Macrophyte Importance in Contaminant Treatment and Biomonitoring

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Abstract

Constructed wetlands are systems based on nature, in which macrophytes are the main biological community. According to the diversity of wastewaters that these systems can treat, its use has expanded all over the world. Treatment wetlands are successfully used in the removal of emerging contaminants. However, the knowledge of the removal mechanisms is still scarce. The use of macrophytes as biomonitors should be considered as a useful tool for the management of aquatic systems contaminated by different sources. In macrophyte ecotoxicological studies, not only the measurement of antioxidant enzymes but also other basic biological parameters, such as changes in different biomass compartments and the internal morphology, should be considered. This chapter discusses the importance of macrophytes in wetlands constructed for the treatment of different effluents, their use as biomonitors in natural wetlands that receive urban pollutants, and some ecotoxicological aspects applied to phytoremediation.

Keywords

Aquatic plants · Wetland systems · Industrial wastewaters · Emerging contaminants · Toxic effects

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1 Introduction

Macrophytes present a high diversity explained by different life forms and morphological and physiological characteristics. They have evolutionary adaptations that allow them to grow in strictly aquatic environments, in aquatic/terrestrial transition environments, and even in terrestrial environments in periods of low waters (Hadad and Mufarrege 2017).

As a key biological community in natural wetlands, macrophytes produce the highest biomass influencing the system dynamics (Esteves 1988). Similarly, in wetlands constructed for wastewater treatment, the macrophytes are essential components because they accumulate contaminants in their tissues, influence the sediment biogeochemistry, and enhance chemical and biochemical reactions in the root zone improving the contaminant removal (Jenssen et al. 1993). In general, they show fast growth and high tolerance when exposed to different pollutants. The treatment of sewage, agricultural and urban runoff wastewaters using constructed wetlands, has the advantage that nutrients are the main contaminants being assimilated by plants in high proportions. In the case of the treatment of metallurgical effluents, metals affect the biological plant responses to the environmental conditions of the system (Hadad et al. 2010; Maine et al. 2013).

Having into account the diversity of wastewaters that constructed wetlands can treat, its use has expanded all over the world (Avila et al. 2017; Kadlec and Wallace 2009; Maine et al. 2017, 2019; Nivala et al. 2019; Zhang et al. 2020). Because treatment wetlands are subjected to plant biological cycles and environmental changes, they are dynamic systems that should be monitored for a long time during the treatment. Besides, plant role should be studied deeper. More studies focused on the plant tolerance to different contaminants and new emerging contaminants and the search for species that are not commonly used in treatment wetlands should be carried out.

The analysis of the plant functions and their responses in wetland systems are necessary to improve phytoremediation techniques (Fig. 1). This chapter discusses the importance of macrophytes in wetlands constructed for the treatment of different effluents, their use as biomonitors in natural wetlands that receive pollutants, and some ecotoxicological aspects applied to phytoremediation.

2 Constructed Wetlands for Effluent Treatment

Constructed wetlands are wastewater treatment systems based on nature, in which macrophytes are the main biological community. These systems try to reproduce what happens in natural ecosystems, but under controlled conditions to optimize contaminant removal processes. Microorganisms and substrate are involved, in addition to the macrophytes. These interactions are key mechanisms to understand wetland functioning (Ahmed et al. 2017). The selection of the wetland type, the macrophytes, and substrates to be used for the treatment of a specific effluent should be the result of previous studies conducted carefully.

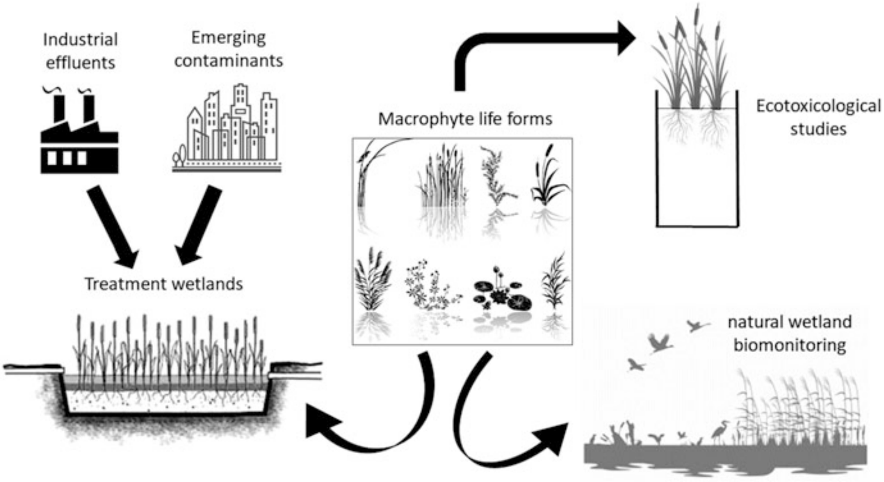

Fig. 1 Macrophyte importance in phytoremediation techniques

The different life forms of aquatic plants acquire great importance with respect to their functions in treatment wetlands. In a natural wetland, a sequence of life forms can be found along a gradient that extends from the coast to the deepest zones. In treatment wetlands, this gradient is attempted to be represented by planting the different types of macrophytes in the areas corresponding to their form of growth. For example, at the edges of a free-surface flow wetland, it would be convenient to plant emergent species, while in the deeper central areas, free-floating species would develop. In order to achieve a higher cover of emergent plants, a strategy to be applied could be the decrease of water depth by the construction of mounds of land (Maine et al. 2009).

On the other hand, since in subsurface flow wetlands emergent species are always used, plants should be capable to grow in the substrate used. Examples of emergent macrophytes commonly used in treatment wetlands are *Typha* spp., *Arundo donax*, and *Phragmites australis* (Kadlec and Wallace 2009). The different macrophytes that inhabit a region can be used in constructed wetlands since they have the advantage of being adapted to the climate and the prevailing water and edaphic conditions. However, not all species have the same efficiency in the removal of contaminants. The plant species used in the treatment will imply differences in removal efficiency (Brisson 2013; Gersberg et al. 1986). It is necessary to find local plant species that have the ability to survive the potentially toxic effects of the effluent to be treated and its variability. During a sanitary effluent treatment where the contaminants to be treated are nutrients, plant growth is assured. However, during the treatment of industrial effluents, the plants not only should achieve a good efficiency in the removal of pollutants but also survive the physical and chemical matrix of the effluent (Hadad et al. 2006; Maine et al. 2009, 2013).

Besides, the basis of wastewater treatment using constructed wetlands is the cooperative growth of plants and microorganisms. It has been thought that most of the responsibility for biodegradation is carried out by microorganisms that live on the plant roots. Once these microorganisms are established in the roots, they generate a symbiotic relationship with the macrophytes. This relationship has a synergistic effect that results in an increase in the rate of degradation and removal of pollutants in the root zone.

For the treatment of effluents containing metals, it is important to highlight that constructed wetlands are final treatments (secondary or tertiary treatments). Therefore, the metal concentrations in the effluents are relatively low. Commonly, free-water surface wetlands are used to treat effluents containing metals. In Argentina, our research group has gained experience during the last decades studying free-water surface wetlands for the treatment of effluents from different metallurgical industries (Hadad et al. 2006, 2018; Maine et al. 2006, 2007, 2009, 2013, 2017, 2019). All the studied parameters in the effluent decreased their variabilities and mean values after the treatment. Metal removal efficiencies were high, and therefore, metal water concentrations were found under the Argentinian law limits for discharge. Due to the chemical composition of the effluent (high pH, alkalinity, Fe, Ca, and ionic concentrations), metals were mainly accumulated in the sediment of the inlet zone and in the roots of the macrophytes (Hadad et al. 2006; Maine et al. 2009, 2017). Di Luca et al. (2011) proposed that if the effluent chemical composition and the environmental conditions of the system are maintained, metals will remain bound to sediment and will not release into the water column. During the long-term study in one of the treatment wetlands, changes in vegetation dominance were observed (Maine et al. 2009). Several macrophytes were transplanted to this wetland initially. Brisson (2013) proposed that a high diversity of plants has advantages, such as a higher efficiency in contaminant removal, disturbance resilience, better habitat, etc. *Eicchornia crassipes* was the dominant species for 2 years. But then, *Typha domingensis* became the dominant species for the last 15 years (Maine et al. 2017). High pH and salinity but not metal concentrations were the cause of disappearance of *E. crassipes* (Hadad et al. 2006). We could see that plant diversity is not easy to maintain for a long time due to the fact that one plant species becomes dominant. pH and salinity are key factors for plant tolerance.

The changes in the dominant plant species determined different contaminant retention mechanisms. Table 1 shows a mass balance of the studied contaminants. During the dominance of *E. crassipes*, metals and P were mainly accumulated in the

Table 1 Contaminant accumulation (%) in plants and sediment during three different stages of macrophyte dominance in a constructed wetland for the treatment of a metallurgical effluent (extracted from Maine et al. 2017)

Dominant species	Cr		Ni		Zn		TP	
	Sediment	Tissues	Sediment	Tissues	Sediment	Tissues	Sediment	Tissues
E. crassipes	11	89	7	93	5	95	2	98
T. domingensis	73	27	87	13	71	29	62	38

plant tissues. Therefore, the plant harvest could remove the metals and P from the system. Contrarily, during the dominance period of the emergent *T. domingensis*, metals were accumulated and phytostabilized in the sediment within the wetland (Hadad et al. 2018).

During the long-term studies, a remarkable fact was that the free-water flow wetlands suffered accidental metal dumps or animal depredation. Since the macrophyte *T. domingensis* showed to be resilient after these events, the systems were capable of recovering their performance, demonstrating its robustness (Maine et al. 2013, 2017).

Among recent works, Xu et al. (2019) who studied a free-water surface wetland which is constructed to remove Cu and Zn from storm runoff water in the Savannah River Site (USA) can be mentioned. Metal removals were 64 and 71% for Cu and Zn, respectively, being their concentrations under the legal limits. Zhang et al. (2020) compared the efficiency of three different substrates in hybrid treatment wetlands planted with *Canna indica*. These wetlands were constructed for the treatment of mixed domestic-industrial effluents discharged into peri-urban water bodies. The wetland systems were satisfactorily efficient in the removal of Pb, Cd, Cu, and Zn, reaching values higher than 85% during the first 24 h of the treatment. The most efficient substrates were gravel and lava rock.

Constructed wetlands can be used for the treatment of different wastewaters, such as municipal, sewage, and domestic effluents. In these cases, macrophyte growth is favored because the wastewaters to be treated contain nutrients in large proportions. García-Ávila et al. (2019) compared the efficiency of two vertical flow wetlands planted with different macrophytes. The wetlands receive municipal wastewater with primary treatment and were planted with *Cyperus papyrus* and *Phragmites australis*. The wetlands planted with the first one showed significantly higher removals of biochemical oxygen demand (81%), chemical oxygen demand (70%), ammoniacal nitrogen (70%), total phosphorus (50%), total coliforms (98%), and fecal coliforms (96%). Contrarily, the wetland planted with *P. australis* showed significantly higher retention of solids. Nivala et al. (2019) compared the removal efficiency of 15 subsurface flow pilot-scale wetlands with different designs. The wetlands were used to treat domestic wastewaters and were planted with *P. australis*. The macrophyte importance on the removal efficiencies was also assessed by using wetlands without plants. There were observed differences among the different wetland designs for the removal efficiencies of the parameter measured. Therefore, it was concluded that design complexity enhanced treatment efficiency. The presence of macrophytes enhanced the contaminant removals in the wetlands.

Landfill leachate treatment is one of the most difficult environmental problems due to its great variability in its composition and in the expected volume that depends on the age of the landfill, the climatic conditions, and the degradation rate of solid wastes. High concentrations of ammonia, recalcitrant COD, and the presence of metals in low concentrations are the main characteristics of leachate (Wojciechowska et al. 2016). Ammonium concentration found in landfill leachate is 0.01–1900 mg L^{-1} NH_4^+-N (Kadlec and Wallace 2009). Clarke and Baldwin (2002) proposed that the toxic ammonium concentration for plants is above

200 mg L^{-1} N. Natural wetlands were often the landfill leachate fate because they were located on land adjacent to them. It was observed that the chemical quality of the leachate improved after crossing them (Higgins and Lugowski 1996; La Forge 1997). Based on these results, the use of constructed wetlands for the treatment of this type of wastewater began to develop (Kadlec and Wallace 2009). Vertical flow wetlands have proven effective and are the most commonly used for the treatment of leachate (Kadlec and Zmarthie 2010; Stefanakis et al. 2014). Yalcuk and Ugurlu (2009) compared vertical flow and horizontal flow wetlands for the treatment of landfill leachate and obtained better ammonium removals in the first one. A et al. (2017) studied at laboratory scale a vertical flow wetland for the treatment of synthetic leachate and found ammonium removals of 44–73% in systems planted with *Juncus effusus* and 46–76% in systems planted with *P. australis*. Lavrova (2016) studied the treatment efficiency of leachate through two laboratory-scale vertical flow wetlands with and without additional carbon source. The significant removal efficiency of chemical and biochemical oxygen demand was achieved: 95% and 96%, respectively. Camaño Silvestrini et al. (2019) compared the system performance of wetlands constructed for the treatment of landfill leachate at mesocosms scale using the species *T. domingensis* and *C. indica*. There were no observed differences for COD, ammonia, nitrate, and TN removals between the systems planted with the two macrophytes. Although both species were tolerant to the wastewater studied, *T. domingensis* presented a significantly higher growth rate in comparison with *C. indica*.

3 Constructed Wetlands for the Treatment of Emerging Contaminants

Emerging contaminants are a current concern related to the quality of water bodies. Among others, these contaminants can be organic compounds such as agrochemicals, medicines, antibiotics, hormones, drugs, and personal care products (Galindo-Miranda et al. 2019). Emerging contaminants are commonly found at trace concentrations in waters, ranging in a scale magnitude from ng L^{-1} to μg L^{-1} (Luo et al. 2014). From an ecological point of view, wildlife and flora are in potential risk due to emerging contaminants that could negatively affect their metabolism and behavior. Treatment wetlands have demonstrated high efficiency in reducing not only common contaminants, such as nitrogen, phosphorus, and metals, but also emerging pollutants (Avila et al. 2017; Carvalho et al. 2014; Chaves-Barquero et al. 2018; Chen et al. 2016, 2019; Pei et al. 2019; Vymazal 2005; Yi et al. 2017). However, the knowledge of the removal mechanisms of emerging contaminants in treatment wetlands is still scarce. As it occurs in different types of treatment wetlands during the treatment of different wastewaters, operation and design factors, such as the types of wetlands, substrates, plant species, hydraulic retention time, hydraulic loading, solar irradiation, and temperature, could affect the performance of the treatment. Further studies should be carried out in order to gain knowledge to optimize the efficiency and operation of the treatment wetlands.

During the last years, the study of the treatment of emerging contaminants has increased significantly. Avila et al. (2015) studied a hybrid wetland system constructed for the treatment of wastewater and reuse in small communities. The hybrid system was composed of a vertical flow, a horizontal flow, and a free-water surface flow wetland. These authors reported a removal of above 80% of endocrine-disrupting compounds and pharmaceuticals and personal care products, which was explained by photodegradation, biodegradation, sorption, volatilization, and hydrolysis. The treatment wetland operation should be taken into account to obtain high removal efficiencies. Regarding hydraulic and carbon loading rates, Sharif et al. (2014) concluded that to obtain a high contaminant removal, the carbon loading rates should be changed without increasing the cumulative hydraulic loading rates. Matamoros et al. (2008) studied a free-water flow wetland for the removal of pharmaceuticals and personal care products, obtaining a removal of 90% when increasing the hydraulic retention time. A hybrid constructed wetland system was studied by Avila et al. (2014) to compare the removals of emerging contaminants using different hydraulic loading rates. These authors concluded that the full-scale system was adequately efficient for the removal of the emerging contaminants from the wastewater at high hydraulic loading rates.

Arginine is widely used as a dietary supplement. Its removal in treatment wetlands is affected by retention time and hydraulic load (Miller et al. 2015). Besides, Decamp and Warren (2000) reported that, in comparison with free-water surface wetlands, subsurface flow wetlands are more efficient in the inactivation and elimination of arginines. Chen et al. (2016) compared six different flow wetlands and obtained a significantly higher arginine removal (64–84%) in wetlands with subsurface flow in comparison with free-water flow wetlands. Previous works reported that zeolite is an efficient substrate to be used in vertical flow wetlands for the treatment of wastewaters containing arginines (Liu et al. 2013). Treatment wetlands have shown removal efficiencies near to 100% of the analgesics ibuprofen, diclofenac, and naproxen. For these compounds, free-water surface wetlands have demonstrated to be the most effective (Matamoros et al. 2008). Li et al. (2014) reported that studies focused on the use of constructed wetlands for the treatment of emerging contaminants are carried out only at microcosms or mesocosms-scales. Also, these authors suggested that different types of treatments, such as tidal flow and dewatered alum-sludge-based wetlands, could be suitable for the treatment of pharmaceuticals. Further studies are necessary because new emerging pollutants are being determined in different wastewaters.

Plants used in constructed wetlands for the treatment of wastewaters containing emerging contaminants affect different environmental variables, favoring indirectly the contaminant removal. For example, macrophytes provide substrate areas for the development of microorganisms (Fang et al. 2017) and oxygenate the microorganisms that are present in the system, which will carry out the arginine removal (Huang et al. 2015). In comparison with other macrophytes, *Phragmites* spp. are widely used in treatment wetlands due to enhanced reduction of arginines and 16sRNA (Yi et al. 2017). Chen et al. (2016) proposed that macrophytes such as *Pontederia cordata*, *Myriophyllum verticillatum*, and *Cyperus alternifolius* are capable to enhance the arginine removal from wastewaters. Rühmland et al. (2015) studied a subsurface flow wetland and a pond with floating macrophytes for the treatment of 18 different pharmaceuticals and 11 different human metabolites. Despite the diversity of the treated compounds, these authors reported good performance of both studied systems. Christofilopoulos et al. (2019) studied the removal of bisphenol A and the antibiotics ciprofloxacin and sulfamethoxazole in a treatment wetland planted with the macrophyte *Juncus acutus*. At the end of the study, the system showed a removal efficiency of 76 and 94% for bisphenol A and ciprofloxacin, respectively, while sulfamethoxazole was not efficiently removed from the wastewater. De la Paz et al. (2019) compared systems planted with *P. australis* with systems without plants constructed for the removal of benzotriazole, sulfamethoxazole, carbamazepine, bisphenol A, and diclofenac. In comparison with the unvegetated systems, the vegetated wetlands showed removals 200% higher. These authors concluded that the higher efficiency of systems with plants was due to root exudates which are released that enhances the metabolism of microorganisms.

4 Contaminant Biomonitoring Using Macrophytes in Natural Wetlands

Natural wetlands receive different sources of pollution, such as industrial effluents, sewage, urban, and road runoff waters (Rodak et al. 2019), among others. The wastewaters that reach the wetlands may contain contaminants of different types, such as metals, organic matter, nutrients, and emerging contaminants. Wetland biomonitoring is an effective tool for contamination assessment (Zhou et al. 2008). Macrophytes are efficient contaminant accumulators being tolerant to the different contaminants; whereby, they are used as efficient contaminant biomonitors (Bonanno et al. 2017; Cardwell et al. 2002). The contaminant accumulation in plant tissues of different macrophytes was studied in wetlands of different parts of the world (Alonso et al. 2018; Batzias and Siontorou 2008; Costa et al. 2018; Bertrand et al. 2019; Farias et al. 2018; Mechora et al. 2014; Wach et al. 2019; Wang et al. 2014; Zhou et al. 2008). The knowledge acquired is key to be applied in the management of contaminated wetlands and in phytoremediation techniques (Bonanno et al. 2017; Hadad et al. 2018).

Alonso et al. (2018) studied peri-urban wetlands located in the Middle Paraná River floodplain (Argentina). In that work, nutrients and metals in tissues of *T. domingensis*, *E. crassipes*, *Alternanthera philoxeroides*, and *Pistia stratiotes* were measured. The roots of *A. philoxeroides* showed the highest phosphorus concentrations in the tissues (Table 2). Also, the tissues of this macrophyte showed higher metal concentrations than that of measured in sediment. Therefore, these authors proposed this scarcely studied macrophyte as an efficient metal hyperaccumulator. These authors also studied root anatomical parameters, concluding that of *T. domingensis* and *E. crassipes* roots which were sensitive to nitrate and ammonium concentrations in water. Having into account the contaminant plant

Table 2 TP and metal concentrations (mg g^{-1} d.w.) in tissues of plants monitored at peri-urban wetlands and a control wetland

Plant species	Leaves	Roots	Leaves	Roots	Leaves	Roots
	TP		Cr		Cu	
T. domingensis (Rincón)	2.05–2.78	1.15–4.36	ND (0.002)	ND (0.002)–0.008	ND (0.002)	0.036–0.072
E. crassipes (Arroyo Leyes)	1.14–1.79	3.15–3.93	ND (0.002)	ND (0.002)–0.003	ND (0.002)	ND (0.002)–0.046
A. philoxeroides (Cayastá)	4.41–5.45	3.09–3.94	ND (0.002)	ND (0.002)	ND (0.002)	0.05
P. stratiotes (RECU)	1.01–1.31	0.923–2.27	ND (0.002)	ND (0.002)–0.005	ND (0.002)	0.028–0.031
	Ni		Pb		Zn	
T. domingensis (Rincón)	ND (0.002)	ND (0.002)–0.008	ND (0.002)	0.029–0.089	ND (0.003)	0.056–0.209
E. crassipes (Arroyo Leyes)	ND (0.002)	ND (0.002)–0.004	ND (0.002)	0.029–0.107	ND (0.003)	0.029–0.059
A. philoxeroides (Cayastá)	ND (0.002)	ND (0.002)	ND (0.002)	0.05	ND (0.003)	0.133
P. stratiotes (RECU)	ND (0.002)	ND (0.002)–0.001	ND (0.002)	0.031–0.032	ND (0.003)	0.043–0.094

ND = not detected, values in parentheses are the detection limits of the method (extracted from Alonso et al. 2018)

tolerance, Alonso et al. (2018) proposed that macrophytes from the Middle Paraná River floodplain are efficient biomonitors.

Baldantoni et al. (2018) carried out the biomonitoring of the Sarno River (Italy) using the macrophytes *Apium nodiflorum* and *Potamogeton pectinatus*. As a result of the measured contaminants in tissues, these authors concluded that this river has a severe pollution degree caused by Cd, Cr, Pb, and V, followed by Cu, Fe, Mn, Na, Ni, and Zn originated from agricultural and urban activities. Some plant taxonomical groups are scarcely studied. This is the case of aquatic bryophytes. Favas et al. (2018) studied the accumulation of 46 elements in tissues of *Fontinalis squamosa*, *Brachythecium rivulare*, *Platyhypnidium riparioides*, and *Thamnobryum alopecurum* in Góis mine region (Central Portugal). These mosses were efficient biomonitors due to high contaminant concentrations in their tissues. However, due to their low biomass, they are not efficient bioaccumulators to be considered in phytoremediation techniques. Farias et al. (2018) measured the concentrations of As, Cd, Cu, Pb, Se, and Zn in 12 macrophyte species from the Derwent estuary (Australia). Due to the different species that showed different metal concentrations in tissues, these authors concluded that the biomonitoring of multiple macrophyte species is needed to understand the metal pollution of the wide estuary. Similar work was carried out by Bonanno et al. (2017) in different wetlands from Sicily (Italy). These authors measured the concentrations of As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn in the seagrasses *Posidonia oceanica* and *Cymodocea nodosa* and in the wetland plants *P. australis*, *Arundo donax*, *T. domingensis*, *A. nodiflorum*, and

Nasturtium officinale. Their results demonstrated that metal biomonitoring was species-specific, and it was not dependent on the type of environment and the plant life forms. Therefore, each plant species responses are different faced with metal exposure in the different water bodies.

Based on the above, the use of macrophytes as biomonitors should be considered as a useful tool for the management of aquatic systems contaminated by different sources and in the design and operation of treatment wetlands.

5 Ecotoxicology Applied to Phytoremediation

Commonly, the changes in different antioxidant enzymes involved in the protection against metal stress are evaluated to assess the macrophyte tolerance to metal exposure. Among the works focused on free-floating macrophytes, it can be mentioned the study of the enzymatic detoxification strategies of *E. crassipes* when it was exposed to different water concentrations of Ni (González et al. 2015) and Zn (González et al. 2018). During the first hours of the Ni exposure, a significant increase of the photosynthetic pigment concentrations was observed indicating the continuity of the metabolic functions. Malondialdehyde (MDA) concentration in leaves increased significantly with a simultaneous increase of the antioxidant enzyme activities demonstrating a compensatory protective mechanism. In the Zn exposure, Chl-b and carotenoid concentrations increase at the end of the study. MDA in plant tissues did not change during the study; however, a significant increase in the enzymatic activity of the antioxidant system was observed. The results reported by Gonzalez et al. (2015, 2018) indicate that *E. crassipes* is tolerant to Ni and Zn exposure increasing the activity of its antioxidant system against metal stress. The physiological effects of Cu on *P. pectinatus* were studied by Brandão Costa et al. (2018). After an exposure of 96 h, chlorophyll and carotenoid concentrations decreased, affecting the photosystem II and causing photosynthesis inhibition. According to the sensitivity to Cu, these authors propose that this macrophyte can be a suitable species for monitoring water bodies contaminated with this metal. Cosio and Renault (2020) compared the physiological effects of methyl-Hg, inorganic Hg, and Cd on the submerged species *Elodea nuttallii*. The exposure to these contaminants did not affect photosynthetic mechanisms nor antioxidant enzymes. These authors reported that the exposure to methyl-Hg affects aminoacyl-tRNA biosynthesis, glycine, serine and threonine metabolism, nitrogen metabolism, arginine and proline metabolism, and cyanoamino acid metabolism. The Cd exposure produced changes in aminoacyl-tRNA biosynthesis and branched-chain amino acid pathways. Contrarily, inorganic Hg did not show metabolic effects. Cosio and Renault (2020) stated that their work was the first one that applied metabolomics in *E. nuttallii* concluding that these data complement the knowledge gained by transcriptomics and proteomics.

Vidal Ribeiro et al. (2019) studied the toxicity of hexazinone, an herbicide commonly applied in sugarcane production, on the free-floating macrophytes *P. stratiotes* and *E. crassipes*. These authors concluded that concentrations of

111 and 333 μg L^{-1} were toxic to the studied species, having into account the changes in biomass and foliar morphological parameters (adaxial and abaxial epidermis, palisade parenchyma, aerenchyma, and leaf blade). The toxic effects of toluene, ethylbenzene, and xylene on the submerged macrophyte *Hydrilla verticillata* were studied by Yan and Zhou (2011). After the exposure at different concentrations of these organic compounds, chlorophyll concentration, lipid peroxidation, and antioxidant enzymes showed changes. Using linear regression analyses, the changes observed indicated that the contaminant concentrations expected to protect the studied macrophyte were 7.30, 1.15, and 2.36 mg L^{-1}, for toluene, ethylbenzene, and xylene, respectively.

Parameters such as foliar injury, chlorophyll concentrations, and biomass changes can indicate the toxic effects of metal exposure on macrophytes. Besides, root morphological plasticity is an important mechanism to regulate the contaminant uptake and to enhance the plant tolerance (Hadad et al. 2007; Kapitonova 2002; Kolotov et al. 2003; Mufarrege et al. 2018; Zhou et al. 2008). The Cr, Ni, and Zn toxic effects on the productivity and root morphology of the free-floating macrophyte *E. crassipes* were studied by Hadad et al. (2011). This species was exposed to 1 mg L^{-1} of each contaminant. Biomass did not decrease in any treatment; however, the three studied metals produced a decrease in the leaf chlorophyll concentrations. Figure 2 shows that the cross-sectional area of roots (CSA) and the number of metaxylematic vessels increased, while the root length decreased during the Zn exposure.

Without a doubt, *Typha* spp. are one of the most studied wetland plants, being also one of the most used macrophytes in treatment wetlands all over the world. Hadad et al. (2010) studied the tolerance and the root morphology of *T. domingensis* in a constructed wetland for the treatment of a metallurgical effluent. In the inlet zone of the constructed wetland, plant height, dry biomass, the cross-sectional area of the roots and stele, and the number of metaxylematic vessels were significantly higher than the measured in the outlet zone and in a natural control wetland. However, the chlorophyll concentration measured in the plants from the inlet zone was significantly lower than the obtained in the plants from the outlet and control wetland (Fig. 3). The morphological root modifications observed in the plants sampled from the inlet zone enhanced the contaminant tissue accumulation demonstrating the adaptability of the studied species to the environmental wetland conditions.

In wetlands studied at mesocosms scale, Mufarrege et al. (2014) exposed *T. domingensis* plants to a metal-combined solution constituted by 100 mg Cr L^{-1} + 100 mg Ni L^{-1} + 100 mg Zn L^{-1}. The studied metals caused a decrease in chlorophyll concentration and growth inhibition. Regarding the root anatomical parameters, they were significantly lower in comparison with the obtained in the control wetlands. These authors concluded that *T. domingensis* could accumulate metals efficiently in its tissues and tolerate the conditions imposed by the presence of contaminants in treatment wetlands, despite the sublethal effects observed. The toxic effects of the chemical species Cr(VI) on *T. domingensis* in treatments with the addition of organic matter were studied by Mufarrege et al. (2018). Plants were exposed to Cr(VI) solutions of 15, 30, and 100 mg L^{-1}. Despite that the organic

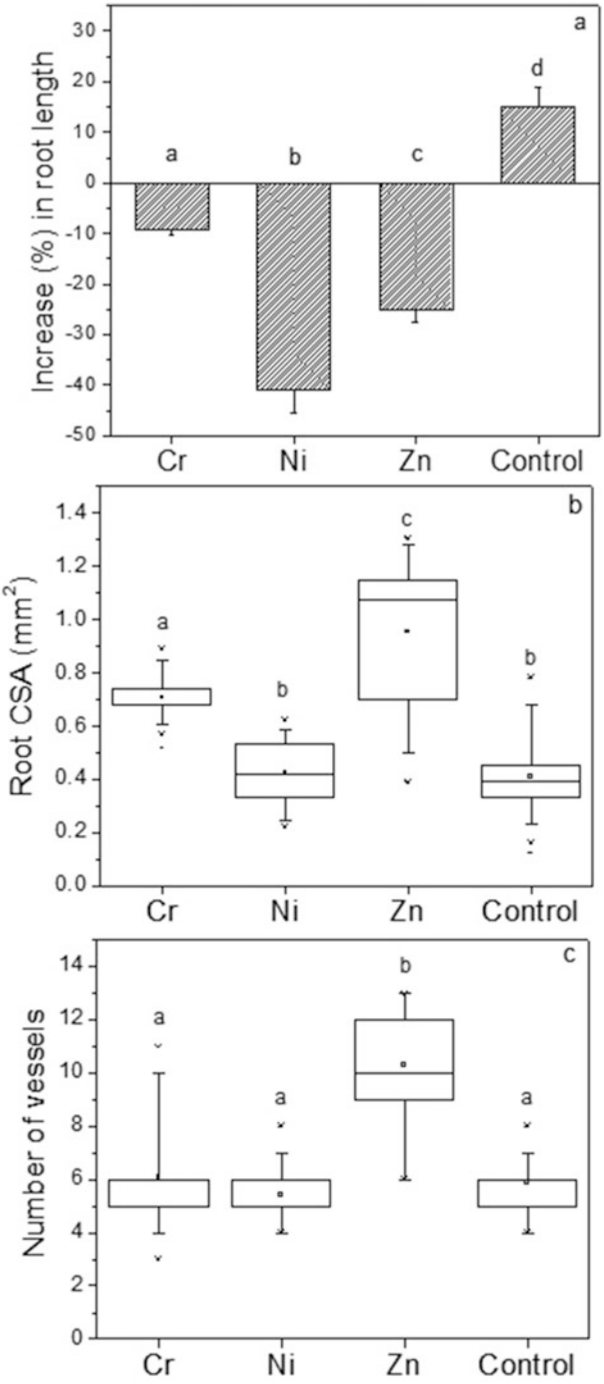

Fig. 2 Percent increase of the root length (a), box and whisker plots of root cross-sectional areas, and number of vessels in roots (c) of *E. crassipes* exposed to Cr, Ni, and Zn. Different letters represent statistically significant differences among treatment (extracted from Hadad et al. 2011)

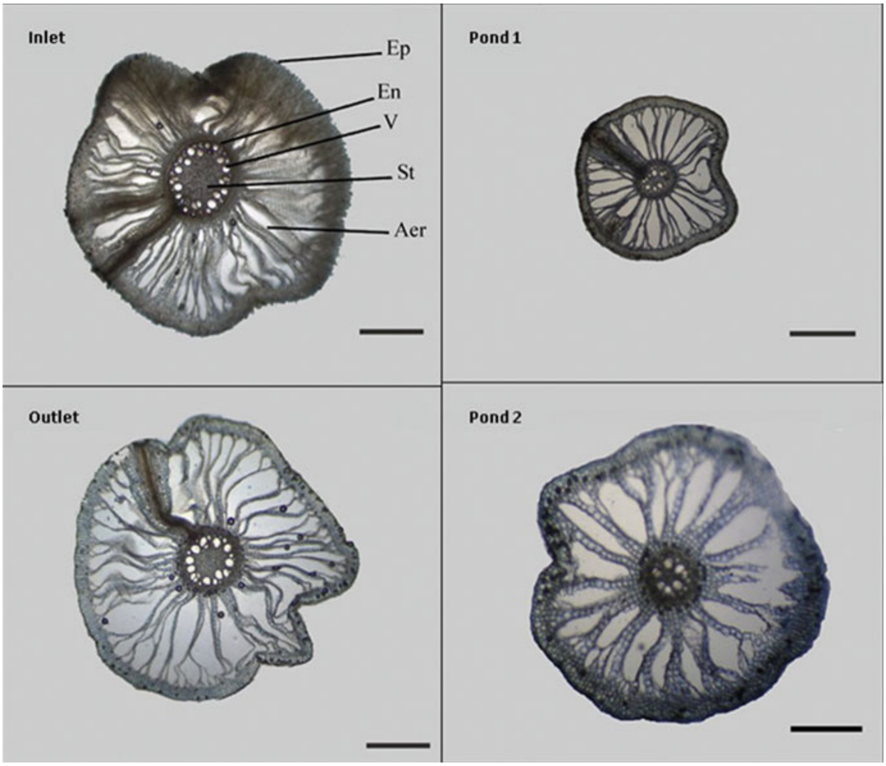

Fig. 3 Optical microscopy image of cross-sectional *T. domingensis* roots from the inlet and outlet areas of a treatment wetland constructed for the treatment of a metallurgical effluent and from natural ponds. *Ep* epidermis, *En* endodermis, *V* metaxylem vessels, *St* stele, *Aer* aerenchyma. Bar = 650 μm (extracted from Hadad et al. 2010)

matter addition increased the plant biomass and the chlorophyll concentrations, the exposure to Cr(VI) affected the root morphology. The ecotoxicological studies focused on the plant root morphology indicated that macrophytes have a considerable root morphological plasticity, which enhances the contaminant uptake and tolerance in wetland systems such as constructed wetlands.

To assess the effects of toxic compounds, different living organisms are used at a laboratory-scale, from bacteria and algae to organisms of higher biological complexity level (Guilizzoni 1991). Generally, studies using submerged and low biomass species are carried out, being focused on the enzymatic activity faced to the exposure to specific contaminants. However, it should be necessary to carry out studies focused on wetland plants that are commonly used in phytoremediation techniques such as constructed wetlands. Besides, these studies should consider other biological variables, such as changes in biomass or growth rate, in order to obtain basic information to be applied not only in wastewater treatments by phytoremediation but also in biomonitoring studies at field scale.

6 Final Considerations

Aquatic plants are key tools to monitor environmental pollution and to carry out the suitable management of natural wetlands. For the ecotoxicological study of macrophytes, it should be taken into account that not only the measurement of antioxidant enzymes involved in the protection against contaminant stress should be evaluated. Other basic biological parameters such as changes in different biomass compartments (roots, leaves, rhizomes, etc.), growth rates, and the internal morphology of roots and leaves should be considered.

The plant role in treatment wetlands has been discussed in a large number of works. However, nowadays, there is no doubt about the key function of plants during the treatment of pollutants in constructed wetlands. The selection of the wetland type, the macrophytes, and substrates to be used in the treatment of a specific effluent should be the result of previous studies conducted carefully.

Studies focused on the search for plant species tolerant for emerging contaminant treatment in CW and for their biomonitoring in natural wetlands are needed.

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