatic pollutant in several Brazilian regions. Nevertheless, there are still few studies conducted in order to evaluate the risk to the exposed populations in Brazil (Hermes et al. 2013) and in the world (Oulhote et al. 2014; Carvalho et al. 2018). On the other hand, the effects of acute or chronic exposure to manganese are well established in different animal/cellular models (Table 19.1).

Table 19.1 Studies that report effects caused by exposure to manganese in different biological models

Authors	Observed effects	Study model
McDougall et al. (2008)	Changes in the release of dopamine and learning deficit of associative and non-associative behavior	Rats (Sprague Dawley)
Yoon et al. (2011)	Increased oxidative stress in endoplasmic reticulum and mitochondrial dysfunction	Cell culture (SK–N–MC)
Khalid et al. (2011)	Changes in the regulation of dopamine release	Mice (C57BL/6)
Huang et al. (2011)	Liver injury (congestion, hypertrophy)	Rats (Sprague Dawley)
Lebda et al. (2012)	Neurotoxicity and hepatotoxicity	Rats (Sprague Dawley)
Vieira et al. (2012)	Generalized oxidative stress in gills, kidneys, liver, and brain	Fish (*Carassius auratus*)
Gabriel et al. (2013)	Oxidative stress in gills, kidney, liver, and brain	Fish (*Colossoma macropomum*)
Liu et al. (2013a, b)	Oxidative stress and testicular apoptosis	Cocks (Hy-line)
Liu et al. (2013a, b)	Oxidative damage to the immune system and apoptosis of immune cells	Cocks (Hy-line)
O'Neal et al. (2014)	Disruption of neurotransmitter systems; locomotor deficits	Rats (Sprague Dawley)
Lu et al. (2015)	Immunosuppression of lymphocytes	Cocks (Hy-line)
Okada et al. (2016)	Oxidative stress in brains of pups from mothers exposed to Mn	Mice
Altenhofen et al. (2017)	Changes in long-term memory and locomotor deficits	Fish (zebrafish adult and larvae)
Silva et al. (2018)	Sperm concentration and quality reduced and hepatic degeneration	Rats (Wistar)
Sarkar et al. (2018)	Neuroinflammation and mitochondrial dysfunction	Cell culture of mice astrocytes (C57BL/6)
Rodrigues et al. (2017)	Intestinal inflammatory damages	Fish (zebrafish adult)
Alcon et al. (2018)	Necrosis in microglial cells by lysosomal dysfunction	Microglial cell of mice BV-2
Foster et al. (2018)	Inflammation of the olfactory and submucous epithelium of the sinuses	Rats F344
Guo et al. (2018)	Cell damage and neuronal apoptosis, through the inhibition of histone acetylation	Cell culture (PC12)
Coppo et al. (2018)	Genotoxicity	Fish (*Oreochromis niloticus*)

Among the effects caused in animal and cellular models by exposure to manganese, neurotoxicity is undoubtedly the most frequent damage, followed by liver changes. This may occur due to the fact that inside the cell, mitochondria are one of the main target organelles for manganese, which enters the cell through the calcium channels (Gunter 2017), the liver and brain being organs rich in mitochondria.

In humans, the reported effects do not differ from those reported in Table 19.1; neurotoxicity, in general, including cognitive and learning deficits and childhood hyperactivity are among the major damages reported in the most recent studies of occupational and environmental exposure to manganese (Hermes et al. 2013; Carvalho et al. 2018; Bouchard et al. 2018).

19.4 Conclusion

Environmental contamination by manganese is a problem that is becoming more and more relevant due to the increasing demand for this metal and the consequent greater release into the atmosphere, soil, and water. Several bodies of water around the world, especially in regions under direct influence of industrial and agricultural activities and mining and in urban areas, have high levels of manganese, and the same situation occurs in Brazil. This can become a public health and ecological problem because of the inefficiency of most water and sewage treatment systems in removing this metal. Many studies have shown that this metal has pathological effects in humans and in animal models when continuous exposure occurs at high concentrations. Despite this, few studies have assessed the risks that populations have been subjected to after being exposed to this type of contamination, and little is known about the ecological impacts on aquatic ecosystems. These are areas of research that deserve attention in future studies aiming at the problem of manganese in the aquatic environment.

References

Alcon SP, Gorojod RM, Kotler ML (2018) Regulated necrosis orchestrates microglial cell death in manganese-induced toxicity. Neuroscience 393:206–225

Alonso SG, Esteban-Hernández J, Rivera YV, Hernández-Barrera V, Miguel AG (2010) Contaminación del agua en Fuentes cercanas a campos petrolíferos de Bolivia. Rev Panam Salud Publica 28:235–243

Altenhofen S, Wiprich MT, Nery LR, Leite CE, Vianna MRM, Bonan CD (2017) Manganese (II) chloride alters behavioral and neurochemical parameters in larvae and adult zebrafish. Aquat Toxicol 182:172–183

Alvarez-Bastida C, Martínez-Miranda V, Solache-Ríos M, Linares-Hernández I, Teutli-Siqueira A, Vázquez-Mejía G (2018) Drinking water characterization and removal of manganese. Removal of manganese from water. J Environ Chem Eng 6:2119–2125

Alves RIS, Machado CS, Nadal M, Schuhmacher M, Domingo JL, Segura-Muñoz SI (2014) Metal concentrations in surface water and sediments from Pardo River, Brazil: human health risks. Environ Res 133:149–155

Ayandiran TA, Fawole OO, Dahunsi SO (2018) Water quality assessment of bitumen polluted Oluwa River, South-Western Nigeria. Water Resources Ind 19:13–19

Bacquart T, Frisbie SH, Mitchell E, Mitchell E, Grigg L, Cole C, Small C, Sarkar B (2015) Multiple inorganic toxic substances contaminating the groundwater of Myingyan township, Myanmar: arsenic, manganese, fluoride, iron, and uranium. Sci Total Environ 517:232–245

Benson NU, Adedapo AE, Fred-Ahmadu OH, Williams AB, Udosen ED, Ayejuyo OO, Olajire AA (2018) New ecological risk indices for evaluating heavy metals contamination in aquatic sediment: a case study of the Gulf of Guinea. Reg Stud Mar Sci 18:44–56

Bhowmik AK, Alamdar A, Katsoyiannis I, Shen H, Ali N, Ali SM, Bokhari H, Schäfer RB, Musstjab ASQ (2015) Mapping human health risks from exposure to trace metal contamination of drinking water sources in Pakistan. Sci Total Environ 538:306–3016

Bouchard MF, Surette C, Cormier P, Foucher D (2018) Low level exposure to manganese from drinking water and cognition in school-age children. Neurotoxicology 64:110–117

BRASIL. Conselho Nacional do Meio Ambiente – CONAMA, 2005. Resolução CONAMA no 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de efluentes, e dá outras providencias. Diário Oficial da República Federativa do Brasil, Brasilia, 18 mar. pp 58–63. Disponível em: <http://www.mma.gov.br/port/conama/res/res05/res35705.pdf> Acesso em: 05/05/2018

BRASIL. Ministério da Saúde – PORTARIA N° 2.914, DE 12 DE DEZEMBRO DE 2011. Dispõe sobre os procedimentos de controle e de vigilância da qualidade da água para consumo humano e seu padrão de potabilidade. Disponível em: <http://bvsms.saude.gov.br/bvs/saudelegis/gm/2011/prt2914_12_12_2011.html> Acesso em 10/08/2018

Brito IA, Garcia JRE, Salaroli AB, Figueira RCL, Martins CC, Neto AC, Gusso- Choueri PK, Choureri RB, Araujo SBL, Ribeiro CAO (2018) Embryo toxicity assay in the fish species *Rhamdia quelen* (Teleostei, Heptaridae) to assess water quality in the upper Iguaçu basin (Parana, Brazil). Chemosphere 208:207–218

Carvalho CF, Oulhote Y, Martorelli M, Carvalho CO, Menezes Filho JA, Argollo N, Abreu N (2018) Environmental manganese exposure and associations with memory, executive functions, and hyperactivity in Brazilian children. Neurotoxicology 69:253–259

Chen H, Teng Y, Lu S, Wang Y, Wang J (2015) Contamination features and health risk of soil heavy metals in China. Sci Total Environ 512–513:143–153

Coppo GC, Passos LS, Lopes TOM, Pereira TM, Merçon J, Cabral DS, Barbosa BV, Caetano LS, Kampke EH, Chippari-Gomes AA (2018) Genotoxic, biochemical and bioconcentration effects of manganese on *Oreochromis niloticus* (Cichlidae). Ecotoxicology 27(8):1150–1160

Couper J (1837) On the effects of black oxide manganese when inhaled into the lungs. Brit Ann Med Pharm Vital Stat Gen Sci 1:41–42

Farrag AEHA, Moghny TA, Mohamed AMG, Saleem SS, Fathy M (2016) Abu zenima synthetic zeolite for removing iron and manganese from Assiut governorate groundwater, Egypt. Appl Water Sci 6:3087–3094

Foster ML, Rao DB, Francher T, Traver S, Dorman DC (2018) Olfactory toxicity in rats following manganese chloride nasal instillation: a pilot study. Neurotoxicology 64:284–290

Frisbie SH, Mitchell EJ, Dustin H, Maynard DM, Sarkar B (2012) World Health Organization discontinues its drinking-water guideline for manganese. Environ Health Perspect 20:775–778

Gabriel D, Riffel APK, Finamor IA, Saccol EMH, Ourique GM, Goulart LO, Kochhann D, Cunha MA, Garcia LO, Pavanato MA, Val AL, Baldisserotto B, Llesuy SF (2013) Effects of subchronic manganese chloride exposure on Tambaqui (*Colossoma macropomum*) tissues: oxidative stress and antioxidant defenses. Arch Environ Contam Toxicol 64:659–667

González-Merizalde MV, Menezes-Filho JA, Cruz-Erazo CT, Bermeo-Flores SA, Saánchez-Castillo MO, Hernández-Bonilla D, Moran A (2016) Manganese and mercury levels in

water, sediments, and children living near gold-mining areas of the Nangaritza River Basin, Ecuadorian Amazon. Arch Environ Contam Toxicol 71:171–182
Gunter TE (2017) Manganese and mitochondrial function, Molecular, genetic, and nutritional aspects of major and trace minerals. Collins, J.F. Elsevier, London, 514p
Guo Z, Zhang Z, Wang Q, Zhang J, Wang L, Zhang Q, Li H, Wu S (2018) Manganese chloride induces histone acetylation changes in neuronal cells: its role in manganese-induced damage. Neurotoxicology 65:255–263
Hatje V, Pedreira RMA, Rezende CE, Schettini CAF, Souza GC, Marin DC, Hackspacher PC (2017) The environmental impacts of one of the largest tailing dam failures worldwide. Sci Rep 7:1–13
Hermes N, Schneider RCS, Molin DD, Riegel GZ, Costa AB, Corbellini VA, Torres JPM, Malm O (2013) Environmental pathways and human exposure to manganese in southern Brazil. An Acad Bras Cienc 85(4):1275–1288
HSDB (2001) Manganese compounds. National Library of Medicine, Hazardous Substances Data Bank, Bethesda. Available at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB
Huang P, Chen C, Wang H, Li G, Jing H, Han Y, Liu N, Xiao Y, Yu Q, Liu Y, Wang P, Shi Z, Sun Z (2011) Manganese effects in the liver following subacute or subchronic manganese chloride exposure in rats. Ecotoxicol Environ Saf 74:615–622
Islam MS, Ahmed MK, Habibullah-Al-Mamum M, Hoque MF (2015) Preliminary assessment of heavy metal contamination in surface sediments from a river in Bangladesh. Environ Earth Sci 73(4):1837–1848
Kakoi B, Kaluli JW, Ndiba P, Thiong'o G (2016) Banana pith as a natural coagulant for polluted river water. Ecol Eng 95:699–705
Kassim A, Rezayi M, Ahmadzadeh S, Rounaghi G, Mohajeri M, Yusof NA, Tee TW, Heng LY, Abdullah AH (2011) A novel ion– selective polymeric membrane sensor for determining thallium (I) with high selectivity. IOP Conf Series Mater Sci Eng 17(1):1–7
Khalid M, Aoun RA, Mathews TA (2011) Altered striatal dopamine release following a sub-acute exposure to manganese. J Neurosci Methods 15(202):182–191
Khan MYA, Gani KM, Chakrapani GJ (2017) Spatial and temporal variations of physicochemical and heavy metal pollution in Ramganga River—a tributary of river Ganges, India. Environ Earth Sci 76:231–244
Lebda MA, El-Neweshy MS, El-Sayed YS (2012) Neurohepatic toxicity of subacute manganese chloride exposure and potential chemoprotective effects of lycopene. Neurotoxicology 33(1):98–104
Lei K, Giubilato E, Critto A, Pan H, Lin C (2016) Contamination and human health risk of lead in soils around lead/zinc smelting areas in China. Environ Sci Pollut Res 23:13128–13136
Li Z, Guo Q, Li Z, Fan G, Xiong DB, Su Y, Zhang J, Zhang D (2015) Enhanced mechanical properties of graphene (reduced graphene oxide)/ aluminum composites with a bioinspired nanolaminated structure. Nano Lett 15:8077–8083
Liu X, Zhang L, Guan H, Zhang Z, Xu S (2013a) Effects of oxidative stress on apoptosis in manganese-induced testicular toxicity in cocks. Food Chem Toxicol 60:168–176
Liu X, Li Z, Tie F, Liu N, Zhang Z, Xu S (2013b) Effects of manganese-toxicity on immune-related organs of cocks. Chemosphere 90(7):2085–2100
Liu X, Yang J, Lu C, Jiang S, Nie X, Han J, Yin L, Jiang J (2017) Downregulation of Mfn_2 participates in manganese-induced neuronal apoptosis in rat striatum and PC12 cells. Neurochem Int 108:40–51
Lu X, Zhu Y, Bai R, Li S, Teng X (2015) The effect of manganese-induced toxicity on the cytokine mRNA expression of chicken spleen lymphocytes in vitro. Res Vet Sci 101:165–167
Lynam DR, Roos JW, Pfeifer GD, Fort BF, Pullin TG (1999) Environmental effects and exposures to manganese from use of methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline. Neurotoxicology 20:145–150
Machado CS, Fregonesi BM, Alves RIS, Tonani KAA, Sierra J, Martinis BS, Celere BS, Mari M, Schuhmacher M, Nadal M, Domingo JL, Segura-Muñoz S (2017) Health risks of environ-

mental exposure to metals and herbicides in the Pardo River, Brazil. Environ Sci Pollut Res 24(25):20160–20172

Marsidi N, Hasan HA, Abdulah SRS (2018) A review of biological aerated filters for iron and manganese ions removal in water treatment. J Water Process Eng 23:1–12

Martin JAR, Arana CD, Ramos-Miras JJ, Gil C, Boluda R (2015) Impact of 70 years urban growth associated with heavy metal pollution. Environ Pollut 196:156–163

McDougall SA, Reichel CM, Farley CM, Flesher MM, Der-Ghazarian T, Cortez AM, Wacan JJ, Martinez CE, Varela FA, Butt AE, Crawford CA (2008) Postnatal manganese exposure alters dopamine transporter function in adult rats: potential impact on nonassociative and associative processes. Neuroscience 154(2):848–860

Mena I (1980) Manganese. In: Waldron HA (ed) Metals in the environment, 1st edn. Academic Press, London, pp 199–220

Mena I, Marin O, Fuenzalida S, Cotzias GC (1967) Chronic manganese poisoning. Clinical picture and manganese turnover. Neurology 17:128–136

Nascimento CA, Staggemeier R, Bianchi E, Rodrigues MT, Fabres R, Soliman MC, Bortoluzzi M, Luz RB, Heinzelmann LS, Santos EL, Fleck JD, Spilki FR (2015) Monitoring of metals, organic compounds and coliforms in water catchment points from the Sinos River basin. Braz J Biol 75(2):50–56

O'neal SL, Lee JW, Zheng W, Canhão JR (2014) Subacute manganese exposure in rats is a neurochemical model of early manganese toxicity. Neurotoxicology 44:303–313

Okada MA, Neto FF, Nosso CH, Voigt CL, Campos SX, Ribeiro CAO (2016) Brain effects of manganese exposure in mice pups during prenatal and breastfeeding periods. Neurochem Int 97:109–116

Oulhote Y, Mergler D, Bellinger DC, Bouffard T, Brodeur ME, Saint-Amour D, Legrand M, Suavé S, Bouchard MF (2014) Neurobehavioral function in school-age children exposed to manganese in drinking water. Environ Health Perspect 122(12):1343–1350

Patil DS, Chavan SM, Oubagaranadin JUK (2016) A review of technologies for manganese removal from wastewaters. J Environ Chem Eng 4:468–487

Quadra GR, Roland R, Barros N, Malm O, Lino AS, Azevedo GM, Thomaz JR, Andrade-Vieira LF, Praça-Fontes MM, Almeida RM, Mendonça RF, Cardoso SJ, Guida YS, Campos JMS (2019) Far-reaching cytogenotoxic effects of mine waste from the Fundão dam disaster in Brazil. Chemosphere 215:753–757

Rietzler AC, Fonseca AL, Lopes GP (2001) Heavy metals in tributaries of Pampulha reservoir, Minas Gerais. Braz J Biol 61(3):363–370

Rodrigues GZP, Souza MS, Silva AH, Zwetsch BG, Gehlen G (2017) Evaluation of intestinal histological damage in zebrafish exposed to environmentally relevant concentrations of manganese. Ciência e Natura 40(e52):1–8

Sarkar S, Malovic E, Harischandra DS, Ngwa HA, Ghosh A, Hogan C, Rokad D, Zenitsky G, Jin H, Anantharam V, Kanthasamy AG, Kanthasamy A (2018) Manganese exposure induces neuroinflammation by impairing mitochondrial dynamics in astrocytes. Neurotoxicology 64:204–218

Silva APG, Santiago MSA, Maranho LA, Oliveira RP, Constantino DHJ, Pereira CDS, Silva CS, Perobelli JE (2018) Could male reproductive system be the main target of subchronic exposure to manganese in adult animals? Toxicology 409:1–12

Simpson SL, Spadaro DA (2016) Bioavailability and chronic toxicity of metal sulfide minerals to benthic marine invertebrates: implications for deep sea exploration, mining and tailings disposal. Environ Sci Technol 50(7):4061–4070

Smith D, Woodall GM, Jarabek AM, Boyes WK (2018) Manganese testing under a clean air act test rule and the application of resultant data in risk assessments. Neurotoxicology 64:177–184

Vieira MC, Torranteras R, Córdoba F, Canalejo A (2012) Acute toxicity of manganese in *goldfish Carassius auratus* is associated with oxidative stress and organ specific antioxidant responses. Ecotoxicol Environ Saf 78(1):212–217

Wang Z, Ren J, Zhang G, Liu S, Zhang X, Liu Z, Zhang J (2015) Behavior of dissolved aluminum in the Huanghe (Yellow River) and its estuary: impact of human activities and sorption processes. Estuar Coast Shelf Sci 153:86–95

Winkel L, Berg M, Amini M, Hug SJ, Johnson A (2008) Predicting groundwater arsenic contamination in Southeast Asia from surface parameters. Nat Geosci 1:536–542

World Health Organization (1981) WHO, Manganese, IPCS, Geneva

World Health Organization. WHO (2011) Guidelines for drinking-water quality, 4th edn, IPCS, Geneva

Wu Q, Zhou H, Tham NFY, Tian Y, Tan Y, Zhou S, Li Q, Chen Y, Leung YS (2016) Contamination, toxicity and speciation of heavy metals in an industrialized urban river: implications for the dispersal of heavy metals. Marine Pollut Bull 104:153–161

Xiao R, Wang S, Li R, Wang JJ, Zhang Z (2017) Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi, China. Ecotoxicol Environ Saf 141:17–24

Yoon H et al (2011) Apoptosis induced by manganese on neuronal SK-N-MC cell line: endoplasmic reticulum (ER) stress and mitochondria dysfunction. Environ Health Toxicol 26:1–7

Chapter 20
Genotoxic Effect of Amoxicillin on Peripheral Blood of Common Carp (*Cyprinus carpio*)

Armando Elizalde-Velázquez, Martínez-Rodríguez Héctor, Marcela Galar-Martínez, Luis Gerardo Bernadac Villegas, Octavio Dublán-García, and Leobardo Manuel Gómez-Oliván

20.1 Introduction

Along the history, mankind has created many different products to prolong and improve their quality of life, and most of them have resulted in efficiently accomplishing the task; however, a few of them have resulted in catastrophic results like thalidomide, methyl parathion, DDT, etc., which have triggered an international concern about the products we have created and the products we actually use daily that could represent a risk for the environment. In the light of the potential impact of these substances, a new definition has raised, emerging pollutants. The emerging pollutants are defined as synthetic or naturally occurring chemicals that are not commonly monitored in the environment but which have the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects. In some cases, release of emerging pollutants to the environment has likely occurred for a long time, but may not have been recognized until new detection methods were developed. In other cases, synthesis of new chemicals or changes in

A. Elizalde-Velázquez · M.-R. Héctor · O. Dublán-García · L. M. Gómez-Oliván (✉)
Laboratorio de Toxicología Ambiental, Facultad de Química,
Universidad Autónoma del Estado de México, Toluca, Estado de México, Mexico
e-mail: lmgomezo@uaemex.mx

M. Galar-Martínez
Laboratorio de Toxicología Acuática, Departamento de Farmacia, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional Adolfo López Mateos, Mexico City, Mexico

L. G. B. Villegas
Departamento de Ingeniería Civil y Ambiental, Instituto de Ingeniería y Tecnología de la Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, Mexico

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8_20

use or disposal of existing chemicals can create new sources of emerging pollutants (Geissen et al. 2015).

Emerging pollutants are categorized in more than 20 classes related to their origin; one of the most prominent classes is the pharmaceutical. Within the pharmaceutical class, we found a humongous group, the antibiotics, composed of a diverse amount of structures, functional groups, uses, and spectra. The occurrence and fate of antibiotics in the environment has become the subject of recent investigations due to their potential health effects on nontarget species as well as the increased emergence of resistant pathogenic bacteria (Mojica and Aga 2019). Therefore, it is not a surprise that they have been detected in the μg/L range in different water bodies around the world (Johnson et al. 2015; Kummerer 2003; Zheng et al. 2012; Watkinson et al. 2009; Gibs et al. 2013).

This work particularly focuses in a semi-synthetic broad-spectrum antibiotic used worldwide in high amounts. Amoxicillin, an antibiotic, can enter into the aquatic environment through diverse pathways; it could be from point sources as effluents of manufacturing plants and effluents from hospitals but also from nonpoint sources as municipal effluents from households, effluents from wastewater treatment plants, disposal of unused or expired compounds in the drainage, runoff from fields where animals have been treated, direct dosage of antibiotics in the water for aquaculture, and so on (Elizalde-Velázquez et al. 2017; Sarmah et al. 2006).

Besides its global high consumption (Elizalde-Velázquez et al. 2016), amoxicillin is excreted unchanged in urine ($\leq$ 85%) and feces ($\leq$ 10%) of humans and animals treated with the bactericide compound, which means a huge and continual disposal of this emerging pollutant in environmental waters. In a past work, we described the oxidative damage produced by amoxicillin in different organs of *Cyprinus carpio* once it was transformed by biotic factors into amoxicilloic acid (Elizalde-Velázquez et al. 2017); our work sums to other past studies that also described the toxic effects of amoxicillin in diverse aquatic organisms. Andreozzi et al. (2004) described that amoxicillin induced high toxicity in the blue algae *S. leopoliensis*; Oliveria et al. (2013) report that the antibiotic induces premature hatching, oedemas, and malformations in embryos of *Danio rerio*, as well as alterations in its normal enzymatic activity; and Liu et al. (2015) found that amoxicillin induced toxicity in the cyanobacteria *Microcystis aeruginosa* after a short-term exposure.

Due to its oxidative damage, it may be possible that this bactericide compound could also be potentially genotoxic. In fact some previous studies that test the genotoxic activity of this compound have reported positive results in mammalian cells. Arabski et al. (2005) reported that amoxicillin induces strand breaks and base modification in DNA of human peripheral blood lymphocytes and in *Helicobacter pylori* using the conventional comet assay. Li et al. (2007) described the damage produced by this beta lactam antibiotic in the DNA of human AGS and NB4 cell lines and in Chinese hamster cell lines, possibly by intracellular induction of reactive oxygen species, and more recently, it was reported the genotoxic activity of this compound in an aquatic species. Anlas and Ustuner (2019) reported that amoxicillin induces

DNA damage in *Oncorhynchus mykiss* erythrocytes due to an induction of reactive oxygen species and a deficient DNA repair activity of rainbow trout; however, some other studies differ with this results. Cahill et al. (2004) reported negative result measuring the genotoxic effect of amoxicillin using the green screen assay, and Istifli and Topaktas (2009) reported that amoxicillin does not exert genotoxic effects in human peripheral blood lymphocytes in vitro.

The aim of this study was to evaluate the in vivo genotoxic effect induced by three different concentrations of amoxicillin (10 ng/L, 10 μg/L, 10 mg/L) on the peripheral blood of the freshwater teleost fish *C. carpio* using the comet assay, so the genotoxic information of this emerging pollutant in aquatic species could be extended.

20.2 Materials and Methods

20.2.1 Test Substance

Amoxicillin trihydrate (CAS number 61336-70-7, >98.0% purity) ($C_{16}H_{19}N_3O_5S{\cdot}3H_2O$), 365.40 Da, was purchased from Tokyo Chemical Industry Co., LTD.

20.2.2 Specimen Collection and Maintenance

The test was carried out on 330 healthy adult common carps (*Cyprinus carpio*) with an average length of 17.93 ± 0.46 cm and an average weight of 48.72 ± 6.4 g, obtained from the aquaculture facility in Tiacaque, State of Mexico. Carps were acclimated to test conditions for 30 days prior to the experiment, temperature between 20 ± 2 °C, oxygen concentrations between 85 and 90%, water pH between 7.5 and 7.8, and natural light/dark photoperiods. During acclimation, carp were fed with Pedregal Silver™ fish food, and three-fourths of the tank water was replaced every 24 hours in order to maintain a healthy environment.

20.2.3 Experimental Design

For the test, we used static systems maintained at room temperature with natural light/dark photoperiods, provided with constant aeration and no food to specimens during the exposure period of time. For the genotoxic evaluation, amoxicillin was tested in three different concentrations (10 ng/L, 10 μ/L, 10 mg/L). The ng/L and μg/L concentrations are based on environmental reported data, while the mg/L concentration was

used to monitor the amoxicillin behaviour by analytical techniques (Elizalde-Velázquez et al. 2017). For each concentration, a kinetic of 12, 24, 48, 72, and 96 h was run, each system with six carps, and the assays were performed in triplicate. A free amoxicillin system with six carps was set up for each exposure time as control group, and a cyclophosphamide system was used only at 10 mg/L as a positive control. At the end of each exposure period of time, peripheral blood was obtained from anesthetized specimens with clove oil (Yamanaka et al. 2011) by puncture of the caudal vessel.

20.2.4 Analytical Measurements

The analytical analysis of amoxicillin and its degradation products in the blood of *Cyprinus carpio* was performed using a LC-10 AD system coupled to a L-ECD-6A electrochemical detector and a SPD-M10A diode array detector (Shimadzu, Kyoto, Japan) fitted to a Rheodyne injection valve (20 µL sample loop), using a Phenomenex Synergi Hydro-RP HPLC column (150 × 4.60 mm, 4 lm). The procedure was adapted from Gozlan et al. (2013a, b), with the following settings: injection volume 20 µL, flow rate 1 mL/min, column temperature 28 °C, and mobile phase isocratic 95/5 (v/v) water [pH adjusted to 2.5 with 99% trifluoroacetic acid (TFA, spectrophotometric grade)] and methanol (HPLC grade). Data was recorded at 230 nm UV absorption. The settings for the EC detection were adapted from Brooks et al. (1981): EC detector was operated in direct mode at +1.17 V vs. an Ag/AgCl reference electrode.

20.2.4.1 Water

Samples of 10 mL were collected from each test systems and frozen at −20 °C until its analysis by the analytical techniques. Samples were brought to room temperature 1 hour before its analysis and mixed in a vortex mixer. For the water analysis, we do not perform any treatment prior its injection in the HPLC. Amoxicillin was used as the standard for the determination of the retardation factors (RFs). Gozlan et al. (2013a) use this technique since the RFs of amoxicillin and amoxicilloic acid are very similar. Based on this assumption, two calibration curves (0.01–10 mg/L) were constructed for amoxicillin and its analysis in the UV and EC detectors.

20.2.4.2 Blood

For the analytical analysis of the blood of *Cyprinus carpio*, we used the homogenization-deproteinization method of Brooks et al. (1981); fresh blood samples were homogenized with buffer phosphate solution and stored protected from the light at −20 °C. One hour before the analysis, frozen samples were brought to room temperature; each sample of 0.2 µL was mixed with 150 µL of Milli-Q water and 50 µL of perchloric acid at 70% (Merck, Germany). Each mixture was centrifuged after a thorough mixing on a vortex mixer; the centrifugation setting was

2000 × g and 4 °C for 5 min on a Hermle Z233 MK2 centrifuge. The supernatant was removed and then analysed by HLPC-EC-UV.

20.2.5 Comet Assay (Tice method et al. 2000)

The comet assay was performed according to Tice et al.'s (2000) methodology with some modifications. Peripheral blood was diluted with cold phosphate buffer solution (1:15); then 25 μL of the past solution was mixed with 75 μL of normal melting point agarose (0.7%); this mixture (100 μL) was then spread into the slides. To solidify the agarose, the slides were immediately protected from light and kept at 4 °C for 15 min; once the agarose was solidified, the slides were immersed in cold lysing solution (2.5 M NaOH, 10 M ethylenediaminetetraacetic acid (EDTA), 10 mM Tris, 10% dimethyl sulphoxide (DMSO), and 1% Triton, at pH 10) for 1 hour at 4 °C and protected from light.

Then slides were placed in an electrophoresis chamber for 20 minutes with a cold alkaline solution (300 mM NaOH and 1 mM EDTA) at pH 13, protected from light, to allow the unwinding. Electrophoresis was performed at 300μAmp, 25 V, and pH > 13 for 20 min. Slides were then stopped and washed three times with a neutralization buffer (0.4 M trizma base) at pH 7.4. Finally, the slides were stained with 20 μL of ethidium bromide and were examined with an epifluorescence microscope attached to an image analyser equipped with a program for measurement of the cell nucleus. A total of 100 measurements per triplicate per sample were made, and the %DNA damage in the tail (T/N) was obtained measuring the length of the tail (T) and the width of the nucleus (N). Measuring was done with a Zeiss Axiophot KS400 microscope equipped with epifluorescence and a 510–560 nm filter.

20.2.6 Statistical Analysis

Results of the comet assays were statistically evaluated by one-way analysis of variance (ANOVA), and differences between means were compared using the nonparametric tests Kruskal-Wallis and Dunn, with P set at <0.05. The differences with respect to time were tested. Statistical determinations were performed with SPSS v10 software (SPSS, Chicago IL, USA).

20.3 Results

Figure 20.1. It shows the results of the comet assay in lymphocytes of *Cyprinus carpio* exposed to 10 ng/L, 10 μ/L, and 10 mg/L of amoxicillin. The figure shows clearly a concentration-dependent increase in the groups exposed at 12 and 48 hours. For the lowest concentration (10 ng/L), an increase compared to the control group

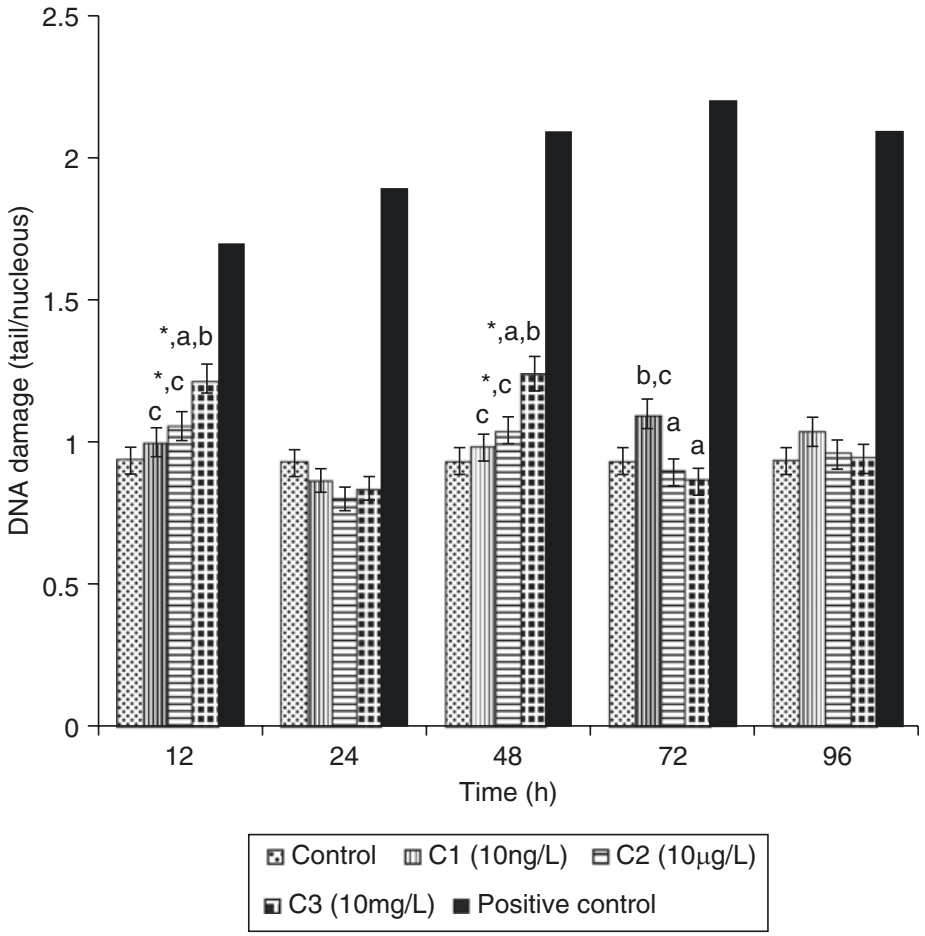

Fig. 20.1 Determination of the DNA damage via the comet assay in blood cells of *Cyprinus carpio* exposed to AMX. The bars represent the mean ± SEM of the index values of damage of five specimens by concentration and by exposure time. The assay was carried out in triplicate. Significantly different from ∗control group (*Kruskal-Wallis and Dunn*, $p < 0.05$)

was recorded at 12, 48, 72, and 96 hours of 7, 6, 18, and 11%, respectively (without significant differences), and at 24 hours, a decrement was obtained compared to the control group of 6% (without significant differences). The middle concentration (10 μ/L) shows a significant increase compared to the control group of 8 and 7% at 12 and 48 hours, but a decrement was obtained compared to the control group at 24 hours of 7% (without significant differences). Finally, for the higher concentration (10 mg/L), an increase compared to the control group was recorded at 12 and 48 hours of 31 and 32%, respectively, but again at 24 hours, a decrement was recorded compared to the control group of 11% (without significant differences)

Table 20.1. It shows the results of the analytical analysis of the water looking for amoxicillin and its main degradation product amoxicilloic acid. It shows clearly that amoxicillin was not detected in any test system at any concentration (10 ng/L, 10 μ/L, 10 mg/L) and at any time (12, 24, 48, 72, 96 h). Amoxicillin completely disappears from the water since the beginning of the experiment, even at 12 hours, which was our shorter time of analysis. On the other hand, amoxicilloic acid, present

Table 20.1 Amoxicilloic acid quantification/detection in water systems

E. time (h)	10mg/L		10 µg/L		10ng/L	
12	AMA1:**3.9 ± 0.88**	AMA2:**6.0 ± 0.95**	AMA1:**D**	AMA2:**D**	AMA1:**D**	AMA2:**D**
24	AMA1:**4.8 ± 0.9**	AMA2:**4.2 ± 0.98**	AMA1:**D**	AMA2:**D**	AMA1:**D**	AMA2:**ND**
48	AMA1:**5.1 ± 0.66**	AMA2:**2.6 ± 0.63**	AMA1:**D**	AMA2:**ND**	AMA1:**D**	AMA2:**ND**
72	AMA1:**4.8 ± 1.02**	AMA2:**0.9 ± 0.99**	AMA1:**ND**	AMA2:**ND**	AMA1:**ND**	AMA2:**ND**
96	AMA1:**4.7 ± 0.75**	AMA2:**0.4 ± 0.74**	AMA1:**ND**	AMA2:**ND**	AMA1:**ND**	AMA2:**ND**

Values are the mean of five replicates ± SE
ND no detected

Table 20.2 Amoxicilloic acid quantification/detection in blood

E. time (h)	10 mg/L		10 µg/L		10 ng/L	
12	AMA1:**D**	AMA2:**ND**	AMA1:**D**	AMA2:**ND**	AMA1:**D**	AMA2:**ND**
24	AMA1:**D**	AMA2:**ND**	AMA1:**D**	AMA2:**ND**	AMA1:**D**	AMA2:**ND**
48	AMA1:**D**	AMA2:**ND**	AMA1:**ND**	AMA2:**ND**	AMA1:**ND**	AMA2:**ND**
72	AMA1:**ND**	AMA2:**ND**	AMA1:**ND**	AMA2:**ND**	AMA1:**ND**	AMA2:**ND**
96	AMA1:**ND**	AMA2:**ND**	AMA1:**ND**	AMA2:**ND**	AMA1:**ND**	AMA2:**ND**

D detected, *ND* no detected

as two isomers 5R6R and 5S6R, was detected and quantified in all the concentrations and at all the times for the higher concentration (10 mg/L) and only detected until the 48 hours for the two lower concentrations (10 ng/L, 10 µ/L).

Table 20.2. It shows the results of the analytical analysis of the blood of *Cyprinus carpio* looking for amoxicillin and its main degradation product amoxicilloic acid. Considering the results in the water systems previously analysed, amoxicillin was neither detected nor quantified in the blood at any exposure time and at any concentration. However, one of the stereoisomers (5S, 6R) of amoxicilloic acid was detected but not quantified in the blood at the highest concentration tested (10 mg/L) but only until 48 hours after exposure and also detected but not quantified for the two lower concentrations (10 ng/L, 10 µ/L) but only until 24 hours after exposure.

20.4 Discussion

In a previous paper, we assess the oxidative stress that amoxicillin produces in *Cyprinus carpio* as well as its transformation in water and within some tissues as the brain, gills, the liver and the kidney, concluding that amoxicillin was transformed

into amoxicilloic acid by abiotic factors due to the presence of bacteria capable to produce beta-lactamase enzymes which lead to the opening of the beta-lactam ring, producing the transformation of amoxicillin into amoxicilloic acid. Furthermore, as this metabolite was detected and even quantified in the water and in all of the tissues analysed, at all concentrations and at all the times tested, it was concluded that the amoxicilloic acid was responsible for the oxidative stress produced in the different tissues of *Cyprinus carpio* (Elizalde-Velázquez et al. 2017).

Now in this research, we are investigating if the amoxicillin represents a genotoxic compound for the environment, assessing its potential to produce damage in the DNA of *Cyprinus carpio* using the comet assay. In the past, some studies have been performed to assess the genotoxic effects of amoxicillin in different species; however, the scientific literature has diverse results: some of them state that amoxicillin is not a genotoxic compound, while some others state the contrary. Istifli and Topaktas (2009) tested the genotoxic effect of amoxicillin in vitro using human peripheral blood lymphocytes, and they concluded that amoxicillin did not induce any sister chromatid exchange (SCE), did not increase chromosomal aberration (CA), and did not induce micronucleus (MN), and Cahill et al. (2004) reported also a negative result measuring the genotoxic effect of amoxicillin in the yeast *Saccharomyces cerevisiae* using the green screen assay.

On the other hand, some other research studies have reported genotoxic effects of amoxicillin in different species. For example, Arabski et al. (2005) tested the genotoxic potential of amoxicillin in human peripheral blood lymphocytes and gastric mucosa cells using the alkaline comet assay, and they conclude that amoxicillin can induce DNA damage in both cell lines causing strand breaks and base modifications, as a result of the production of reactive oxygen species; however, they suggest that amoxicillin needs a cellular activation before it can induce any DNA damage. Interestingly, Li et al. (2007) reported similar results as Arabski et al. (2005). They tested the genotoxic potential of amoxicillin in human and hamster culture cells using a modified comet-assay technique (comet nuclear extract or NE), concluding that amoxicillin induces DNA damages by action of reactive oxygen species, causing oxidation and opening rings of purine and pyrimidine bases. Finally, Anlas and Ustuner (2019) reported a study of the genotoxic activity of amoxicillin in *Oncorhynchus mykiss*; they tested the genotoxic potential of amoxicillin in rainbow trout erythrocytes using the comet assay and the micronucleus test, concluding that amoxicillin has genotoxic effects on fish, increasing the micronuclei frequency and the percent of tail DNA in cells, as well as suggesting that amoxicillin does not directly affect the DNA. Rather, it induces the DNA damage indirectly by increasing the production of reactive oxygen species, which leads to apoptosis and oxidative stress in fish species.

From the above, it is important to remark that three different scientific researches conclude that amoxicillin induce DNA damage indirectly by increasing the production of reactive oxygen species (Arabski et al. 2005; Anlas and Ustuner 2019; Li et al. 2007). As stated at the beginning of the discussion, in a previous work we suggest that amoxicillin induce oxidative stress in the brain, gill, liver and kidney of *Cyprinus carpio* by action of a hypersensitivity reaction, which induces an increase in the reactive oxygen species (Elizalde-Velázquez et al. 2017). Blood is another

susceptible tissue of oxidative damage since, in addition to fulfilling diverse functions such as the transport of xenobiotics throughout the body, it also transports proteins like albumin, lymphocytes, and haemoglobin, as well as other biomolecules to all body tissues, which are target of the free radical attack (Sanjuan-Reyes et al. 2013). Figure 20.1, as we described in the results section, shows that amoxicillin indeed has genotoxic effects in the lymphocytes of peripheral blood of *Cyprinus carpio*, increasing the tail of DNA cells particularly at 12 and 48 hours after its exposure to this bactericide chemical. Gathering the information of the works described above, with our previous work results and the current results of this research paper, it can be said that amoxicillin induces DNA damage in the lymphocytes of the peripheral blood of *Cyprinus carpio*, due to an increase in the reactive oxygen species.

Arabski et al. (2005) suggest that amoxicillin needs a cellular activation to induce the DNA damage, since amoxicillin did not induce DNA strand breaks in isolated plasmid DNA; therefore, a cellular activation of the drug might be associated with the free radical generation. About this point in our past work, we also demonstrate that amoxicillin was transformed into amoxicilloic acid by the cleavage of the beta-lactam ring due to the presence of beta-lactamase enzymes. Amoxicilloic acid in contrary to amoxicillin has reports of toxicity, specifically by inducing the activation of the immune response, since its structure is capable to create adducts with proteins, which after being recognized as strange for the body can trigger a hypersensitivity reaction that could explain the generation of reactive oxygen species, and as a result, it may induce oxidative stress or a genotoxic effect in cells (Elizalde-Velázquez et al. 2017). Table 20.1 and Table 20.2 show the results of the analytical analysis of all the water systems and the blood of *Cyprinus carpio*, and it is evident that amoxicillin was completely transformed into amoxicilloic acid since amoxicillin was not even detected at the shorter time of analysis (12 h) for both water and blood. Therefore, we suggest that the activation process that Arabski et al. (2005) refer could be the transformation of amoxicillin into amoxicilloic acid; furthermore, as described above amoxicilloic acid is responsible for the elevation of the intracellular reactive oxygen species, which also could explain what Anlas and Ustuner (2019) suggest that amoxicillin does not directly affect the DNA. Rather, it induces the DNA damage indirectly by increasing the production of reactive oxygen species, which leads to apoptosis and oxidative stress in fish species.

Finally, Arabski et al. (2005) and Li et al. (2007) reported that the DNA lesions of amoxicillin could be repaired within 60 min and 6 hours after the exposure to the antibiotic; however, both experiments were performed in human cell lines. Particularly Li et al. (2007) report that mammalian glycosylated enzymes OOG1 and OOG2 are enzymes capable to repair the damage caused by amoxicillin in the DNA by excision of the damaged bases. However, amoxicillin DNA damage may pose potential genotoxic problems to those that are genetically or physiologically deficient in the capacity to remove the oxidative DNA damage (Arabski et al. 2005). In fact, Anlas and Ustuner (2019) remark that fish cells have a low DNA repair activity compared to mammalian cells and therefore may be more susceptible to genotoxic agents.

20.5 Conclusion

Amoxicillin transformed by biotic factors into amoxicilloic acid may be capable to induce oxidative DNA damage to blood lymphocytes of *Cyprinus carpio* by generation of reactive oxygen species. Available data is not enough to conclude whether amoxicillin has genotoxic activity or not as well as if it represents a risk for the environment; therefore, scientific guild must strive to generate more genotoxic studies in different species to assess its genotoxic effects and to assess the DNA repair capacity in different bioindicators.

References

Andreozzi R, Caprio V, Ciniglia C, de Champdoré M, Lo Giudice R, Marotta R, Zuccato E (2004) Antibiotics in the environment: occurrence in Italian STPs, fate, and preliminary assessment on algal toxicity of amoxicillin. Environ Sci Technol 38:6832–6838

Anlas C, Ustuner O. Genotoxic assessment of amoxicillin in rainbow trout (Oncorhynchus Mykiss) by comet assay and micronucleus test. [cited 15 March 2019]. Available from https://www.researchgate.net/profile/Ceren_Anlas/publication/311587561_Genotoxic_assessment_of_amoxicillin_in_rainbow_trout_oncorhynchus_mykiss_by_comet_assay_and_micronucleus_test/links/58c78549aca27232ac90ea33/Genotoxic-assessment-of-amoxicillin-in-rainbow-trout-oncorhynchus-mykiss-by-comet-assay-and-micronucleus-test.pdf

Arabski M, Kazmierczak P, Wisniewska-Jarosinska M, Poplawski T, Klupinska G, Chojnacki J, Drzewoski J, Blasiak J (2005) Interaction of amoxicillin with DNA in human lymphocytes and H. pylori-infected and non-infected gastric mucosa cells. Chem Biol Interact 152:13–24

Brooks MA, Hackman MR, Mazzo DJ (1981) Determination of amoxicillin by high-performance liquid chromatography with amperometric detection. J Chromatogr A 210:531–535

Cahill PA, Knight AW, Billinton N, Barker MG, Walsh L, Keenan PO, Williams CV, Tweats DJ, Walmsley RM (2004) The GreenScreen(R) genotoxicity assay: a screening validation programme. Mutagenesis 19:105–119

Elizalde-Velázquez A, Gómez-Oliván LM, Galar-Martínez M, Islas-Flores H, Dublán-García O, SanJuan-Reyes N (2016) Amoxicillin in the aquatic environment, its fate and environmental risk. In: Environ. Heal. Risk – Hazard. Factors to Living Species. InTech. https://doi.org/10.5772/62049

Elizalde-Velázquez A, Martínez-Rodríguez H, Galar-Martínez M, Dublán-García O, Islas-Flores H, Rodríguez-Flores J, Castañeda-Peñalvo G, Lizcano-Sanz I, Gómez-Oliván LM (2017) Effect of amoxicillin exposure on brain, gill, liver, and kidney of common carp (*Cyprinus carpio*): the role of amoxicilloic acid. Environ Toxicol 32:1102–1120

Geissen V, Mol H, Klumpp E, Umlauf G, Nadal M, van der Ploeg M, van de Zee SEATM, Ritsema CJ (2015) Emerging pollutants in the environment: a challenge for water resource management. Int Soil Water Conserv Res 3:57–65

Gibs J, Heckathorn HA, Meyer MT, Klapinski FR, Alebus M, Lippincott RL (2013) Occurrence and partitioning of antibiotic compounds found in the water column and bottom sediments from a stream receiving two wastewater treatment plant effluents in Northern New Jersey, 2008. Sci Total Environ 458–460:107–116

Gozlan I, Rotstein A, Avisar D (2013a) Amoxicillin-degradation products formed under controlled environmental conditions: identification and determination in the aquatic environment. Chemosphere 91:985–992

Gozlan I, Rotstein A, Avisar D (2013b) Investigation of an amoxicillin oxidative degradation product formed under controlled environmental conditions. doi:https://doi.org/10.1071/EN10037

İstifli ES, Topaktaş M (2009) Cytogenetic genotoxicity of amoxicillin. Environ Mol Mutagen 51:NA-NA

Johnson AC, Keller V, Dumont E, Sumpter JP (2015) Assessing the concentrations and risks of toxicity from the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim and erythromycin in European rivers. Sci Total Environ 511:747–755

Kummerer K (2003) Significance of antibiotics in the environment. J Antimicrob Chemother 52:317–317

Li P-Y, Chang Y-C, Tzang B-S, Chen C-C, Liu Y-C (2007) Antibiotic amoxicillin induces DNA lesions in mammalian cells possibly via the reactive oxygen species. Mutat Res Toxicol Environ Mutagen 629:133–139

Liu Y, Wang F, Chen X, Zhang J, Gao B (2015) Cellular responses and biodegradation of amoxicillin in Microcystis aeruginosa at different nitrogen levels. Ecotoxicol Environ Saf 111:138–145

Mojica E-R, Aga DS. Antibiotics pollution in soil and water: potential ecological and human health issues. [cited 14 March 2019]. Available from http://elmericomojica.weebly.com/uploads/1/1/8/3/11833807/antibiotics_pollution_in_soil_and_water_potential_ecological.pdf

Oliveira R, McDonough S, Ladewig JCL, Soares AMVM, Nogueira AJA, Domingues I (2013) Effects of oxytetracycline and amoxicillin on development and biomarkers activities of zebrafish (Danio rerio). Environ Toxicol Pharmacol 36:903–912

Sanjuan-Reyes N, Gómez-Oliván LM, Galar-Martínez M, Vieyra-Reyes P, García-Medina S, Islas-Flores H, Neri-Cruz N (2013) Effluent from an NSAID-manufacturing plant in Mexico induces oxidative stress on Cyprinus Carpio. Water Air Soil Pollut 224

Sarmah AK, Meyer MT, Boxall ABA (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65:725–759

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35:206–221

Watkinson AJ, Murby EJ, Kolpin DW, Costanzo SD (2009) The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. Sci Total Environ 407:2711–2723

Yamanaka H, Sogabe A, Handoh IC, Kawabata Z (2011) The effectiveness of clove oil as an anaesthetic on adult common carp, Cyprinus carpio L. J Anim Vet Adv 10:210–213

Zheng Q, Zhang R, Wang Y, Pan X, Tang J, Zhang G (2012) Occurrence and distribution of antibiotics in the Beibu Gulf, China: impacts of river discharge and aquaculture activities. Mar Environ Res 78:26–33

Final Conclusions

Leobardo Manuel Gómez-Oliván

Water

Water is a vital resource for the survival of living beings, covering approximately 75% of the Earth's surface, is fundamental for both environmental and social processes, and is indispensable for the emergence and development of life.

As long as this resource is in a clean form, it will allow the proper functioning of ecosystems, communities, and economies around the world. Currently in Latin America and around the world, we are altering aquatic systems at an accelerated rate, and we have very serious problems related to the use and maintenance of this valuable resource.

Water quality is increasingly threatened by the impact of the growth of human populations and because anthropogenic activities expand. Both human activities and natural phenomena can change the physical, chemical, and biological characteristics of water, for example, changes in temperature, pH, biological factors, contamination by heavy metals, toxins, persistent organic compounds, pesticides, and emerging pollutants (among those that emphasize the pharmaceutical products and of personal care), among others. This affects the human health and the life of the organisms that inhabit all the ecosystems.

Water Pollution

Water pollution is defined as the introduction of chemical, physical, or biological agents or factors that induce a deterioration in the body of water. Likewise, these agents can produce deleterious effects in the organisms found in these aquatic ecosystems. The degree of contamination necessary to cause a body of water to be damaged depends on the type of water body, its location, and the sources of contamination surrounding them.

L. M. Gómez-Oliván
Laboratorio de Toxicología Ambiental, Facultad de Química,
Universidad Autónoma del Estado de México,
Toluca, Estado de México, México
e-mail: lmgomezo@uaemex.mx

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8

Although natural conditions can cause water pollution, this effect is also largely due to anthropogenic sources of pollution, that is, pollution derived from human activities.

Among the main water pollutants are:

(a) Garbage, chemical waste from factories, and industries
(b) Waste water and other waste (organic matter)
(c) Pathogens, such as bacteria, viruses, protozoa, and parasites
(d) Chemical products
(e) Petroleum
(f) Inorganic minerals and chemical compounds
(g) Sediments formed by soil particles and minerals washed away by storms and runoff from farmland, unprotected soils, mining operations, roads, and urban demolitions
(h) Radioactive substances from residues from the mining and refining of uranium and thorium, nuclear power plants, and the industrial, medical, and scientific use of radioactive materials
(i) Heat
(j) Noise

Water Pollution in Latin America

The American continent has 33% of the world's renewable water resources, and especially Latin America has the highest availability. Approximately, 3100 m^3 of water per capita per year doubles the world average. Most of the countries of Latin America have availability of water cataloged between high and very high due to its surface and population.

In the last two decades, the pollution of the rivers of Latin America has increased by 50%, warned the United Nations for the Environment Program.

The main causes of the alarming increase in surface water pollution in Latin America are population growth, increased economic activity, the expansion and intensification of agriculture, and the greater amount of untreated wastewater discharged into rivers and lakes.

The contamination of surface and groundwaters (rivers, lakes, reservoirs, aquifers, and the sea) in Latin America is a consequence mainly of anthropogenic activities. The contamination of the water is given by the introduction to the water of substances foreign to its composition, modifying its quality.

This contamination has its origin in various factors such as:

1. Pathogens: bacteria, viruses, protozoa, and parasites that enter the water from organic waste.
2. Waste that requires oxygen: Organic waste can be decomposed by bacteria that use oxygen to biodegrade them. If there are large populations of these bacteria, they can deplete the oxygen in the water, thus killing the aquatic life forms.

3. Inorganic chemical substances: acids and metals.
4. Vegetable nutrients: They can cause the excessive growth of aquatic plants that later die and decompose, exhausting the oxygen of the water and in this way cause the death of the marine species (dead zone).
5. Organic chemicals: petroleum, plastic, pesticides, and detergents that threaten life.
6. Sediments or suspended matter: insoluble soil particles.
7. Radioactive substances.

In Latin America, the problem is not the lack of fresh drinking water but, rather, the poor management and distribution of water resources and their methods. Most of the freshwater is used for agriculture, while a substantial amount is lost in the irrigation process.

Some other water problems in Latin America are as follows:

(i) 77 million people lack access to clean water.
(ii) 100 million people lack health services.
(iii) Lack of wastewater treatment or inefficient processes to treat it.
(iv) Great inequality between water rates.
(v) Serious financial limitations for the hydraulic sector.
(vi) Overexploitation of groundwater.
(vii) Pollution of lakes and rivers.
(viii) Environmental disasters such as hurricanes.
(ix) In addition, we must consider that wealth does not mean clean water. There are many countries that, although they are rich, do not necessarily have clean water and serious contamination problems.

Consequences of Water Pollution in Latin America

The effects of water pollution are serious, and each one of us has the duty to make efforts to reduce it.

The World Health Organization has stated on several occasions that 85% of the causes of diseases and deaths in the world are associated with contaminated water and lack of access to it. Annually, dysentery, diarrhea, and other waterborne diseases claim the lives of 3 million people.

The disappearance of biodiversity and aquatic ecosystems. The most serious problem of water pollution is that it kills the life of fish and aquatic animals. Fish, crabs, birds, gulls, dolphins, and many other animals are often found dead on beaches due to water pollution. Its habitat is polluted.

The human being is greatly harmed because of the alteration in the food chain. Pollution in water alters the natural food chain, since contaminants such as metals, pesticides, and hydrocarbons are ingested by small animals generating toxicity and at the same time these organisms are consumed by fish and shellfish, and then there is a transfer of toxicity to the humans.

The human being contracts diseases by drinking or using contaminated water.

Destruction of ecosystems. Due to water pollution, many ecosystems can be modified or destroyed, as animals die or modify their habits to survive. This contamination is caused by the carelessness of humans to dispose of contaminated waste in the water.

The main consequences of water pollution in Latin America can be summarized as follows:

- Alteration and destruction of the environment
- Alteration and destruction of the resource or vital liquid water
- Depletion of natural resources
- Loss of biodiversity and ecosystems
- Consumption of contaminated water not potable for humanity and living beings
- Respiratory, cardiac, chronic, and gastrointestinal diseases, among many others, that weaken physical, mental, and emotional health
- Deaths of children and the elderly
- Scarcity of drinking water
- Proliferation of pathogenic organisms
- Poverty and hunger
- Erosion and salinization
- Destruction of the landscape
- Loss of quality of life
- Other social, economic, and environmental consequences

Main Conclusions and Recommendations of the Water Pollution Problem in Latin America

In this book, the situation of Latin America and the impact of pollution are summarized. It shows both a review of the state of the art of the contamination of bodies of water in Latin America and some specific investigations that show the damages generated by the main pollutants that are present in superficial and groundwaters on various organisms. As a summary, we can determine that the waters of the bodies of water are contaminated principally by metals, pesticides, hydrocarbons, plastics, and emerging pollutants of various chemical nature. The concentrations of these pollutants fluctuate in magnitudes from ng/L to mg/ L.

These pollutants have the following effects: toxic effects (acute, subchronic, and chronic toxicity), specific effects (oxidative stress, genotoxicity, and cytotoxicity), and, in addition to embryotoxicity and teratogenicity, functional damages (neurotoxicity, nephrotoxicity, hepatotoxicity, hemotoxicity, and other manifestations).

These effects were identified in various hydrobionts including algae, crutaceous, amphipods, amphibians, and fish, of various gender.

In Latin America, less than 20% of wastewater is adequately treated, meaning serious social, economic, and environmental problems in the region; this is basically

a problem of financial capacity, due to the difficulty of internalizing the costs of treating this wastewater. Efficient strategies are required at the administrative, educational, and research level, as well as innovation in technologies and economic instruments, which are socially equitable.

Several aspects are identified, such as the need for alternative financing sources, the demand for the rehabilitation of the current infrastructure, the indispensable increase in the installed capacity to cover the deficit in the service, environmental education, training and awareness of all the sectors and at all levels, including administrative ones, together with the effective availability of information and ensuring the participation of all actors. All these are premises to advance toward an integral management of water bodies.

Indicator studies are required to evaluate comparatively and in transparent databases directed to the entire population and to environmental authorities.

More legislation is required, as well as compliance with existing legislation to ensure the adoption of mitigation measures for water pollution and prevention of water pollution.

Index

L. M. Gómez-Oliván (ed.), *Pollution of Water Bodies in Latin America*,
https://doi.org/10.1007/978-3-030-27296-8