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THEMATIC ISSUE

Distribution of high Zn concentrations in unvegetated and *Typha domingensis* Pers. vegetated sediments

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Abstract An experiment was carried out to determine if the presence of *Typha domingensis* affects the accumulation and speciation of high concentrations of Zn in sediments of aquatic systems. Reactors containing sediment and two plants of *T. domingensis* were disposed in triplicate. The treatments were: (1) 100 mg L^{-1} Zn (Zn100); (2) 500 mg L^{-1} Zn (Zn500) and (3) control, without Zn. The same treatments without plants were disposed. The experiment lasted 30 days. Zn was efficiently removed from water in all treatments (greater than 75 %). In Zn500 treatments, Zn removal was significantly high in vegetated reactors. Zn accumulation was significantly high in the superficial sediment layer (0–3 cm). *T. domingensis* influenced Zn accumulation and its distribution in sediments. The unvegetated sediment accumulated higher Zn concentrations than vegetated sediments. Zn was accumulated in less available fractions in unvegetated than vegetated sediments. Submerged parts of leaves accumulated higher Zn concentration than the other plant organs. Plants did not show toxic symptoms, so it could be assumed that they would continue taking up Zn. In the occasion of a dump of high Zinc concentration, constructed wetlands could retain it, minimizing the environmental impact. Further investigation is needed to evaluate the role of macrophytes in the sustainability of Zn retention in constructed wetlands.

Keywords Phytoremediation · Speciation · Macrophytes · Sediment

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Introduction

Constructed wetlands (CWs) are systems that have been developed to take advantage of the natural processes implying wetland vegetation, sediments and associated microbial assemblages for treating wastewaters (Vymazal and Krópfelová 2005). Its use for successful metal removal from several types of industrial effluents has been increasing around the world (Maine et al. 2009; Marchand et al. 2010; Vymazal 2011). Metal removal processes in CWs are extremely complex and have been well described by many authors such as Guilizzoni (1991), Sheoran and Sheoran (2006) and Marchand et al. (2010). They are the result of biotic and abiotic interactions that can produce metal precipitation (with phosphates, sulfides, hydroxides, organic salts, etc.), mechanical retention (when they are included in suspended matter), complexation (with organic matter), adsorption/desorption (on Fe and Mn oxides, clay particles, root surfaces, etc.) or their absorption (by plants or microorganisms). On the whole, a general consensus in the scientific literature is that sediment/substrate acts as the primary sink for metals (Maine et al. 2009; Weis and Weis 2004; Ye et al. 2001). However, the sediment can retain or release such pollutants depending on the environmental conditions. Sediment characteristics as redox conditions, pH, organic matter content, etc., affect the chemical form in which metals are retained. The fractionation of metals in

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different forms is necessary to understand the mechanisms of exchanging at the sediment–water–plant system and their potential removal from water. Typical forms of metals in sediments, ranging from more to less available, are: (a) easily available: exchangeable and dissolved; (b) potentially available: metal carbonates, oxides and hydroxides, metals occluded with or adsorbed on iron and manganese oxides, metals adsorbed or chelated with insoluble high molecular weight humic compounds and metal sulfides; (c) unavailable: metals within the crystal lattice structure of the clay and other trace minerals.

Nevertheless, concerning the role of plant uptake in metal, removal opinions differ. Rooted macrophytes are able to stabilize and oxidize the bottom sediments (Brix 1994; Jacob and Otte 2003). Through their roots, emergent macrophytes supply oxygen to sediment producing oxidizing conditions with subsequent oxidation of Fe and Mn that result in the formation of amorphous oxyhydroxides which adsorb and/or co-precipitate metals. However, the organic material from the decay of plants is often responsible for the generation of low O_2 and sediment-reduced conditions in areas covered by vegetation. Under reducing conditions, according to the redox potential and pH values, metals can be precipitated as sulfides, carbonates or bound to insoluble humic compounds, resulting in long-term immobilization of these metals in the sediment. Therefore, the accumulation and speciation of contaminants in the sediment may differ in the presence of vegetation. The importance of plants in aquatic systems has been studied, often leading to controversial results (Doyle and Otte 1997; Caçador et al. 1996; Cambrollé et al. 2008; Lee and Scholz 2007; Marchand et al. 2010; Teuchies et al. 2013). Emergent macrophytes, such as *T. domingensis*, are widely used in constructed wetlands because they affect sediment biogeochemistry and have high capacity to accumulate contaminants in their biomass (Maine et al. 2007).

Zinc is a common contaminant in wastewater of industries such as galvanization, paint, batteries, smelting, fertilizers and pesticides, fossil fuel combustion, pigment and polymer stabilizers (Harte et al. 1991). In many cases, this wastewater was not purified satisfactorily. Therefore, the use of constructed wetlands could enhance Zn removal from these effluents.

In aquatic environments as in the case of a constructed wetland, Zn occurs mainly as a free ion in water. Increases of pH, alkalinity or natural organic matter would decrease Zn bioavailability through complexation or competition with other positively charged ions (Ca^{2+}, Mg^{2+}, Na^{+}, etc.). Zn could complex with organic matter or be adsorbed on Fe and Mn oxides (minerals), or could precipitate with sulfides and/or carbonates according to environmental conditions, increasing Zn concentration in the sediment. Hence, the cycling of Zn among different geochemical forms is strongly influenced by changes in the pH and redox potential in sediment–water systems. Soluble forms of Zn in water and sediment are easily available to plants. Zn distribution in plant tissues typically follows the pattern: roots $>$ rhizomes $\gg$ leaves. There is a general agreement that Zn species which is absorbed by roots are both hydrated Zn and Zn^{2+}. However, several other complex ions and Zn-organic chelates may also be absorbed.

We hypothesized that *T. domingensis* play an important role in Zn removal in constructed wetlands receiving high Zn water loads, as in the case of an accidental Zn dump. An experiment was performed to determine whether the presence of *T. domingensis* affects speciation and accumulation of high Zn concentrations in aquatic sediments and finally its retention in the system. The concentrations applied attempts to simulate an accidental dump of high Zn concentrations. This species was chosen for this study, since it is a widespread and often dominant macrophyte in aquatic wetland systems in Argentina. Furthermore, *T. domingensis* remained as the long-term dominant species in a wetland constructed for wastewater treatment in a metallurgic industry in which several locally abundant macrophytes were transplanted (Maine et al. 2007, 2009).

Materials and methods

Experimental design

Sediment, *T. domingensis* plants and water were collected from an unpolluted pond of the Paraná River floodplain near Santa Fe city, Argentina (31° 32′ 45″ S; 60° 29′ 37″ W). Only healthy plants of uniform size and weight were selected. Macrophytes were pruned before their transport to the greenhouse.

Eighteen PVC reactors of 10 L (20 cm diameter and 30 cm height) capacity were set outdoors under a semi-transparent plastic roof. After an acclimation of 15 days, the plants were pruned to a height of 20 cm approximately. Nine reactors contained two plants and 4 kg of sediment (vegetated treatments) and another nine reactors without plants (only sediment) (unvegetated treatments) were arranged. Zn solutions (5 L) were added to the reactors to obtain the following treatments: 100 mg L^{-1} Zn (Zn100), 500 mg L^{-1} Zn (Zn500) and control (water, without Zn addition). The experiment lasted 30 days and was performed in triplicate.

A stock solution of $ZnCl_2$ and water from the sampling site was used to prepare Zn solutions. The chemical composition of the water from the sampling site employed in the study was (mean ± standard deviation) (APHA 2012): pH 7.8 conductivity = 223 ± 1 µS cm^{-1}; dissolved oxygen (DO) = 6.71 ± 0.10 mg L^{-1}; soluble reactive phosphorus (SRP) = 0.023 ± 0.002 mg L^{-1}; NH_4^+ = 0.990 ± 0.005 mg L^{-1}; NO_3^- = 0.410 ± 0.005 mg L^{-1}; NO_2^- = non detected (detection limit = 5 µg L^{-1}); Ca^{2+} = 9.8 ± 0.1 mg L^{-1}; Mg^{2+} = 2.2 ± 0.2 mg L^{-1}; Na^+ = 36.8 ± 0.5 mg L^{-1}; K^+ = 16.1 ± 0.5 mg L^{-1}; Fe = 0.291 ± 0.005 mg L^{-1}; Cl^- = 14.6 ± 1.0 mg L^{-1}; Zn = non detected (detection limit = 5 µg L^{-1}); SO_4^{2-} = 10.5 ± 1.0 mg L^{-1}; total alkalinity = 104.2 ± 1.2 mg L^{-1}. The pH of the solution was controlled with HCl addition to prevent Zn precipitation.

The water level in the reactors was maintained by adding water from the sampling site. The temperature ranged from 21.1 to 31.3 °C during the experimental period.

Chemical analysis

In each reactor, water was sampled at 0, 1, 2, 4, 7, 14 and 30 days. Water samples were acidified to pH <2 with concentrated nitric acid and Zn was determined directly by atomic absorption spectroscopy (flame or electrothermal vaporization, Perkin Elmer AAnalyst 200) (APHA 2012).

Zn concentrations in plants and sediment were determined at the beginning and the end of the experiment. Sediment samples were collected using a plastic corer of 3 cm diameter. Sediment cores were sliced in situ with a plastic cutter at the following depth layers: 0–3 (surface), 3–7 (medium) and 7–10 cm (deep), and stored at 4 °C until analysis. Redox potential (Eh) (Ag/AgCl electrode) and pH of the sediment samples were measured in situ with an Orion pH/mV-meter in triplicate. Organic matter content (OM) was determined by weight loss on ignition at 550 °C for 3 h. Sediment samples were oven dried at 45 °C until constant weight, ground using a mortar and pestle and sieved through a 53 µm sieve. In sediment, organic matter was 5.41 %, pH was 7.03 and Eh was 280 mV.

Due to the fact that root development was mainly observed in the surface sediment layer, only these sediment samples were analyzed by the sequential extraction proposed by Tessier et al. (1979) to evaluate the chemical association of metals in sediment. The sequential extraction includes five steps: (1) exchangeable (1 M $MgCl_2$; pH 7); (2) bound to carbonates (1 M NaOAc; pH 5); (3) bound to Fe–Mn oxides (0.04 M NH_2OH–HCl in 25 % (v/v) HOAc); (4) bound to OM (0.02 M H NO_3 + 30 % H_2O_2; pH 2; 3.2 M NH_4OAc in 20 % (v/v) HNO_3); (5) residual. To diminish sediment loss, acid-washed polyethylene centrifuge tubes (50 mL) were used for the extraction. Separation between each extraction was achieved by centrifugation at 3000 rpm for 20 min. The supernatant was removed with a pipet and analyzed for metals. The residue was rinsed with distilled water and centrifuged at 3000 rpm for 20 min. This second supernatant was dismissed and the residue extracted further. For total and residual metal analyses, 1 g of dried samples was digested with a $HClO_4$:HNO_3:HCl (7:5:2) mixture. The resulting solution from the total and residual digestion and the extracts obtained from the sequential extraction procedure were analyzed for Zn by atomic absorption spectrometry (Perkin Elmer AAnalyst 200) using an air–acetylene flame.

Plants were sampled and separated into roots, rhizomes, and submerged and aerial parts of leaves. They were rinsed with tap and distilled water and later oven dried at 60 °C for 48 h. 0.5 g of dried macrophyte samples were ground and digested with an HClO4:HNO3:HCl (7:5:2) mixture. The total Zn in the sediment was determined by atomic absorption spectrometry (Perkin Elmer AAnalyst 200). In the sediment, Zn initial concentration was 0.045 mg g^{-1}. In macrophyte tissues, Zn initial concentration was: roots = 0.071 mg g^{-1}; rhizomes = 0.030 mg g^{-1} and leaves = 0.049 mg g^{-1}.

Zn amounts (mg) were estimated by multiplying Zn concentration in plant tissues, sediment (mg g^{-1} dry weight) or water (mg L^{-1}) by mass (g dry weight) or volume (L).

Plant study

Plant height was measured and the external appearance of plants was observed daily to detect possible senescence.

Relative growth rate (RGR) (cm cm^{-1} day^{-1}) was calculated according to Hunt's equation (1978):

$$\mathrm{RGR} = \frac{\ln H_2 - \ln H_1}{T_2 - T_1},$$

where H_1 and H_2 are the initial and final plant height (cm), respectively, and $(T_2 - T_1)$ is the experimental period (days).

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine whether significant differences existed in the relative growth rate among samples and in plant tissue concentrations (aerial parts, submerged parts of leaves, rhizomes and roots). Two-way ANOVA (factors: vegetation and sampling date (for Zn in water); depth and vegetation; vegetation and Zn fractions) was achieved to determine whether significant differences existed in Zn concentrations in water and sediment. The normality of residuals was analyzed graphically and homogeneity of variances was checked using Bartlett's test. Plant tissue data were transformed (log) to achieve homogeneity of variances and normality. Duncan's test was used to differentiate means where appropriate. A level of $p < 0.05$ was used for all comparisons.

QA/QC

All glassware was pre-cleaned and washed with 2 N HNO_3 prior to each use. All reagents were of analytical grade. Certified standard solutions were used. Replicate analyses (at least ten times) of the samples showed a precision of typically less than 4 % (coefficient of variation). The detection limit was 3 µg g^{-1} for Zn for sediment and plant tissues.

Results and discussion

Zn was efficiently removed from water during the experiment (Fig. 1). The highest metal removal was achieved in both, vegetated and unvegetated treatments of 100 mg L^{-1} (95 and 97 %, respectively), without significant differences. In the treatments of 500 mg L^{-1} Zn, the final removal was significantly higher in vegetated (87 %) than in unvegetated treatments (77 %) (Fig. 1), suggesting that *T. domingensis* was directly involved in metal removal. The present results are in agreement with those of Ning et al. (2014) and Kumari and Tripathi (2014) who reported high Zn removal in vegetated treatments.

Flooding generated a decrease of Eh in our experiment. Usually, pH decreases when Eh increases, and reciprocally due to the development of H^+ ions during the oxidation process (Kaplan and Knox 2007; Yu et al. 2007). At the end of the experiment, the vegetated sediments presented significantly lower pH values and significantly higher Eh values than unvegetated sediments in all treatments (Zn100, Zn500 and controls) (Table 1). The lowest pH and the highest Eh values were measured in the surface layer (0–3 cm). In all cases, the sediments were reduced (Eh < 100 mV). The pH of the sediment in the vegetated treatments was different from that of the unvegetated sediments by up to 1 pH unit; this reduction suggests that more cations than anions are take in and consequently results in the release of H^+. Other acidifying processes that may also contribute to the decrease of sediment pH is the microbial breakdown of organic matter with release of CO_2 (Tinker and Barraclough 1988). Emergent macrophytes alter local sediment pH conditions through incorporation/production of anions/cations by root exudates and stabilize and oxidize the sediment by oxygen translocation from their aerial parts to the roots and then the rhizosphere (Brix 1994; Dunbabin et al. 1988; Jacob and Otte 2003). Organic matter related to plant development, senescence and root exudates directly impacts microbial activity, increases sediment oxygen demand and decreases sediment pH. Since the acidic zone of the root may coincide with the location of radial oxygen loss (ROL), the net pH decrease may result from the combined effect of iron oxidation and root hydrogen release. Negrin et al. (2011) argued that the low pH values in the vegetated sediment in the lower marsh of the Bahía Blanca Estuary could reflect local CO_2 production via aerobic oxidation of organic matter in the rhizosphere. On the other hand, the root tissues release H^+ to the pore water to maintain electro-neutrality after taking up ammonium ions.

The complete mechanism of phytoextraction of heavy metals has five elementary points: mobilization of the metals in the substrate, sorption of the metal ions by plant roots, translocation of the accumulated metals from roots to leaves, sequestration of the metal ions in plant material and metal tolerance. Metal tolerance is a crucial prerequisite for

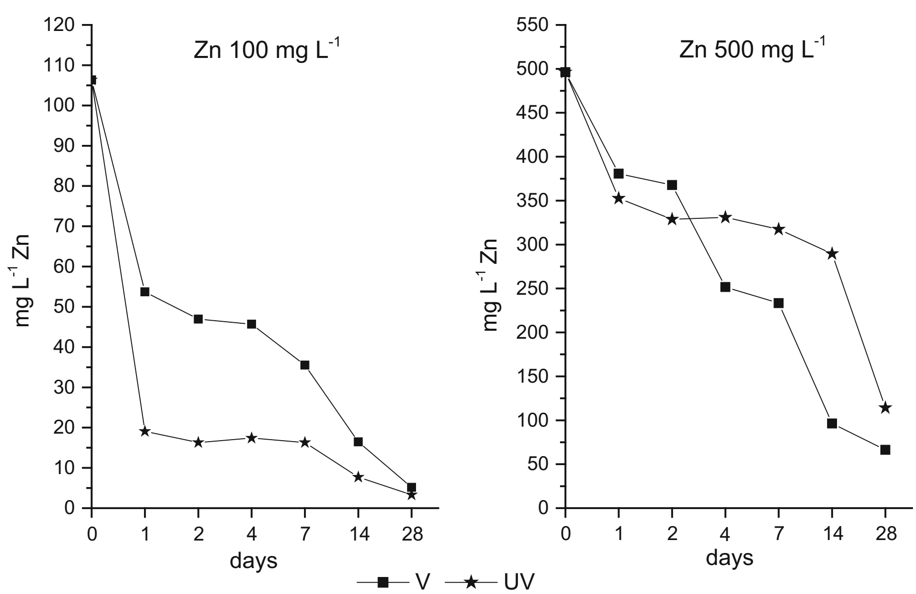

Fig. 1 Zn removal percentage in vegetated (*V*) and unvegetated (*UV*) reactors during the experiment

Table 1 pH and Eh (mV) values measured in different sediment layers of vegetated (V) and unvegetated (UV) treatments

	pH			Eh (mV)		
	Surface sediment (0–3 cm)	Medium sediment (3–7 cm)	Deep sediment (7–10 cm)	Surface sediment (0–3 cm)	Medium sediment (3–7 cm)	Deep sediment (7–10 cm)
Initial	7.03 ± 0.10	7.03 ± 0.10	7.03 ± 0.10	280 ± 11	280 ± 11	280 ± 11
Zn100 V	6.7 ± 0.16	7.08 ± 0.2	7.11 ± 0.18	91 ± 10	44 ± 13	25 ± 11
Zn100 UV	7.15 ± 0.15	7.38 ± 0.15	7.42 ± 0.14	65 ± 10	46 ± 15	20 ± 15
Zn500 V	6.62 ± 0.13	7.03 ± 0.12	7.01 ± 0.13	69 ± 13	37 ± 10	15 ± 12
Zn500 UV	6.95 ± 0.17	7.06 ± 0.18	7.18 ± 0.16	45 ± 10	27 ± 14	12 ± 15
Control V	7.08 ± 0.13	7.15 ± 0.16	7.2 ± 0.15	517.5 ± 15	500 ± 14	496 ± 15
Control UV	7.67 ± 0.17	7.78 ± 0.14	7.92 ± 0.17	152.2 ± 13	148.7 ± 16	148 ± 10

metal accumulation and therefore phytoremediation (Clemens 2001; Tong et al. 2004). It has been widely reported that metals are accumulated in root tissues as a tolerance strategy and that for most plants the main uptake pathway of metals from the sediment is via the root system (Chandra and Yadav 2010; Hadad et al. 2007, 2011; Stoltz and Greger 2002; Vymazal 2011). However, there is a high variability among metals and among plant species. In our experiment, *T. domingensis* submerged parts of leaves presented, in both, Zn100 and Zn500 treatments, significantly higher Zn concentration than the other parts of the plant, namely aerial parts leaves and rhizomes, up to 20 (Zn100) or 10 (Zn500) times higher, and roots up to 10 (Zn100) or 5 (Zn500) times higher than the submerged parts of leaves (Fig. 2). These results indicate that Zn was accumulated in the plant tissues mainly by sorption by direct contact with the solution, probably due to the high concentrations used. Zinc is an important micronutrient for plants. It is implicated in a diversity of critical functions related to gene control, oxygen transport and metabolic enzymes (Bonilla 2008). Nevertheless, when metal concentrations achieve a threshold value, they become first inhibitory and then toxic for most plants. In both treatments, RGR was significantly lower than the control, but positive, demonstrating that although *T. domingensis* growth was affected by the exposure of high Zn concentrations, this macrophyte survive to the experimental conditions (Fig. 3).

Metal accumulation was produced mainly in the surface layer of sediments (0–3 cm) in both vegetated and unvegetated treatments (Fig. 4). This is in agreement with other works and suggest a scarce metal mobility (Chagué-Goff and Rosen 2001; Di Luca et al. 2011; Maine et al. 2009; O'Sullivan et al. 2004; Yu et al. 2001, etc.). Zn was accumulated even at the deeper layer (7–10 cm) in both unvegetated and vegetated sediments. However, vertical profiles of Zn concentrations indicate that metal incorporation was different in vegetated sediments from that observed in unvegetated sediments. After the experiment, unvegetated sediments accumulate higher Zn concentration

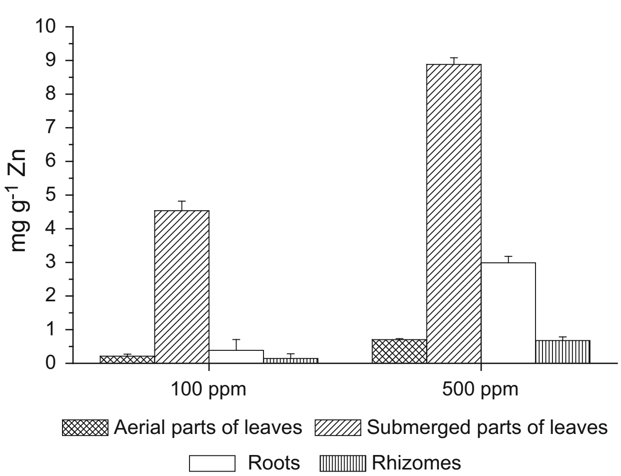

Fig. 2 Zn concentration (mg g^{-1}) in *T. domingensis* tissues (leaves, submerged leaves, roots and rhizomes) in the different treatments at the end of the experiment

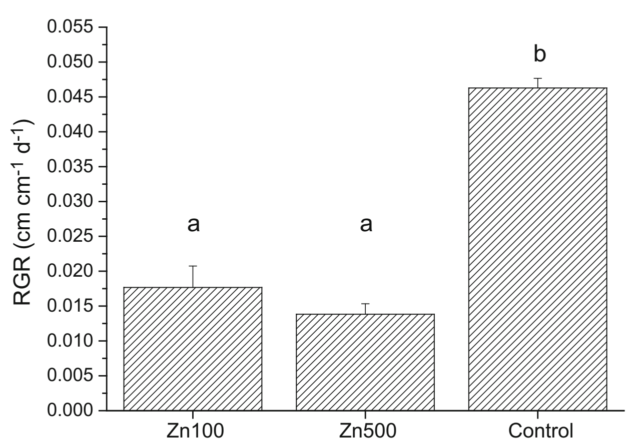

Fig. 3 Relative growth rates (cm cm^{-1} day^{-1}) obtained at the end of the experiment compared with the control. *Different letters* represent statistically significant differences among the treatments. *Bars* represent standard deviations

than vegetated sediments (Fig. 4). Also, an increase was observed in the contaminant concentration in the deeper sediment layers in unvegetated treatments as compared to the vegetated treatments. Variances in metal concentrations between unvegetated and vegetated sediments appear to be linked to the presence and activity of roots. Zn is incorporated and retained by *T. domingensis*, which acts as a Zn extraction pump. Some authors found an opposite situation, i.e., higher metal concentration in vegetated than in unvegetated sediments. Almeida et al. (2004) found that sediment colonized by *Juncus maritimus* presented significantly higher metal concentrations than unvegetated sediment in the Douro River estuary (Portugal). Caçador et al. (1996) have also found an enrichment of Zn concentration in sediments vegetated by the marsh plants, *S. maritima*, *H. portulacoides* and *A. fruticosum*, grown at the Tagus estuary, Portugal. This exposes the impact of plant species on metal retention. The metal uptake capacity of the total plant biomass mainly depends on the biological traits of the selected plant species that are planted, but also on management practices. In fact, the quantity of metals exported in plants is linked to the mean absorption efficiency of metal into plants and to the plant biomass production.

Figure 5 shows Zn fractionation in the surface sediments of all treatments. In the control treatments, Zn was mainly found in the residual fraction (48 and 60 % in vegetated and unvegetated treatments, respectively) and bound to Fe–Mn oxides (30.69 and 20.21 % in vegetated and unvegetated treatments, respectively). However, in Zn 100 and Zn 500 treatments, distribution of Zn in chemical phases changed, since Zn was accumulated mainly bound to carbonates in all treatments. Several authors have

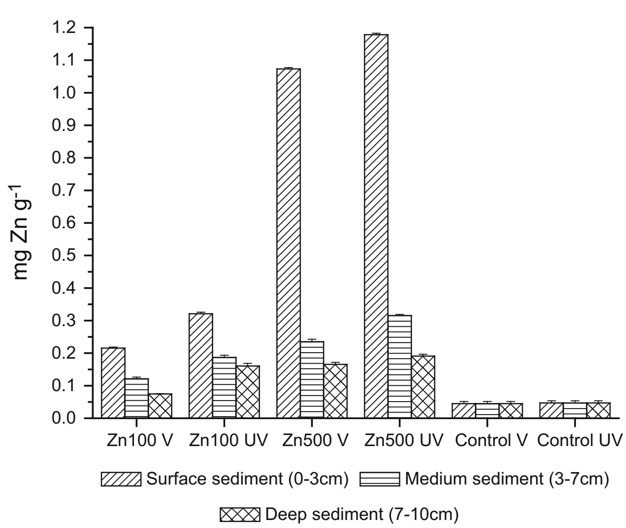

Fig. 4 Zn concentration (mg g^{-1}) in different sediment layers of vegetated (*V*) and unvegetated (*UV*) treatments at the end of the experiment

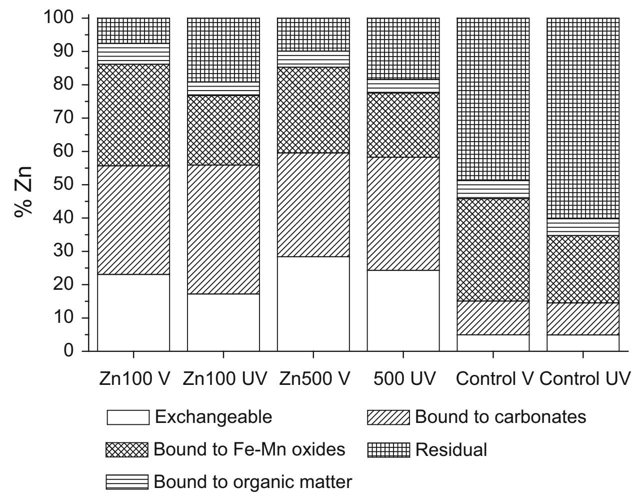

Fig. 5 Zn fractionation in surface sediment layer (0–3 cm) of vegetated (*V*) and unvegetated (*UV*) treatments at the end of the experiment

reported Zn bound to carbonates as a Zn retention mechanism (Banerjee 2003; Lee et al. 2005; Stone and Marsalek 1996). Characterization of Fe plaques and metals from the rhizosphere of *Phalaris arundinacea* using XAS showed that Zn forms nodules of hydrozincite in close spatial association with rhodochrosite and a mixed Mn/Zn carbonate (Hansel et al. 2001). The formation of hydrozincite and/or a mixed Mn/Zn carbonate in the dense rhizosphere of the *T. domingensis* treatment in this study is probable. Furthermore, in neutral sediment, the Zn amount associated with Fe–Mn oxides was replaced by Zn associated with carbonates while Eh decreases (Guo et al. 1997) as observed in the present study.

The existence of the macrophyte also changed the metal fractionation in the sediment (Fig. 5). The percentages of the metal in the residual fraction were significantly lower in the vegetated sediments than in the respective unvegetated sediments from both treatments, which is compatible with higher metal availability. Almeida et al. (2004) also found that Zn percents in the residual fraction were significantly lower in the sediments vegetated with *Juncus maritimus* than in the unvegetated sediments. Contrary to that observed in this study, Caçador et al. (1996) have reported that Zn in vegetated sediments (*S. maritime*, *H. portulacoides*, and *A. fruticosum*) was mostly associated with the residual fraction. Accordingly, the influence of plants on the sediment surrounding roots may depend on the plant species (and presumably also on sediment composition). Zinc is a chalcophile element, forming discrete ZnS phases under anoxic conditions that can be found in the residual fraction (Achterberg et al. 1997; Bostick et al. 2001; Davison 1993; Huerta-Diaz et al. 1993, 1998; Morse and Luther 1999; Peltier et al. 2003). *T. domingensis* vegetated sediments were less anoxic than unvegetated sediments; this high redox potential could cause ZnS oxidation and release of Zn that was be subsequently taken by plants or re-precipitated in other sediment fractions.

Under suboxic conditions, Zn is usually associated with Fe–Mn oxides (Bostick et al. 2001; Doyle and Otte 1997; Peltier et al. 2003). Zn has also been found to associate with Fe-rich concretions formed in the rhizosphere (Hansel et al. 2001; Sundby et al. 1998). Accordingly, our results show a higher association of metals to Fe and Mn oxides in sediments with a higher redox potential (vegetated sediments). The highest Eh in vegetated sediments was likely due to ROL from active roots (Armstrong et al. 2000) of *T. domingensis*. This species as a monocot plant has a well-developed aerenchyma, favoring the transport of oxygen from the aerial parts to the roots and the surrounding sediment. It was expected that the increased oxidation with the addition of macrophytes would reduce the pore water Fe concentrations, via oxidation and precipitation, and also Zn through coprecipitation reactions.

The exchangeable fraction, which is the most labile and bioavailable, was significantly higher in vegetated than in unvegetated sediments in all treatments (Fig. 5). The presence of plant (and microbe) can increase Zn mobility by developing soluble complexes and chelates (Kiekens 1995), especially at neutral pH (above pH 7.2; Yin et al. 2002), via various root exudates or phytosiderophores (Zhang et al. 1991). Zn mobility increased with the incorporation of plants to mine tailings, probably because of acidification and complexation that result from the release of organic acids from roots (Banks et al. 1994). Kirk and Bajita (1995), experimenting with rice plants, concluded that root-induced oxidation of reduced Fe caused a substantial solubilization of Zn. The solubilized Zn is re-adsorbed in more soluble fractions, so that Zn can be taken up by the plants. Wright and Otte (1999) found that the roots of *T. latifolia* may release organic acids into the surrounding tailings, thereby increasing Zn solubility by chelation (Kiekens 1995). Alternatively, a competing soil cation not included in this study may have inhibited Zn adsorption (McBride and Blasiak 1979), again increasing the solubility. For these reasons, it is likely that a longer experimentation time would change this result, i.e., Zn concentration in the exchangeable fraction would decrease due to the plant uptake; this would be possible because plants were in a healthy state after 30 days of exposure. Further research is needed to have a better understanding of the root–sediment interactions.

Hence, the results obtained in this study proved that *T. domingensis* was capable of modifying the characteristics of the sediment surrounding its roots, altering Zn fractionation (Zn more weakly bound to the vegetated sediment than the unvegetated sediment) and metal accumulation (concentrating metals in unvegetated sediments). Such changes can influence the metal mobility, availability and toxicity, particularly to organisms that live and feed in the surrounding environment. It is assumed that the removal capacity of the substrate mainly depends on its sorption capacity (conditioned by its specific surface, mineral and/or organic composition) and on the physical and chemical conditions in the constructed wetland. The latter mainly depends on the system dimensions (shape, depth, surface) and on the physico-chemical properties of wastewater (Sheoran and Sheoran 2006). However, the management practices of plants can also affect the retention capacity of the substrate.

Conclusions

Despite the high concentrations studied, *T. domingensis* did not show toxic symptoms during the study and Zn was efficiently removed from water in all treatments (above 75 %), suggesting that in a case of a possible dump of high Zn concentrations, constructed wetlands could act as a cushion, minimizing the environmental impact. Sediment was the compartment which exhibited the largest Zn accumulation. Nevertheless, the presence of *T. domingensis* influences the accumulation and speciation of Zn in the sediments. Unvegetated sediments accumulated higher Zn concentration and its accumulation was higher in less available fractions than vegetated sediments. However, plants accumulated Zn in their tissues preventing that the metal reaches deeper sediment areas. High Zn concentrations in the submerged part of leaves were determined, suggesting the Zn sorption by direct contact. At the end of the experiment, plants were in a healthy state, so it could be assumed that they would continue uptaking Zn. These results stress the role of *T. domingensis* in metal accumulation, availability and uptake. Further investigation is needed to evaluate the role of plant in the sustainability of Zn retention in constructed wetlands.

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