Chapter 3
Metal(loid)s in Macrophytes from the Americas

Magdalena V. Monferrán, Iara da C. Souza, Hiulana P. Arrivabene, and María V. Amé

Abstract Pollution from metals and metalloids is a major concern due to the persistence of these elements in the environment and the impacts on ecological and human health. Evaluation of the distribution, bioaccumulation and toxicity of metal(loid)s in aquatic organisms has been studied for many years. In consequence, research on plants with a high tolerance to metals, and therefore, of use as bioindicators of theses contaminants, has become a subject of interest in recent years. This chapter presents a brief analysis of the bioaccumulation patterns and physiological responses of aquatic plants to pollution from metals and metalloids, with a focus on describing what we have learned from our studies conducted in the laboratory and in the field with the emergent macrophyte, *Potamogeton pusillus* and with the mangrove species, *Avicennia schaueriana*, *Laguncularia racemosa* and *Rhizophora mangle*, all of which are native to South America. Furthermore, the capacity of *P. pusillus* to be used for active and passive biomonitoring in aquatic ecosystems highly impacted by environmental degradation is discussed. In addition, there is a discussion of the bioaccumulation and translocation of metals from interstitial water and sediment to the roots and leaves of mangroves inhabiting estuaries in Brazil with different levels of pollution, correlating metal bioaccumulation with differences in macrophyte anatomy. Finally, the use of stable isotopes in mangroves as biomarkers of environmental pollution is demonstrated.

M. V. Monferrán · M. V. Amé (✉)
Facultad de Ciencias Químicas, Departamento de Bioquímica Clínica, Centro de Investigaciones en Bioquímica Clínica e Inmunología-CIBICICONICET, Universidad Nacional de Córdoba, Córdoba, Argentina
e-mail: valeria.ame@unc.edu.ar

M. V. Monferrán
e-mail: mmonferran@unc.edu.ar

I. da C. Souza · H. P. Arrivabene
Departamento de Ciências Biológicas, Universidade Federal do Espírito Santo, Espírito Santo, Brazil
e-mail: iara.csouza@ufscar.br

M. L. Menone and C. D. Metcalfe (eds.), *The Ecotoxicology of Aquatic Macrophytes*, Environmental Contamination Remediation and Management,
https://doi.org/10.1007/978-3-031-27833-4_3

3.1 Introduction

In recent years, there has been increasing evidence of the widespread occurrence of contamination by metals and metalloids in aquatic ecosystems from all around the world. These elements, unlike organic contaminants, which can be degraded to less harmful products by biotic or abiotic processes, are non-degradable and persistent, with high toxicity, and can be bioaccumulated in aquatic organisms, resulting in sublethal concentrations affecting the biota. In some cases, these elements can even be biomagnified in the food chain, thus threatening human health (Cordoba-Tovar et al. 2022).

There are different sources of metals and metalloids in the environment. These can be of natural origin (geogenic) or derived from human activities (anthropogenic). The most important natural sources are mineral weathering, erosion and volcanic activity, while anthropogenic sources include mining, smelting, electroplating, pesticide and fertilizer use, as well as biosolids from agriculture, sludge dumping from industrial and domestic sources, among others (Shi et al. 2022). Although some metal(loid)s can be strongly adsorbed onto the suspended particles and sediments, they can be released into the water under suitable conditions of pH and redox potential, leading to further contamination (Wang et al. 2022). Some metals, including cadmium (Cd), zinc (Zn), lead (Pb), chromium (Cr), nickel (Ni), copper (Cu), vanadium (V), platinum (Pt), silver (Ag), tin (Sn) and titanium (Ti) are highly toxic to aquatic organisms (Zaynab et al. 2022).

Many aquatic ecosystems in South America are contaminated with metals and metalloids. Their distribution is quite variable, reflecting contamination from both point and non-point sources. For example, the upper and middle reaches of the Cachapoal River in Chile are characterized by elevated concentrations of Cu, Mo, As, Pb, Cr that reflect inputs of material from mining activities (Lacassie and Ruiz-Del-Sola 2021). Also, the Río de La Plata between Argentina and Uruguay shows strong features of sediment retention, favoring pollutant accumulation. Sediments from Montevideo Bay are highly polluted with Zn, Pb, Cu, Cr and mercury (Hg) and moderately contaminated with Ni and Ag (Barletta et al. 2019). The presence of metals was also documented in the main water bodies of the Province of Córdoba, Argentina, in the Suquía, Xanaes and Ctalamochita Rivers and in the San Roque, Los Molinos and Río Tercero Reservoirs, which is mainly associated with urban pollution (Contardo-Jara et al. 2009; Monferrán et al. 2011, 2016a, b; Griboff et al. 2017, 2018b, 2020; Bertrand et al. 2019). Moreover, high levels of aluminum (Al), Cr, manganese (Mn), iron (Fe), Ni, Cu, Zn, arsenic (As), selenium (Se), Ag, Cd, Hg and Pb were found in water and sediment in two neotropical estuaries located in the State of Espírito Santo, Brazil, namely, Vitória Bay and Santa Cruz, which are areas affected by different pollution sources and marine processes (Souza et al. 2014a, 2015).

The accumulation of metals and metalloids in biota depends on the chemical properties of each element, the characteristics of individual organisms and the role played by different organs and tissues in the processes of absorption, regulation, storage and excretion. Some essential elements, such as Zn, Fe and Cu are necessary for cellular functions, but exposure to high and/or prolonged doses of these elements can lead to neurotoxic, genotoxic and carcinogenic alterations (Geng et al. 2019). Exposure to high concentrations of metals and metalloids can induce oxidative stress by producing reactive oxygen species (ROS), resulting in DNA damage, lipid peroxidation, depletion of protein sulfhydryl groups, impairment of cell signaling, altered Ca homeostasis, changes in expression of the Ca regulation gene, etc. In addition, they can replace essential metals in pigments or enzymes, disrupting their normal function (Kolarova et al. 2021).

To detect environmental pollution by using biological materials as indicators is a reliable and simple alternative to conventional sampling methods. The distribution and condition of many aquatic macrophytes are often correlated with water quality. Some macrophytic species can accumulate considerable amounts of metals in their tissues. Therefore, aquatic macrophytes stand out as having the potential to be useful indicators of metal contamination in the aquatic environment (i.e., biomonitors, bioindicators) as documented by Farias et al. (2018), due to their high tolerance to metal pollution, convenience for sampling and easy culturing in the laboratory. As we document in this chapter, our previous studies have shown that monitoring of the macrophyte species, *Potamogeton pusillus*, *Avicennia schaueriana*, *Laguncularia racemosa* and *Rhizophora mangle* can be an effective tool for studying contamination by metals and metalloids in both fresh and brackish waters.

Potamogeton pusillus is an aquatic plant of 20–30 cm length, with thin and branched stems (Fig. 3.1). Its leaves are shaped like a ribbon, flattened and subphyliform when the plant is young, and they are between 3 and 6 cm long and 0.6–1 cm wide, hyaline, with 1–3 vascular bundles and air channels in the central region of the sheet. These freshwater plants have a fully developed root system that is completely submerged in the river sediment. It is considered a sentinel native macrophyte, having ecological importance within sub-tropical aquatic ecosystems, providing shelter and habitat for young fishes and other aquatic animals (Novara 2003. http://www.iucnredlist.org). This species is present in aquatic environments that are moderately to highly polluted in the main rivers of the state of Córdoba in Argentina, which makes this macrophyte very useful as a biomonitoring species (Harguinteguy et al. 2016; Bertrand et al. 2019).

Avicennia schaueriana (Stapf & Leechm. ex Moldenke) of the plant family of Acanthaceae, *Laguncularia racemosa* (L., C. F. Gaertn) of the Combretaceae family and *Rhizophora mangle* (L.) of the Rhizophoraceae family are three true mangrove species (Tomlinson 2016). *A. schaueriana* has extensive underground roots, supporting pneumatophores and absorption roots. Its bark is variably rough, dark, rigid and fissured. It has an entire leaf blade, ranging from ovate to elliptical. The leaf blade is leathery or slightly fleshy, with inconspicuous veins and a prominent mid-rib below (Fig. 3.2). It has salt glands on both sides of the leaf blade and water storage parenchyma underlying the adaxial surface of the epidermis. *L. racemosa* has extensive underground roots, cable-like, supporting pneumatophores and absorbing roots. Its bark is rough, fissured and grayish. Its branches have numerous slightly prominent lenticels. Its leaf blade is somewhat fleshy, elliptical to oblong (Fig. 3.2). They have leaves with a petiole containing a pair of extrafloral nectaries

Fig. 3.1 *Potamogeton pusillus* in the Suquía River at La Calera, Córdoba, Argentina (A). *P. pusillus* leaves shaped like a flattened ribbon (B and C)

on their adaxial surface. The leaf blade has salt glands distributed on the abaxial and adaxial surfaces of the epidermis. A cross-section of the blade reveals a water storage parenchyma in its middle portion. Finally, *R. mangle* presents rhizophores with positive geotropism, responsible for providing stability in the sediment, which, when in contact with the soil, form the roots. Its leaves are entire, elliptical, glabrous and with numerous cork warts on the abaxial surface, visible on older leaves as dark spots (Fig. 3.2). They have evident but not prominent veins. Its seeds are viviparous, germinated by the extension of the hypocotyl, with propagules measuring about 20–30 cm. The analysis of oxygen isotopes ($^{18}O/^{16}O$) in *R. mangle* showed that this mangrove plant use surface soil and seawater rather than groundwater as a water source, even when the water has a high salinity (Lin and Sternberg 1994).

This chapter describes the responses of these structurally and physiologically different macrophyte species that grow in freshwater and under saline conditions when they are exposed to metals and metalloids both in the laboratory and in the environment under natural conditions.

Fig. 3.2 Mangroves from Santa Cruz estuary, State of Espírito Santo, Brazil (A). Leaves of mangrove plants: *Laguncularia racemose* (B)*, Avicennia schaueriana* (C) *and Rhizophora mangle* (D)

3.2 Laboratory Studies

3.2.1 Accumulation of Metal(loid)s in P. pusillus *and Impact on Biochemical Parameters*

We have carried out several laboratory studies with *P. pusillus* to assess bioaccumulation and effects from exposure to different metals. The concentrations used in the laboratory experiments were selected according to environmentally relevant data found in the literature (Cheung et al. 2003; Smolders et al. 2003; Monteiro et al. 2010; Hashem et al. 2020; Francisca et al. 2006).

In one series of experiments, we collected macrophytes from a reference site, placed them into a 40 L tank containing 10% Hoagland's solution and sediment (1/4) from the same sampling area and grew them for two weeks under a light/dark photoperiod of 14 h:10 h before starting the exposures (Monferrán et al. 2012a). For exposure to metals and metalloids, organisms were relocated into 1 L beakers (three plants per beaker, 5–8 g wet weight-w.w. per liter) containing 10% Hoagland's solution prepared without the element to be tested. After the exposure time, which depended on the element studied in each case, plants were washed three times with ultra-pure water, frozen with liquid nitrogen and kept at −80 °C until analysis. Concentrations of metals and metalloids in exposure media were measured by inductively coupled plasma-mass spectrometry (ICP-MS) and accumulation in plant tissues was determined by atomic absorption spectroscopy (AAS) after digestion of the samples with aqua regia (Monferrán et al. 2012a), with the exception of Hg and As, which were also analyzed by ICP-MS.

A laboratory bioassay testing the kinetics of Cu^{+2} and Cr^{+6} uptake by *P. pusillus* from water solutions (individual exposure) demonstrated that accumulation of these metals is in a concentration and time-dependent manner, where the most significant increase in concentration observed was at 5-day exposure, although the metal content continued to increase gradually up to 15 days. Time-dependent (kinetic) studies on the uptake of metals by aquatic plants have shown an initial rapid accumulation phase, followed by a slower linear phase. The initial phase represents a rapid, reversible, metal binding process (i.e., biosorption), while the subsequent slower phase is governed by metal transport across the plasma membrane into the plant cytoplasm (i.e., bioaccumulation), as described by Monferrán et al. (2012a).

Another experiment was conducted to assess the effect of Cu^{+2} on the bioaccumulation of Cr^{+6} by *P. pusillus*. These assays showed that the presence of Cu^{+2} drastically increased the phytoextraction of Cr^{+6}, particularly at the lowest Cu^{+2} concentrations of 0.1 mg/L and 0.5 mg/L, where the phytoextraction of Cr^{+6} by the plant rose 3.5-fold when Cu^{+2} concentration was increased from 0 to 0.5 mg/L, keeping Cr^{+6} concentration constant. These observations are clear evidence of enhanced phytoextraction of Cr^{+6} by *P. pusillus* from binary solutions containing Cu^{+2}.

The accumulation of both metals in the plant resulted in toxic effects. This was seen when NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) values were calculated, based on changes in chlorophyll-a (Chl-a) and protein contents in *P. pusillus* exposed to different concentrations of Cu^{+2} and Cr^{+6}. The NOEC for Chl-a was 0.5 mg/L for Cu^{+2} and 2 mg/L for Cr^{+6} (Monferrán et al. 2012a). This indicates that Cu^{+2} is more toxic to the plant than Cr^{+6}. The same trend was found when protein contents were used to calculate the NOECs, which were 1 mg/L for Cu^{+2} and 2 mg/ L for Cr^{+6}. This indicated that Chl-a is the more sensitive toxic endpoint for Cu^{+2} toxicity than the protein level (i.e., NOEC = 0.5 mg/L for Chl-a and NOEC = 1 mg/L for proteins), while Cr^{+6} showed the same NOEC for both toxic endpoints.

We also demonstrated that *P. pusillus* was able to accumulate significant concentrations of Hg after 7, 14 and 20 days of hydroponic treatment (Griboff et al. 2018a). The maximum rate of metal accumulation was found after day 7 in a treatment with 2 mg/L Hg, when 96% of the total accumulated metal was taken up by the plant (2,372 μg/g dw). Metal accumulation continued through days 14 and 20, although the bioaccumulation rate was lower than that reported for day 7. Thus, Hg content was 2,034 μg/g dw (83%) after a 14-day exposure, and 2,465 μg/g dw after a 20-day exposure. It is worth mentioning that bioaccumulation also occurred at lower Hg concentrations (i.e., 0.1; 0.5 and 1 mg/L) but at lower rates in comparison with the bioaccumulation observed during exposures at 2 mg/L.

When the capacity of *P. pusillus* to accumulate As was evaluated, we observed that the accumulation of As^{+3} and As^{+5} by *P. pusillus* increased as the exposure concentration increased, but it did not increase as the exposure time increased. Specifically, the concentration of As accumulated by *P. pusillus* when it was exposed for 7 days at different As^{+3} or As^{+5} concentrations was the same or not statistically different from treatments with exposure for 14 or 20 days to the same concentrations (Griboff et al. 2018a). This plant accumulated more As when it was exposed to As^{+3} (281 μg/g dw) relative to accumulation when exposed to relative to As^{+5} at the same concentration (117 μg/g dw). These results are important, given that As^{+5} is more toxic than As^{+3}.

NOEC and LOEC values were calculated for the toxic endpoints of Chl-a and protein content in *P. pusillus* exposed to different concentrations of As^{+3}, As^{+5} and Hg over 15 days. NOEC for chlorophyll-a was 0.1 mg/L for As^{+3}, As^{+5} and Hg (Griboff et al. 2018a). These experiments indicated that the As species were more toxic to the plant than Hg, taking into account that the concentrations of Hg accumulated (46 μg/g dw) at 0.1 mg/L of exposure were much higher than that of As (10 and 6 μg/g dw for As^{+3} and As^{+5} exposure, respectively) exposed in treatments at the same concentration. No statistically significant differences in protein levels were observed in the plants exposed to As^{+3}, As^{+5} or Hg compared to the control group. Comparing these results, it appears that Chl-a is a more sensitive endpoint for the toxic effects of As^{+3}, As^{+5} and Hg than the proteins level (NOEC = 0.1 mg/L). In our experiments, significant damage to the macrophyte pigments were observed in *P. pusillus* after exposure to As^{+3}, As^{+5} and Hg (Griboff et al. 2018a). These changes reflect the diversity of the disorders to cellular metabolism generated by exposure to these elements. Loss of photosynthetic pigments is a common response of plants to environmental stressors such as heat, diseases and pollution.

P. pusillus accumulated large amounts of Pb (2,470 μg/g dw) after exposures of the plants for 10 days to Pb^{+2} at a concentration of 2.0 mg/L, with removal of 74–92% of this metal from solution. In addition, *P. pusillus* accumulated large amounts of Cd^{+2} (2,045 μg/g dw) after exposure of the plants for 10 days to 2.0 mg/L of Cd^{2+}, with removals from solution of 89 to 91% (Rivela Fretes et al. 2021a, b). The accumulation of Pb in *P. pusillus* did not result in changes to the content of Chl-a and b, malondialdehyde (MDA) and sugars in all treatments. However, the content of carotenes increased relative to the control treatment for the plants exposed to 0.5 mg/L of Pb for 7 days. Carotenoids belong to the plant's non-enzymatic antioxidant defense system and in fact play a key role in protecting the photosynthetic system from the effects of excess metals. Carotenoids trap and then scavenge ROS (Krayem et al. 2021). In contrast, the accumulation of Cd resulted in changes in levels of Chl-a and Chl-b. Chlorophyll-a and b decreased as Cd concentration increased and the NOEC for Chl-a and b toxic endpoints was 1 mg/L. The content of carotenoids, sugars and MDA were not affected by exposure to Cd in all treatments.

Finally, to determine the potential use of *P. pusillus* as a bioindicator of aquatic contamination with Zn, the macrophyte was experimentally exposed to this metal and the response of biomarkers of exposure and effect were evaluated (Bertrand et al. 2016). In this study, both the biomarkers and the accumulation of Zn were evaluated in different tissues of the plant (leaf, stem and root). The experimental treatments were: Control (not metal exposed) and plants exposed to 5, 50 and 500 μg/L Zn. The biomarkers of toxicity monitored were hydrogen peroxide (H_2O_2) concentration, lipid peroxidation measured as thiobarbituric acid-reactive substances (TBARs), antioxidant enzymes activities and concentrations of chlorophyll (Chl) and pheophytins (Pheo) concentrations, consistent with Bertrand et al. (2016). In these experiments, Zn bioaccumulation and biological responses (i.e., oxidative stress biomarkers and pigments) indicated a differential response pattern among leaf, stem and roots in *P. pusillus*. This macrophyte showed rapid Zn accumulation, with a significant increase in tissue concentrations in treatments with 50 μg/L in the leaf and in treatments with 5 μg/L in the stem and root. Although Zn accumulation in the leaf occurred at higher exposure concentrations than in the other plant tissues, the bioaccumulation in the treatment with 500 μg/L was greater than those in stem and root. Specifically, the Zn concentration in leaf exposed at 500 μg/L was five times higher than in the control treatment (i.e., leaf in control = 198 ± 34 μg/g d.w.; leaf in 500 μg/L treatment = 1,063 ± 208 μg/g d.w.), while Zn concentrations in other plant tissues increased only three times relative to the control (i.e., stem in control = 177 ± 56 μg/g d.w.; stem in 500 μg/L treatment = 676 ± 165 μg/g d.w.; root in control = 146 ± 83 μg/g d.w.; root in 500 μg/L treatment = 554 ± 120 μg/g d.w.).

The induction of cellular changes often goes along with the bioaccumulation of metals in higher plants, some of which directly contribute to metal tolerance of plants. In *P. pussilus*, even though a significant rise in H_2O_2 was observed in leaf and root in treatments with Zn at 5 μg/L, no significant variations in TBARs concentration were detected in any of the plant tissues (Bertrand et al. 2016). Higher levels of H_2O_2 were measured in the root from the Zn treatment at 500 μg/L, at levels three times greater than in the control condition (i.e., root in control = 0.33 ± 0.06 mg/g H_2O_2 w.w.; root in 500 μg/L treatment = 0.92 ± 0.19 mg/g H_2O_2 w.w.). When this macrophyte was exposed to Zn, the levels of Chl-a in the leaf decreased significantly in the 500 μg/L treatment (i.e., leaf in control = 399 ± 35 μg/g w.w.; leaf in 500 μg/L treatment = 191 ± 55 μg/g w.w.). However, no significant differences between the control and other treatments were observed in the Chl-b, Pheo-a or Pheo-b levels measured either in the leaf or the stem. Among pigments, Chl-a is well-known to be the most sensitive to oxidative stress. Therefore, the decrease in Chl-a concentrations in the leaf could be related to the increased H_2O_2 levels in the same plant tissue. In the stem, concentrations of Chl-a remained constant. The Zn concentration at which a significant effect on pigments is detected is species-dependent. In *P. pectinatus*, significant Chl-a loss was observed in the treatment with 6.5 mg/L Zn after 24 h exposure (Tripathi et al. 2003), while similar changes in *Ceratophyllum demersum* required concentrations higher than 13 mg/L Zn to affect the photosynthetic system, including the pigments (Aravind and Prasad 2004).

Regarding antioxidant enzyme activities, there was significant inhibition of glutathione peroxidase (GPx) activity in the leaf and root in the Zn treatment with 5 μg/L, the lowest Zn concentration tested, while in the stem, the enzyme activity increased in the treatments with 5 and 50 μg/L. The activity of glutathione reductase (GR) in leaf diminished in the 500 μg/L treatment, while in the root, the same effect was observed at 50 and 500 μg/L (Bertrand et al. 2016). The capacity of Zn to inhibit the activity of GR in plants has been reported by other authors (Schaedle and

Bassham 1977). This response could indicate a negative effect on enzymatic antioxidant mechanisms. However, no variations were observed in guaiacol peroxidase activity (POD). This lack of response could indicate that exposure concentrations were not sufficient to affect this enzyme of the antioxidant system. No change in enzymatic activity was detected for glutathione-S-transferase (GST) in microsomal or cytosolic fractions, as well as for GR in the stems.

All results considered, the leaf of *P. pusillus* showed a higher resistance to the inhibition of antioxidant enzymes in comparison to the root. The increase in H_2O_2 levels (i.e., ROS) in the leaf was not enough to activate the enzymes from the antioxidant and protective system. This could be due to non-enzymatic antioxidant mechanisms being able to neutralize the ROS. Non-enzymatic antioxidant mechanisms such as glutathione were not measured in the present study. The biomarkers of Chl-a and GR measured in leaf as well as H_2O_2 and GR activity in root were the best parameters to explain the variation in effects from Zn exposure concentrations (Bertrand et al. 2016). However, these biomarkers did not show a good capacity to predict impacts from the different exposure concentration. The levels of Zn accumulated in any tissues of *P. pusillus* were more representative of exposure concentrations.

Submerged plants have very thin cuticles through which metals in the surrounding water can readily pass. The accumulation of metals and metalloids by *P. pusillus* is selectively related to the physiological roles of these elements in the metabolism of the plant. The surfaces of submerged plants such as *P. pusillus* are usually coated with active biofilms, which consist of a complex combination of microorganisms, exudate polymers, absorbed nutrients and metabolites, and particulate materials. Biofilms have been found to exert effective control on metal pollution in aquatic systems. Since they are polyanionic, biofilms can facilitate the biosorption of metal compounds (Geng et al. 2019). In addition, some bacteria species can modify sorption of these elements by increasing the surface area of the plants or root length, or promoting biofilm formation, which can potentially increase their bioavailability (Palansooriya 2019).

The bioaccumulation of metals in *P. pusillus* is often accompanied by the induction of a variety of cellular changes, some of which directly contribute to the metal tolerance of plants. Among the variety of toxicity endpoints for the elements that we studied in *P. pusillus*, the photosynthetic apparatus and protein contents seem the most sensitive. The toxicity of metals also involves oxidative stress, followed by oxidative damage to membranes and pigments.

3.2.2 Distribution of Metal(loid)s in **P. pusillus** *Tissues*

Our previous studies on the accumulation Cu and Cr by *P. pusillus* showed that the amounts accumulated in the plant tissues were consistent with the concentrations of the metals in the aqueous medium, with significantly higher levels of both metals in the root and leaves than in the shoots (Monferrán et al. 2012a). The relative amounts of As and Hg increased in all studied tissues as metal concentration increased, showing significantly higher levels of As in the root than in the shoots or leaves when the aquatic plant was exposed to all concentrations of As^{3+}. On the other hand, when *P. pusillus* was exposed to As^{5+}, the plant tissues with the highest As accumulation were the root at the lowest exposure concentration of 0.1 mg/L and the stems at the exposure concentration of 0.5 mg/L, while no statistically significant differences were observed between the plant tissues in treatments at 1 and 2 mg/L. Finally, when *P. pusillus* was exposed to Hg, it was observed that the highest levels of Hg were found in leaves relative to the shoots or roots at all concentrations evaluated (Griboff et al. 2018a).

When *P. pusillus* was exposed to Cd at concentrations of 0.5 and 1 mg/L, no significant differences were observed in accumulation in the different parts of the plant. However, in a treatment at 2 mg/L of Cd for 10 days, the leaves showed higher accumulation for this metal relative to the stem and root. The *P. pusillus* tissue that accumulated the highest Pb concentrations was the root (Rivela Fretes et al. 2021a, b).

As was described earlier, *P. pusillus* exposed to Zn at 5 and 50 μg/L showed no significant differences in Zn concentration accumulated in the different sections of the plant. However, in a treatment with 500 μg/L Zn for 4 days, the leaves showed a higher accumulation for this metal relative to the stem and root.

Submerged plants have considerable potential to accumulate metals from the surrounding environment. The leaves and roots provide physical support for biofilms, which facilitate both facultative anaerobic and anaerobic microorganisms to absorb nutrients. In addition to the nutrients required by living organisms, plants and biofilms also accumulate non-essential elements (e.g., Cd, Cr and As). The epiphytic biofilms adsorb/absorb metals and transport them to the leaves (Geng et al. 2019).

Roots are the main tissue for the accumulation of various metals by aquatic plants. The sequestration, immobilization and accumulation of metals in the root may be due to the process of rhizofiltration, which is commonly observed in aquatic plants. Roots exudates in the rhizosphere may also cause settling of metals onto the root surface. Moreover, metals can be actively absorbed into root cells via plasmalemma, adsorbed onto cell walls via passive diffusion or moved acropetally in the roots of aquatic macrophytes. Besides, ion exchange with the surrounding solution may also take place rapidly in the “free space” (apoplasm) of the root, which facilitates the penetration process without passing through living membranes (Tibbett et al. 2021).

Metal accumulation in leaves may be largely attributed to ion exchange within this tissue and the surrounding solution and also via passive transport of ions into the peripheral region. Aquatic macrophytes, with a well-developed root/rhizome system and totally submerged foliage, extract elements mostly from sediments. However, uptake by leaves becomes important when the metal concentration in the surroundings is high or when metals are bound to not readily available compounds in the sediment (Rezania et al. 2016).

Stems of *P. pusillus* accumulated much less metals than leaves or roots. This could be due to its lower volume in relation to a large surface area for uptake in leaves. It has been demonstrated that the ratio of total volume to surface area differed significantly between leaves and stems in *P. natans* (Rezania et al. 2016). Additionally, leaves have lower water content than stems, indicating that leaves contain more dry material to which metals can bind. Furthermore, the organic matter content may influence the binding capacity, since metals have a high affinity for organic material. The organic matter consists largely of cell walls containing pectin, which contain a number of negative-charged polygalacturonic acid sites, allowing cation exchange and thus, metal absorption (Rezania et al. 2016).

3.2.3 Accumulation and Translocation of Metal(loid)s in Mangrove Plants

Mangrove ecosystems in tropical and subtropical intertidal zones play a key role in maintaining the coastal ecological balance and species diversity (Souza et al. 2015). Ecotoxicological studies with mangrove plants can be carried out by growing the plants in a greenhouse. In experiments described by Arrivabene et al. (2016), propagules of *A. schaueriana*, *L. racemosa* and *R. mangle* were collected directly from the mother plant in an ecological reserve, transported to a greenhouse and cultivated in pre-cleaned PVC pots (2.8 L each) containing washed sand. Sand pots were stored in receptacles containing Hoagland's nutrient solution, with 0.25 ionic strength and a salt content of 7 g/L. The level of the nutrient medium in the substrate was approximately 3 cm during plant growth, and approximately 7 cm during exposures, simulating mangrove swamp conditions. The nutrient medium was covered with a black PVC film to prevent photo-oxidation. Propagules were developed during eight months and afterward plants were used for metal exposure. Initially, exposures were performed by adding 0 (control), 10, 20 and 100 mg/L Fe(II)SO_4 (to simulate the bioavailable form of Fe), disodium EDTA and MES buffer (1 mM, pH 6) to the nutrient medium (which already contained 0.53 mg L^{-1} Fe as $FeCl_3$). Iron concentrations of 10 and 20 mg/L were selected as they are close to values found in the interstitial water during field studies and the highest concentration (100 mg/L) was selected to simulate a more toxic condition, with iron levels exceeding current environmental levels. Sets of five independent plants from each species (randomly selected) were exposed to different Fe concentrations over a period of eight weeks. After exposure, plants were harvested and analyzed.

In the experiments conducted according to this protocol, it was found that the three plant species were capable of bioaccumulating Fe in their tissues (Arrivabene et al. 2016). L. *racemosa* showed dose-dependent bioaccumulation in root and in iron plaque, in addition to an inhibitory behavior with secretion of Fe through salt glands. This species was judged to be the most appropriate mangrove for biosensing the amount of iron present in estuarine/marine environments due to environmental pollution. A significant decline in translocation factors between aerial parts of the plant and the root was evident, mainly in *R. mangle* and *A. schaueriana*, indicating the impact on the plant transport mechanism induced by high concentrations of added Fe(II). Changes in plant anatomy and histochemistry were not as evident as those observed with bioaccumulation and translocation. Iron plaque proved to be an important site of accumulation of the metal, functioning as a barrier to entry into the plant. Furthermore, Fe elimination was observed by salt glands located in leaves of *A. schaueriana* and *L. racemosa*, although there was no greater Fe elimination in plants subjected to higher doses of Fe in the substrate (Arrivabene et al. 2016).

Using the same experimental design, a study was carried out to evaluate the effects of salinity on the bioaccumulation of Cr, As, Hg and Pb and on the anatomical, physiological and biochemical characteristics of *L. racemosa* and *R. mangle* (Campos 2018). Exposures to these metal(loid)s occurred by adding 28 μg/L of Cr_2O_7, 2 μg/L of As_2O_3, 10 μg/L of $HgCl_2$ and 10 μg/L of $PbCl_2$ into Hoagland's nutrient solution. After 12 weeks of treatment, samples were collected for analysis. The results showed that *L. racemosa* was more responsive to the sublethal toxic effects of Cr, As and Hg than *R. mangle*, especially in the root. In *L. racemosa*, the accumulation of Cr, As and Hg in the root changed stomatal density, stomatal conductance and the vascular bundle area of the mid-rib. Therefore, considering the species studied, *L. racemosa* proved to be most suitable as an environmental bioindicator for the presence of these elements (Campos, 2018).

3.3 Field Studies

3.3.1 Potamogeton pusillus *as a Bioindicator of Elemental Contamination*

Biomonitoring can be conducted by sampling organisms living in an investigated area (i.e., passive biomonitoring), or by exposure of organisms collected from a reference site or from a laboratory culture translocated to the investigated area (i.e., active biomonitoring). Both approaches were applied with *P. pusillus* in our field studies conducted in Córdoba Province: passive biomonitoring in the Suquía River and active biomonitoring in the Ctalamochita River.

3.3.1.1 Suquía River, Córdoba Province, Argentina

The Suquía River basin is the main source of drinking water for the city of Córdoba in Argentina. In recent times, the use of agrochemicals in nearby lands and discharges of metals and poorly treated domestic waste have resulted in increased pollution of its waters. In order to assess whether *P. pusillus* reflects different degrees of pollution generated by anthropogenic sources, the concentrations of a range of metals, metalloids and other elements were evaluated in *P. pusillus*, matching this information with the concentrations in corresponding water and sediment samples from the river basin. The elements studied included Ag, Al, As, gold (Au), barium (Ba), beryllium (Be), bismuth (Bi), boron (B), calcium (Ca), Cd, cerium (Ce), cobalt (Co), Cr, Cu, dysprosium (Dy), europium (Eu), erbium (Er), Fe, gallium (Ga), gadolinium (Gd), hafnium (Hf), Hg, holmium (Ho), potassium (K), lanthanum (La), lithium (Li), lutetium (Lu), magnesium (Mg), Mn, molybdenum (Mo), palladium (Pd), praseodymium (Pr), Pt, sodium (Na), neodymium (Nd), Ni, Pb, rubidium (Rb), Se, samarium (Sm), strontium (Sr), terbium (Tb), thorium (Th), thallium (Tl), thulium (Tm), uranium (U), V, yttrium (Y), ytterbium (Yb) and Zn. Sample preparation and analyses of samples were carried out according to Monferrán et al. (2011, 2012a). Plants were collected during the wet season at two stations along the Suquía River basin, having different degrees of pollution and anthropogenic impacts. One area was located upstream from the provincial capital city of Córdoba, representing a site with low population impact and with less pollution according to previous studies. The second monitoring station was located downstream from Córdoba city. This area is primarily affected by the input of pollutants from domestic sewage, in addition to massive urbanization and intensive agriculture downstream from the city.

Considering the concentrations of the target elements in both water and sediments, most of the elements were at lower concentrations upstream from Córdoba city. Some elements (i.e., Al, B, Ba, Ce, Cr, Cu, Fe, Ga, Hg, Mn, Ni, Pb, Pd, Rb, Rh, Sb, Sn, Sr, V, Y and Zn) were present in sediments and water at significantly higher concentrations downstream of Córdoba, originating from sewage discharge. Chemometrics demonstrated good matching between metal and trace element concentrations found in water and sediment with those observed in aquatic plants collected at each monitoring site, indicating the accumulation of these pollutants from both water and sediment to the plant (Monferrán et al. 2012b). These results demonstrate the capacity of *P. pusillus* to be used as an effective bioindicator of aquatic pollution.

3.3.1.2 Ctalamochita River, Córdoba Province, Argentina

An active biomonitoring approach was used to evaluate the capacity of *P. pusillus* to reflect environmental quality. Plants were translocated from a pristine reference site and exposed during two different seasons at seven sites in the river (i.e., S1–S7) with different land uses, where variations in pollution could be expected due to the impacts of different sources (Fig. 3.3). Before the exposures, individuals of *P. pusillus* were acclimated during two weeks in glass aquaria filled with 10% Hoagland's solution, sediments (1/4) from the same sampling area, and maintained at 25 ± 1 °C under a natural light: dark regime. Then, acclimated plants were transported in tanks to the monitoring area. Perforated plastic envelopes containing groups of 24 macrophytes were deposited at each site. Envelopes were maintained at a water depth of 0.5–0.7 m, simulating environments usually colonized by *P. pusillus*. The plants were exposed for four days. Studies were performed in two seasons, reflecting the rain seasonality and temperature variation for the Ctalamochita River basin; that is, cold in July (CP) and warm in December (WP), according to previous studies (Bertrand et al. 2018). After exposure, macrophytes were collected, counted, washed with ultrapure water, flash frozen in liquid nitrogen and stored at -80 °C until analysis. During each monitoring campaign, water and sediment samples were also collected. A water quality index (WQI) was calculated with physicochemical and bacteriological data from water samples (Pesce and Wunderlin 2000). Residues of 13 pharmaceuticals were also measured in water samples and 20 elements, including 17 metals and three metalloids were quantified in collected water and sediment samples, according to methods described by Valdés et al. (2014) and Bertrand et al. (2018), respectively.

The WQI displayed spatio-temporal variations along the basin, with lower values measured during the WP when compared to the CP (Bertrand et al. 2019). In most cases, a decrease in water quality could be observed at those sites downstream of cities (S2, S4, S6), with S6 being the site with the lowest WQI in both monitored periods. The presence of pharmaceutical compounds in water along the basin (e.g., atenolol and carbamazepine showed the highest levels), as well as metal(loid)s in water (Pb, Al, As, B, Hg) and in sediments (Hg) surpassing local and international environmental guidelines were evidence of discharges of inadequately treated sewage and, possibly, industrial wastewater. In *P. pusillus*, the levels of metals and metalloids measured in plant tissues showed the following order: stem < root < leaf in the CP and the inverse pattern during WP; leaf < root < stem. During the CP, the maximum accumulated concentrations in leaf and root occurred in plants at S4, thus exceeding by 7 and 10 times, respectively, the accumulation values of plants at the reference site, S1. On the other hand, maximum accumulation levels in the stem were observed in plants from S5 (i.e., the site with moderate industrial activities), representing a doubling over levels at S1. In WP, the maximum concentrations of metal(loids) in leaf and

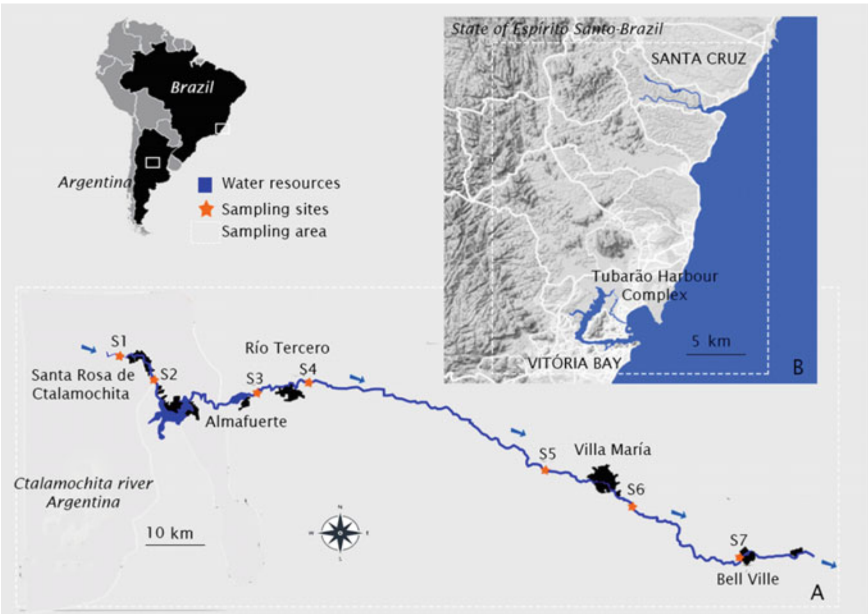

Fig. 3.3 Field study locations that include: (A) Active biomonitoring sites for *P. pusillus* in the Ctalamochita River in Córdoba Province, Argentina; Note that S1 was considered a reference site due to low anthropogenic activities upstream (B) Monitoring sites for mangroves in the State of Espírito Santo in Brazil illustrating the sampling points located in Santa Cruz, Vitória Bay and Tubarão Complex

root were observed in plants from S3, where there is low urban but high agricultural activities, doubling the values in plants from S1. In contrast, the stems showed the highest concentrations of total metal(loids) accumulated in plants from S4 and the lowest in plants from S6. In general, the accumulation of target elements in leaf and stem showed a significant correlation with elemental concentrations in water, for both monitoring periods (i.e., significant for Al, As, Ba, Cr, Pb, Sr and V). In contrast, the accumulation of elements in roots was correlated with the elements measured in the bioavailable and pseudo-total fractions of the sediments (i.e., significant for Cd, Co, Pb, V and Zn).

Throughout the study, biomarker responses in *P. pusillus* showed sensitivity under different environmental scenarios and indicated the most contaminated sites. In CP, Chl-a, Chl-b, Pheo-a and Pheo-b levels decreased significantly or showed a tendency to decline at sites S2, S4, S5 and S6, relative to the reference site (Bertrand et al. 2019). However, this trend was not so clear in the WP. Regarding antioxidant enzymes, different patterns of response were observed at each monitoring site, depending on the season of sampling, of the tissue or the biomarker measured. When comparing the antioxidant enzyme responses in plants from S2 to S7 with plants from S1, a greater number of significant responses were observed in plants deployed in WP than for plants deployed in CP, and they were observed in both leaf and root. Inhibition and/or induction of GPx, POD and SOD activities indicated significant levels of oxidative stress in P. *pusillus* translocated to the Ctalamochita River, particularly at the urban sites (S4 and S6) as well as at those sites with intense industrial and agricultural activities (S5 and S7, respectively).

The individual interpretation of biomarkers in field studies is complex due to the different patterns observed for each of them. Therefore, an integrative biomarker response index (IBR) was used as a tool to integrate and interpret responses obtained along the basin to achieve a comprehensive understanding of the biomonitoring response (Bertrand et al. 2016). During both monitoring periods, the stressor response in *P. pusillus* increased along the basin from S1 to S5 or S6, with a slight or strong decline at S7. The higher values of IBR in plants deployed at S5 and S6 could be associated with an increased complexity of the pollutant mixture originating from multi-sources discharges into the river at both sites. The higher conductivity and salinity observed in the lower basin could be responsible for the IBR decrease in plants at S7, since those physicochemical parameters were described to promote variations in the speciation and bioavailability of pollutants (Luoma and Rainbow, 2008). Through our results, there is strong evidence of the potential to use *P. pusillus* as a biomonitor of pollution hotspots in aquatic ecosystems using both passive and active biomonitoring approaches.

3.3.2 Mangrove Plants as Bioindicators of Metal Contamination

Field studies to measure the concentrations of metals in the abiotic medium and in mangrove plants can be carried out to assess the health of estuarine and marine ecosystems. Our field studies in southeastern Brazil (Fig. 3.3) conducted in contaminated and pristine mangrove areas provide an example of how this can be done. Using *A. schaueriana*, *L. racemosa* and *R. mangle*, it was possible to evaluate the accumulation of 28 metals, metalloids and other elements (i.e., Ag, Al, As, B, Ba, Bi, Cd, Ce, Cu, Cr, Fe, Hg, La, Mn, Ni, Nb, Pb, Rb, Se, Sn, Sr, Ta, Ti, V, W, Y, Zn and Zr) in sediment, interstitial water, roots and leaves, in addition to some anatomical responses of these plants to pollutants and different physical conditions (Arrivabene et al. 2014; Souza et al. 2014a, b, 2015). The studies showed that the elements accumulate in different concentrations in sediment and interstitial water close to the rhizosphere of each species. Our studies also indicated that there is a differential in the bioaccumulation of these elements between the three study species. In general, the elements showed preferential accumulation in roots, but some of the elements were more easily translocated to the shoot, such as Cu, Ag, B and Mn (Arrivabene et al. 2014; Souza et al. 2014a, b, 2015). Comparing the three species, *A. schaueriana* was the mangrove plant that generally accumulated higher levels of the elements in their tissues.

The three mangrove species also showed adaptive plasticity by changing their root anatomy in response to the pollutants, where air gap area, cortex/vascular cylinder ratio, periderm thickness and lignification of the periderm were some of the parameters directly related to the level of environmental contamination. Multivariate analysis revealed that among more than 60 parameters evaluated (between physical, chemical and biological parameters), only 6 to 15 parameters (6 for *A. schaueriana*, 13 for *R. mangle* and 15 for *L. racemosa*) were necessary to identify the study areas according to the anatomical responses, with 100% correct classification. For *L. racemosa*, the multivariate analysis indicated that the cortex/vascular cylinder ratio of pneumatophores, periderm of pneumatophores and air gap area of absorption roots were the parameters showing the maximal discriminating power. For *A. schaueriana*, anatomical parameters evaluated in roots were also the most important for such differentiation. Thus, it is clear that the condition of the plant can indicate differences in pollution between mangrove areas to complement analytical measurements from sediment and interstitial water.

Regarding pollution by sources of atmospheric metals in dust, the leaf structure may or may not favor metal accumulation on the leaf surface. Leaves such as those of *A. schaueriana* and *L. racemosa*, which have salt glands, tend to accumulate dust that easily adheres to the saline secretions deposited on the leaf surface, while glabrous leaves with a large amount of epicuticular wax, such as of *R. mangle*, accumulate less dust (Arrivabene et al. 2015). Leaf analysis of these three mangrove species showed that dust from mining activities deposited on the leaf surface was not capable of generating morphological and anatomical changes. Although gas exchange was not evaluated, it was observed by scanning electron microscopy that the dust particles were large enough to obstruct the stomatal pore and had the potential to alter gas exchange rates. The dust was largely made up of Fe, but it also contained Al, Mn, Zn, Sr, Cr, Ni, V, Cu, Pb, Rb and As. Furthermore, the chemical analyses of Fe in the leaves and in the substrate suggest that there is foliar absorption of this element (Arrivabene et al. 2015).

3.3.3 Stable Isotopes in Mangrove Plants as Bioindicators of Environmental Pollution

Stable isotopic studies to assess the sources of environmental pollution were carried out by Souza et al. (2018) over eight trophic levels, including mangrove plants (*R. mangle, L. racemosa* and *A. schaueriana*). Analysis of isotopes of Sr ($^{87}Sr/^{86}Sr$) showed the influence of marine water on mangrove plants, which also demonstrate the potential for using these plants for estuarine/marine monitoring programs. Lin and Sternberg (1994), based on the analysis of oxygen isotopes ($^{18}O/^{16}O$) in *R. mangle*, also showed that mangrove plants use surface soil and seawater rather than groundwater as a water source, even when the water has a high salinity. Mangrove plants also proved to be good bioindicators of environmental contamination by particulate matter through the analysis of Pb stable isotopes ($^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$), which have higher values in plants located in areas with metallurgical activity as the main contamination source, such as at the Tubarão Complex (Fig. 3.3). Moreover, field research measuring stable nitrogen isotopes, such as $\delta^{15}N$, in mangrove plants, can show the anthropogenic impact of fertilizers (Souza et al. 2018). Changes in the ratios of $\delta^{15}N$ were associated with nitrogen enrichment from fertilizers in the mangrove plants from Vitória Bay when compared with those in Santa Cruz estuary, which is in close proximity (Souza et al. 2018). According to Tanu et al. (2020), the nitrogen ratios in mangroves from less contaminated site are typically lower than the ratios in mangroves from contaminated sites, highlighting how mangroves can be a powerful tool for anthropogenic disturbances. Therefore, the measurement of $\delta^{15}N$ ratios in mangroves allowed us to define Santa Cruz as a quasi-pristine mangrove ecosystem ($-2‰$ $\delta^{15}N$, according to Souza et al. 2018) and this site was used in this study as a reference location to understand metal/metalloid dynamics under natural conditions. Conversely, the higher $\delta^{15}N$ values for mangroves from Vitória Bay represented an anthropogenically impacted site. Consequently, a comparison between $\delta^{15}N$ in these estuarine plants is instructive. Due to the close proximity of the study sites (i.e., just 70 km), the original ecosystems should have been similar but current differences in stable isotopes can be attributed to the intensive development around Vitória Bay (Souza et al. 2018).

3.4 Conclusions

This chapter highlighted the capacity of macrophytes, which grow under different environmental conditions, to adapt to stress caused by exposure to metal(loids), with specific examples from laboratory and field studies with *P. pusillus*, *A. schaueriana*, *L. racemosa* and *R. mangle*. We observed that each of these macrophyte species adapted differently, taking into account their physiology or structure. For instance, *A. schaueriana* showed adaptive changes, leading to reduced amounts of metals and metalloids in roots by limiting the uptake and/or increasing the translocation of the elements, or both. However, it is not fully understood what triggers these anatomical and physiological changes to adapt to the presence of environmental contaminants by preventing the absorption of potentially toxic metals and metalloids. Future studies, evaluating gene expression and epigenetic factors could help to elucidate the research questions arising from the current results.

Analysis of Sr isotopes ($^{87}Sr/^{86}Sr$) in *A. schaueriana*, *L. racemosa* and *R. mangle* demonstrated that they use seawater rather than groundwater as a source of water. Although this was already demonstrated by other authors in *R. mangle* using oxygen isotopes, these studies are the first to demonstrate this mechanism in *A. schaueriana*, *L. racemosa* and *R. mangle* using other isotopes (i.e., $^{87}Sr/^{86}Sr$). These results confirm the high potential of these plants to be used for estuarine/marine quality biomonitoring programs.

Studies carried out on *P. pusillus* exposed to metal(loid)s indicated that this macrophyte species can be used as a bioindicator for these elements, but also, this species shows potential for removing them from solution (e.g., industrial and domestic wastewaters), particularly for those aqueous solutions with high Hg concentrations. Our results are of note since previous studies reported lower Hg accumulation for different plant species than those reported in our work with *P. pusillus*. Further work is in progress to understand the molecular and biological mechanisms by which *P. pusillus* can accumulate large amounts of Hg in its tissues without showing great physiological damage.

Our results could help to understand the responses of different kinds of macrophytes to metal exposure. This will contribute to a more precise risk assessment, helping to predict and prevent toxic effects in these species. These studies will also guide regulatory decisions for the development of national and international plans for conserving biodiversity and protecting wetlands.

References

AEWQG (2003) Niveles Guía Nacionales de Calidad de Agua Ambiente. Subsecretaria de Recursos Hídricos de la Nación, República Argentina, Buenos Aires, Argentina.

Aravind P, Prasad MN (2004) Zinc protects chloroplasts and associated photochemical functions in cadmium exposed *Ceratophyllum demersum* L., a freshwater macrophyte. Plant Sci 166:1321–1327. https://doi.org/10.1016/j.plantsci.2004.01.011

Arrivabene HP, Souza I, Có WLO, Rodella RA, Wunderlin DA, Milanez CR (2014) Functional traits of selected mangrove species in Brazil as biological indicators of different environmental conditions. Sci Total Environ 476:496–504. https://doi.org/10.1016/j.scitotenv.2014.01.032

Arrivabene HP, Costa IC, Có WLO, Conti MM, Wunderlin DA, Milanez CRD (2015) Effect of pollution by particulate iron on the morphoanatomy, histochemistry, and bioaccumulation of three mangrove plant species in Brazil. Chemosphere 127:27–34. https://doi.org/10.1016/j.chemosphere.2015.01.011

Arrivabene HP, Campos CQ, Costa IC, Wunderlin DA, Milanez CRD, Machado SR (2016) Differential bioaccumulation and translocation patterns in three mangrove plants experimentally exposed to iron. Consequences for environmental sensing. Environ Pollut 215:302–313. https://doi.org/10.1016/j.envpol.2016.05.019

Barletta M, Lima ARA, Costa MF (2019) Distribution, sources and consequences of nutrients, persistent organic pollutants, metals and microplastics in South American estuaries. Sci Total Environ 651:1199–1218. https://doi.org/10.1016/j.scitotenv.2018.09.276

Bertrand L, Asis R, Monferrán MV, Amé MV (2016) Bioaccumulation and biochemical response in South American native species exposed to zinc: boosted regression trees as novel tool for biomarkers selection. Ecol Ind 67:769–778. https://doi.org/10.1016/j.ecolind.2016.03.048

Bertrand L, Monferrán MV, Mouneyrac C, Amé MV (2018). Native crustacean species as a bioindicator of freshwater ecosystem pollution: a multivariate and integrative study of multi-biomarker response in active river monitoring. Chemosphere 206: 265e277. https://doi.org/10.1016/j.chemosphere.2018.05.002.

Bertrand L, Monferrán MV, Valdés ME, Amé MV (2019) Usefulness of a freshwater macrophyte (*Potamogeton pusillus*) for an environmental risk assessment in a multi-source contaminated basin. Chemosphere 222:1003–1016. https://doi.org/10.1016/j.chemosphere.2019.02.018

Campos CQ (2018) Efeitos da salinidade no acúmulo de metais pesados e nas características anatômicas, fisiológicas e bioquímicas em plantas do manguezal. PhD Dissertation, Universidade Federal do Espírito Santo, Brazil.

Cheung KC, Poon BHT, Lan CY, Wong MH (2003) Assessment of metal and nutrient concentrations in river water and sediment collected from the cities in the Pearl River Delta, South China. Chemosphere 52:1431–1440. https://doi.org/10.1016/S0045-6535(03)00479-X

Contardo-Jara V, Galanti LN, Ame MV, Monferrán MV, Wunderlin DA, Wiegand C (2009) Biotransformation and antioxidant enzymes of *Limnoperna fortunei* detect site impact in watercourses of Córdoba, Argentina. Ecotoxicol Environ Saf 72: 1871–1880. https://doi.org/10.1016/j.ecoenv.2009.07.001

Cordoba-Tovar L, Marrugo-Negrete J, Ramos Baron P, Díez S (2022) Drivers of biomagnification of Hg, As and Se in aquatic food webs: a review. Environ Res 204:112226. https://doi.org/10.1016/j.envres.2021.112226

Farias DR, Hurd CL, Eriksenc RS, Macleod CK (2018) Macrophytes as bioindicators of heavy metal pollution in estuarine and coastal environments. Mar Pollut Bull 128:175–184. https://doi.org/10.1016/j.marpolbul.2018.01.023

Francisca FM, Cebollada Verdager P, Carro Perez ME (2006) Distribución espacial del arsénico en las aguas subterráneas de la provincia de Córdoba, Argentina. VIII Congreso Latinoamericano de Hidrología Subterránea. Asunción, Paraguay.

Geng N, Wu Y et al (2019) Bioaccumulation of potentially toxic elements by submerged plants and biofilms: a critical review. Environ Int 131:105015. https://doi.org/10.1016/j.envint.2019.105015

Griboff J, Wunderlin DA, Monferrán MV (2017) Metals, As and Se determination by inductively coupled plasma-mass spectrometry (ICP-MS) in edible fish collected from three eutrophic reservoirs. Their consumption represents a risk for human health? Microchem J 130:236–244. https://doi.org/10.1016/j.microc.2016.09.013

Griboff J, Wunderlin DA, Monferrán MV (2018a) Phytofiltration of As^{3+}, As^{5+}, and Hg by the aquatic macrophyte *Potamogeton pusillus* L, and its potential use in the treatment of wastewater. Int J Phytorem 20:914–921. https://doi.org/10.1080/15226514.2018.1448360

Griboff J, Horacek M, Wunderlin DA, Monferrán MV (2018b) 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. https://doi.org/10.1016/j.ecoenv.2017.10.028

Griboff J, Wunderlin HM, DA, Amé MV, Monferrán MV, (2020) $\delta^{15}N$ as an indicator of freshwater systems suitable for edible fish production. Ecol Ind 108:105743. https://doi.org/10.1016/j.ecolind.2019.105743

Harguinteguy CA, Cofré MN, Fernández Cirelli A, Pignata ML (2016) The macrophytes *Potamogeton pusillus* L. and *Myriophyllum aquaticum* (Vell.) Verdc. as potential bioindicators of a river contaminated by heavy metals. Microchem J 124:228–234. https://doi.org/10.1016/j.microc.2015.08.014

Hashem MA, Hasan M, Momen MA, Payel S, Nur-A-Tomal MS (2020) Water hyacinth biochar for trivalent chromium adsorption from tannery wastewater. Environ Sustain Indic 5:100022. https://doi.org/10.1016/j.indic.2020.100022

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soils. California Agricultural Experimental Station, Berkeley, CA, USA.

Kolarova N, Napiórkowski P (2021) Trace elements in aquatic environment. Origin, distribution, assessment and toxicity effect for the aquatic biota. Ecohydrol Hydrobiol 21:655–668. https://doi.org/10.1016/j.ecohyd.2021.02.002

Krayem M, Khatib SE et al (2021) In search for potential biomarkers of copper stress in aquatic plants. Aquat Toxicol 239:105952. https://doi.org/10.1016/j.aquatox.2021.105952

Lacassie JP, Ruiz-Del-Sola J (2021) Integrated mineralogical and geochemical study of the Rapel fluvial system, central Chile: an application of multidimensional analysis to river sedimentation. J South Am Earth Sci 109:103289. https://doi.org/10.1016/j.jsames.2021.103289

Lin G, Sternberg LDS (1994) Utilization of surface water by red mangrove (*Rhizophora mangle* L.): An isotopic study. Bull Mar Sci 54:94–102

Luoma SN, Rainbow PS (2008) Metal contamination in aquatic environments. Cambridge University Press, Cambridge, UK, Science and Lateral Management, p 573

Monferrán MV, Galanti LN, Bonansea RI, Amé MV, Wunderlin DA (2011) Integrated survey of water pollution in the Suquıa River basin (Córdoba, Argentina). J Environ Monit 13: 398–409. https://doi.org/10.1039/c0em00545b

Monferrán MV, Pignata ML, Wunderlin DA (2012a) Enhanced phytoextraction of chromium by the aquatic macrophyte *Potamogeton pusillus* in presence of copper. Environ Pollut 161:15–22. https://doi.org/10.1016/j.envpol.2011.09.032

Monferrán MV, Pignata ML, Wunderlin DA (2012b) Bioaccumulation of heavy metal and trace elements by *Potamogeton pusillus* as bioindicator of aquatic pollution. 6th SETAC World Congress/SETAC Europe 22nd Annual Meeting, May 20–24, 2012, Berlin, Germany.

Monferrán MV, Garnero P, Bistoni M, Anbar AA, Gordon GW, Wunderlin DA (2016a) From water to edible fish. Transfer of metals and metalloids in the San Roque Reservoir (Córdoba, Argentina). Implications associated with fish consumption. Ecol Ind 63:48–60. https://doi.org/10.1016/j.ecolind.2015.11.048

Monferrán MV, Garnero PL, Wunderlin DA, Bistoni MA (2016b) Potential human health risks from metals and As via *Odontesthes bonariensis* consumption and ecological risk assessments in a eutrophic lake. Ecotoxicol Environ Saf 129:302–310. https://doi.org/10.1016/j.ecoenv.2016.03.030

Monteiro DA, Rantin FT, Kalinin AL (2010) Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish, matrinxã, *Brycon amazonicus* (Spix and Agassiz, 1829). Ecotoxicology 19:105–123. https://doi.org/10.1007/s10646-009-0395-1

Novara LJ (2003) Flora del valle de Lerma: Potamogetonaceae Dumort. Aportes Botanicos de Salta. Serie Flora 7:1–4. http://www.unsa.edu.ar/biblio/herbario/flora/vol7/pdf/1.%20POTAMOGETONACEAE.pdf

Palansooriya KN, Wong JTF, Hashimoto Y et al. (2019) Response of microbial communities to biochar-amended soils: a critical review. Biochar 1:3–22. https://doi.org/10.1007/s42773-019-00009-2

Pesce SF, Wunderlin DA (2000) Use of water quality indices to verify the impact of Córdoba City (Argentina) on Suquía River. Water Res 34: 2915e2926. https://doi.org/10.1016/S0043-1354(00)00036-1

Rezania S, Taib SM, Din MF, Dahalan FA, Kamyab H (2016) Comprehensive review on phytotechnology: heavy metals removal by diverse aquatic plants species from wastewater. J Hazard Mater 318:587–599. https://doi.org/10.1016/j.jhazmat.2016.07.053

Rivela Fretes C, Arán DS, Harguinteguy CA, Monferrán MV (2021a) Evaluación de la capacidad de *Potamogeton pusillus* de remover Pb y Cd y su posible aplicación en la remoción de aguas residuales. SETAC Latin America 14th Biennial Meeting, Virtual Meeting, September 26–29, 2021.

Rivela Fretes C, Griboff J, Arán DS, Harguinteguy CA, Monferrán MV (2021b) Utilización de plantas autóctonas para evaluar fitofiltración de cadmio. XXIII SINAQO (Simposio Nacional de Química Orgánica), Virtual meeting, November 15–18, 2021.

Schaedle M, Bassham JA (1977) Chloroplast glutathione reductase. Plant Physiol 59:1011–1012. https://doi.org/10.1104/pp.59.5.1011

Shi W, Li T, Feng Y, Su H, Yang Q (2022) Source apportionment and risk assessment for available occurrence forms of heavy metals in Dongdahe wetland sediments, southwest of China. Sci Total Environ 815:152837. https://doi.org/10.1016/j.scitotenv.2021.152837

Smolders AJP, Lock RAC, Van der Velde G, Medina Hoyos RI, Roelofs JGM (2003) Effects of mining activities on heavy metal concentrations in water, sediment, and macroinvertebrates in different reaches of the Pilcomayo River, South America. Arch Environ Contam Toxicol 44:314–323. https://doi.org/10.1007/s00244-002-2042-1

Souza IC, Morozesk M, Duarte ID, Bonomo MM, Rocha LD, Furlan LM, Arrivabene HP, Monferrán MV, Matsumoto ST, Milanez CRD, Wunderlin DA, Fernandes MN (2014a) Matching pollution with adaptive changes in mangrove plants by multivariate statistics. A case study, *Rhizophora mangle* from four neotropical mangroves in Brazil. Chemosphere 108:115–124. https://doi.org/10.1016/j.chemosphere.2014.02.066

Souza IC, Bonomo MM, Morozesk M, Rocha LD, Duarte ID, Furlan LM, Arrivabene HP, Monferrán MV, Matsumoto ST, Milanez CRD, Wunderlin DA, Fernandes MN (2014b) Adaptive plasticity of *Laguncularia racemosa* in response to different environmental conditions: integrating chemical and biological data by chemometrics. Ecotoxicology 23:335–348. https://doi.org/10.1007/s10646-014-1191-0

Souza IC, Rocha LD, Morozesk M, Bonomo MM, Arrivabene HP, Duarte ID, Furlan LM, Monferrán MV, Mazik K, Elliot M, Matsumoto ST, Milanez CRD, Wunderlin DA, Fernandes MN (2015) Changes in bioaccumulation and translocation patterns between root and leaves of *Avicennia schaueriana* as adaptive response to different levels of metals in mangrove system. Mar Pollut Bull 94:176–184. https://doi.org/10.1016/j.marpolbul.2015.02.032

Souza IC, Arrivabene HP, Craig CA, Midwood AJ, Thornton B, Matsumoto ST, Elliott M, Wunderlin DA, Monferrán MV, Fernandes MN (2018) Interrogating pollution sources in a mangrove food web using multiple stable isotopes. Sci Total Environ 640:501–511. https://doi.org/10.1016/j.scitotenv.2018.05.302

Tanu FZ, Asakura Y, Takahashi S, Hinokidani K, Nakanishi Y (2020) Variation in foliar δ15N reflects anthropogenic nitrogen absorption potential of mangrove forests. Forests 11:133. https://doi.org/10.3390/f11020133

Tibbett M, Green I, Rate A, De Oliveira VH, Whitaker J (2021) The transfer of trace metals in the soil-plant-arthropod system. Sci Total Environ 779:146260. https://doi.org/10.1016/j.scitotenv.2021.146260

Tripathi RD, Rai UN, Vajpayee P, Ali MB, Khan E, Gupta DK, Mishra S, Shukla MK, Singh SN (2003) Biochemical responses of *Potamogeton pectinatus* L. exposed to higher concentrations of zinc. Bull Environ Contam Toxicol 71:255–262. https://doi.org/10.1007/s00128-003-0158-2

Tomlinson PB (2016) The botany of mangroves. Cambridge University Press, Cambridge, UK.

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. https://doi.org/10.1016/j.scitotenv.2013.10.124

Wang Z, Lin K, Liu X (2022) Distribution and pollution risk assessment of heavy metals in the surface sediment of the intertidal zones of the Yellow River Estuary. China. Marine Pollution Bulletin 174:113286. https://doi.org/10.1016/j.marpolbul.2021.113286

Zaynab M, Al-Yahyai R, Ameen A, Sharif Y, Ali L, Fatima M, Khan KA, Li S (2022) Health and environmental effects of heavy metals. J King Saud University Sci 34:101653. https://doi.org/10.1016/j.jksus.2021.101653