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RESEARCH ARTICLE

Long-term study of Cr, Ni, Zn, and P distribution in *Typha domingensis* growing in a constructed wetland

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Abstract

The aim of this work was to study Cr, Ni, Zn, and P bioaccumulation in different compartments of *Typha domingensis* plants and sediment in a free-water surface constructed wetland for the treatment of a metallurgical effluent for 5 years. Removal efficiencies were satisfactory. To increase metal tolerance, its transport from belowground to aboveground tissues is reduced, being metal concentrations in the roots and rhizomes significantly higher than in the aerial and submerged parts of leaves. Regarding belowground tissues, metals were retained in the roots, while P was mainly accumulated in rhizomes. Bioaccumulation factors (BAFs) of Cr and Ni showed values near 1, and BAF of Zn and P were above 1 in several samplings, indicating bioaccumulation in the roots. Translocation factors (TFs) of Cr, Ni, and Zn were below 1, showing a scarce translocation from the roots to the aerial parts of the leaves, while the TF of P were above 1 in many samplings, indicating that this element is necessary for plant metabolism. The study of plant tissues where contaminants are accumulated allows gaining insight into the constructed wetland operation. The high translocation of P in *T. domingensis* makes this species suitable for its phytoextraction, while the low metal translocation makes *T. domingensis* suitable for phytostabilization.

Keywords Industrial effluent · Macrophytes · Metals · Phosphorous · Tissues

Introduction

Plants growing in constructed wetlands (CWs) show several properties that render them an essential component (Brix 1994). There are numerous biological, chemical, and physical processes that occur among contaminants and aquatic plants. Currently, these processes and their relationship are not sufficiently known. Regarding metals, it has been proposed that metal removal mechanisms in plants are not necessarily the same for different macrophyte species and for different metals (Maine et al. 2016; Suñe et al. 2007). Among these mechanisms, root sorption (a combination of physical and chemical processes such as quelation, ionic exchange), biological processes (which include translocation to aerial parts), and precipitation induced by root exudates or microorganisms could be mentioned. Regarding plant tolerance, several species tolerate high metal concentrations in sediment because they limit the absorption and translocation to the leaves maintaining constant and relatively low concentrations in the aerial biomass, independently of the metal concentration in sediment (Bonanno 2011; Hadad et al. 2006; Vymazal 2011).

To understand contaminant dynamics, it is important to study the ecosystem compartments where they are accumulated (Bonanno et al. 2017; Eid and Shaltout 2014). Gupta et al. (2012) studied the dynamics of Cr, Ni, Cu, and Pb among the different ecosystem compartments: water, surface sediments, submerged and free-floating macrophytes, and fishes of two man-made lakes in India. These authors concluded that although a significant metal uptake by macrophytes and fishes was observed, metal concentrations in sediment were significantly higher than those of the other compartments. Regarding plant compartments, Mufarrege et al. (2015) exposed the emergent

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species *Typha domingensis* to high Cr, Ni, and Zn concentrations. These authors observed that metals were accumulated not only in the roots but also in the submerged parts of leaves in direct contact with the solution. On the other hand, macrophytes do not only remove contaminants when they are alive. It was demonstrated that plant dry biomass is able to adsorb metals (André et al. 1999; Dushenkov et al. 1995; Miretzky et al. 2006; Schneider et al. 1999, 2001; Suñé et al. 2007; Wang et al. 1998), which may be an important issue for wetland management.

In Argentina, a CW for the treatment of effluent from a metallurgical industry showed different stages as to vegetation dominance (Maine et al. 2009, 2013). The wastewater contains P, Cr, Ni, and Zn and shows high pH and conductivity. Initially, an assemblage of locally common macrophytes from the Middle Paraná River floodplain was transplanted to the wetland. Plant growth showed three different periods: the first one dominated by the floating macrophyte *Eichhornia crassipes*, followed by its decline and an increase of the emergent macrophyte *T. domingensis* cover period, and finally a *T. domingensis* dominance period (Maine et al. 2007, 2009). Despite the presence of metals in the effluent, the cause of disappearance of the floating macrophytes was the high values of pH and conductivity (Hadad et al. 2006). Since the emergent macrophyte *T. domingensis* was more tolerant to the effluent conditions than the floating species, it became dominant in the wetland over the last 12 years. A predation of *T. domingensis* aerial parts by capybaras (*Hydrochoerus hydrochaeris*) occurred during this period. However, the roots and rhizomes of *T. domingensis* were not damaged. After such event, *T. domingensis* recovered its aerial biomass and covered the entire wetland surface (Maine et al. 2013).

Despite macrophytes being a key component in treatment wetlands, knowledge about their role is still insufficiently understood. The depuration efficiency in different wetland designs, the plant species to be used and their tolerance to contaminants and the characteristics of the effluent to be treated are topics that should be deepened due to their influence on the system optimization (Wu et al. 2015). The study of the compartments where contaminants are accumulated is necessary to understand the contaminant dynamics in CWs. The aim of this work was to study contaminant bioaccumulation in different compartments of *T. domingensis* plants and in sediment in a CW for the treatment of a metallurgical effluent for 5 years. A long-term study of the plant tissues where the contaminants are accumulated allows gaining insight into the CW operation and determining if biomass harvesting is worth performing.

Materials and methods

Description of the wetland system

A free-water surface (FWS) wetland was constructed for the final treatment of industrial wastewater and sewage from a metallurgical factory. Wastewaters from both sources receive a primary treatment (precipitation, sieving, and decantation) and are treated in the same CW. The CW has been operating since 2001. It is 50 m long, 40 m wide, and 0.4–0.5 m deep. The wetland has a central baffle to force the effluent to cover double the distance. Mean wastewater discharge is 100 $m^3\ d^{-1}$. Water residence time ranges from 7 to 12 days. After flowing through the CW, the effluent is discharged into a 1.5-ha pond. Further design details have been explained in a previous study (Maine et al. 2009).

Sampling

This study began when the CW reached stability and maturity. The study period was between 2010 and 2015. Wastewater, sediment, and plants were sampled monthly by triplicated. The contaminant concentrations of the influent and the effluent were used to estimate the system efficiency. The sediment and plant samples were taken in the inlet zone of the CW. Surface sediment samples were collected with a PVC corer at a depth of 0–3 cm and then stored at 4 °C. *T. domingensis* plants were sampled by hand and separated into aerial parts of leaves, submerged parts of leaves, roots, and rhizomes. The roots and rhizomes were washed with tap water in order to separate the attached sediment.

Analytical determinations and calculations

Conductivity, pH, and dissolved oxygen (DO) were measured in situ with an YSI 33 conductivity-meter, Orion pH-meter, and Horiba OM-14 oximeter, respectively. Wastewater chemical analysis was carried out following APHA (1998). Wastewater samples were kept refrigerated during their transport to the laboratory. Soluble reactive phosphorus (SRP) was determined by the colorimetric molybdenum blue method (Murphy and Riley 1962). In the case of total phosphorus (TP), non-filtered water samples were digested with sulfuric acid-nitric acid. SRP was determined in the digested samples (Murphy and Riley, 1962). Nitrite (NO_2^-) was determined by coupling diazotation followed by a colorimetric technique. Nitrate (NO_3^-) and ammonium (NH_4^+) were determined by potentiometry (Orion ion selective electrodes, sensitivity 0.01 mg L^{-1} of N, reproducibility ± 2%). Chemical oxygen demand (COD) was determined by the open reflux method and biochemical oxygen demand (BOD) by the 5-day BOD test. For metal analysis, samples were acidified to pH < 2. Total Fe, Cr, Ni, and Zn concentrations were determined in water samples by atomic absorption spectrometry (by flame or electrothermal atomization, according to sample concentration, Perkin Elmer AAnalyst 200) and previous acid digestion with nitric acid-hydrochloric acid (APHA, 1998).

Cr, Ni, Zn, and P were measured in sediment and plant tissues. Plants and sediment samples were oven-dried at 70 °C for 48 h (APHA 1998). Dried samples were ground, sieved, and digested with a $HClO_4$:HNO_3:HCl (7:5:2) mixture, and then, metals were measured by atomic absorption spectrometry (Perkin Elmer, AAnalyst 200), and P was determined as SRP in the digested samples (Murphy and Riley, 1962).

The bioaccumulation factor (BAF) was calculated to determine the ability of plants to accumulate contaminants from the sediment:

$$\text{BAF} = \text{concentration of a contaminant in roots } (\text{mg g}^{-1}) / \text{concentration of the same contaminant in sediment } (\text{mg g}^{-1})$$

The translocation factor (TF) was calculated to assess the plant capacity to translocate a contaminant from the roots to the aerial parts of leaves:

$$\text{TF} = \text{concentration of contaminant in the aerial parts of leaves } (\text{mg g}^{-1}) / \text{concentration of the same contaminant in roots } (\text{mg g}^{-1})$$

The mass of Cr, Ni, Zn, and P in sediment and tissues was calculated (mg m^{-2}) using the dry plant biomass and the dry sediment mass. To estimate dry biomass, *T. domingensis* stands were sampled with a 0.50×0.50 m^{-2}. Four replicates were collected. Plants were harvested, separated into aerial and submerged parts of leaves, roots, and rhizomes, and dried at 105 °C until a constant weight was reached (APHA 1998). For sediment sampling, it was considered an active sediment layer of 3 cm, according to our previous work (Di Luca et al. 2011). Sediment samples were dried at 105 °C until constant weight. The mean contaminant retention percentage by macrophyte tissues and sediment was calculated.

Statistical analysis

In order to ascertain statistical differences in contaminant concentrations in water, sediment, and plant tissues between the inlet and outlet, paired tests were used. ANOVA was carried out to determine if there were significant differences in contaminant concentrations among the sediment and the different plant tissues, in the BAF and TF among the different contaminants, and among the mass of the different contaminants. To differentiate means where appropriate, Duncan's test was used. Normality of residuals was tested graphically. Bartlett's test was used to verify that variances were homogeneous. In all comparisons, a level of $p < 0.05$ was used.

QA/QC

To wash all glassware, 2 M HNO_3 was used. To prepare all solutions, analytical grade reagents and Milli-Q water were used. A precision lower than 5% (coefficient of variation) was determined in all replicate analyses. Detection limits for water were Cr = 2 μg L^{-1}, Ni = 3 μg L^{-1}, Zn = 3 μg L^{-1}, and P = 5 μg L^{-1}, and for macrophyte tissues and sediment were Cr = 2 μg g^{-1}, Ni = 3 μg g^{-1}, Zn = 3 μg g^{-1}, and P = 0.5 μg g^{-1}.

Results and discussion

Table 1 shows mean values, concentration ranges, and removal efficiencies of the parameters measured in water. High pH and conductivity were determined in the influent. The chemical composition of the wastewater showed significantly high variability, which is a common characteristic of industrial effluents. However, after flowing through the CW, the effluent presented not only significantly lower concentrations (paired test, $p < 0.05$) but also significantly lower variability of the parameters measured compared with that of the influent, which proves the buffer capacity of the system.

Metals were efficiently removed from the wastewater (paired test, $p < 0.05$). It is important to highlight that this CW was meant for a final treatment, and metal concentrations in the inlet were low. Contaminant removal efficiencies were satisfactory, except for SRP and NH_4^+, probably due to low DO concentration. CW performance was steady over the operation period, allowing the effluent to meet law regulatory limits. A more detailed analysis of the removal efficiency was reported by Maine et al. (2017).

The sediment in the inlet zone of the CW showed significantly higher Cr, Ni, Zn, and P concentrations than those registered in the outlet zone (paired test, $p < 0.05$) (Fig. 1), and the concentrations in the latter remained showing no significant differences along the study (paired test, $p < 0.05$). Comparing studied contaminant concentrations in sediment, Zn showed the lowest ones reflecting the low Zn concentration in the influent. Sediment sorption is considered the main long-term contaminant accumulation mechanism (Machemer et al. 1993; Maine et al. 2009; Wood and Shelley, 1999). Di Luca et al. (2011) evaluated metal retention and distribution in the sediment of the studied CW. These authors concluded that sediment may be expected to continue retaining Ni and Zn mainly stored in carbonate fraction, Cr was mainly associated with the Fe–Mn oxide fraction, while Fe was mainly associated with the residual fraction. These metals will not be released into water while the chemical and environmental conditions remain unchanged. Aquatic plants can modify the biogeochemistry of the sediment by altering redox conditions, pH, and organic matter content, influencing contaminant accumulation in this compartment (Di Luca et al. 2016).

Cr and Ni concentrations were significantly higher in sediment than in plant tissues (ANOVA, $p < 0.05$), while in some samplings, the concentrations of Zn and P were significantly higher in roots than in sediment. Cr, Ni, and Zn concentrations in roots and rhizomes were significantly higher than in the aerial and submerged parts of leaves (ANOVA, $p < 0.05$) (Fig. 2). This indicates a scarce metal translocation from belowground to aboveground parts. To increase metal tolerance, transport from belowground to aboveground tissues is reduced. The exclusion of metals in roots is a macrophyte tolerance strategy to avoid the damage of vital tissues (Chaney 1993; Göthberg et al. 2004;

Table 1 Parameters (mean, minimum, and maximum) measured in the influent and effluent and mean removal efficiency (%) (concentrations = mg L^{-1}, conductivity = μmho cm^{-1})

Parameters	Influent	Effluent	Mean removal
pH	10.8 (10.4–12.2)	8.3 (7.9–9.3)	–
DO	3.40 (0–6.2)	2.1 (0.3–5.2)	–
Conductivity	5113 (3890–8700)	1955 (1400–2500)	–
SRP	0.030 (0.005–0.079)	0.026 (0.005–0.334)	13
Total P	0.396 (0.064–1.38)	0.309 (0.129–0.696)	22
NO_2^-	2.22 (0.281–6.21)	0.352 (0.017–0.766)	84
NO_3^-	50.6 (15.4–98.2)	9.9 (3.6–24.2)	80
NH_4^+	0.88 (0.15–2.6)	0.77 (0.05–2.14)	12
Cr	0.092 (0.023–0.204)	0.014 (0.002–0.033)	85
Zn	0.041 (0.022–0.070)	0.020 (0.015–0.050)	51
Ni	0.068 (0.004–0.101)	0.023 (0.004–0.082)	70
Fe	0.824 (0.052–2.54)	0.087 (0.05–0.230)	89
COD	85 (27.9–154.0)	37.1 (13.9–42.9)	75
BOD	31.3 (9.8–30.9)	9.97 (3.0–20.1)	73

Hechmi et al. 2014; Kabata-Pendias and Pendias 2011; Loneragan and Webb 1993; Marchand et al. 2010; Shanker et al. 2005). The tolerance mechanisms consist of metal deposition on the root surface and inside the vacuoles and the root cell walls (Taylor and Crowder 1983).

BAF of Cr and Ni showed values near 1. BAFs of Zn and P were above 1 indicating that roots bioaccumulate them (Fig. 3). In all samplings, metal TFs were lower than 1 showing a scarce translocation of Cr, Ni, and Zn from the roots to the aerial parts of leaves.

In most samplings, P showed BAF and TF values higher than 1, demonstrating that it is taken from the sediment by roots and then it is translocated to the aerial parts of leaves. The BAF values for Zn higher than 1 indicate that this metal is accumulated in root tissues to a higher extent than Cr and Ni. Bragato et al. (2009) reported that Zn was the most accumulated metal in *Phragmites australis* in a CW in North Italy. Zn is a micronutrient for plants and it is both a structural and a catalytic component in many proteins and enzymes (Marschner 1995; Van Assche and Clijsters 1986). Nyquist and Greger (2007) studied the stress effects in response to Zn, Cu, and Cd exposure on *Elodea canadensis*. They reported that most of the accumulated Zn and Cu are transported inside the cell through the cell membrane, whereas Cd remains in the cell membrane. Probably, this could be explained by the essentiality of these elements that would increase uptake rates and translocation within the plant. While in the cell, Zn is quickly subjected to enzyme

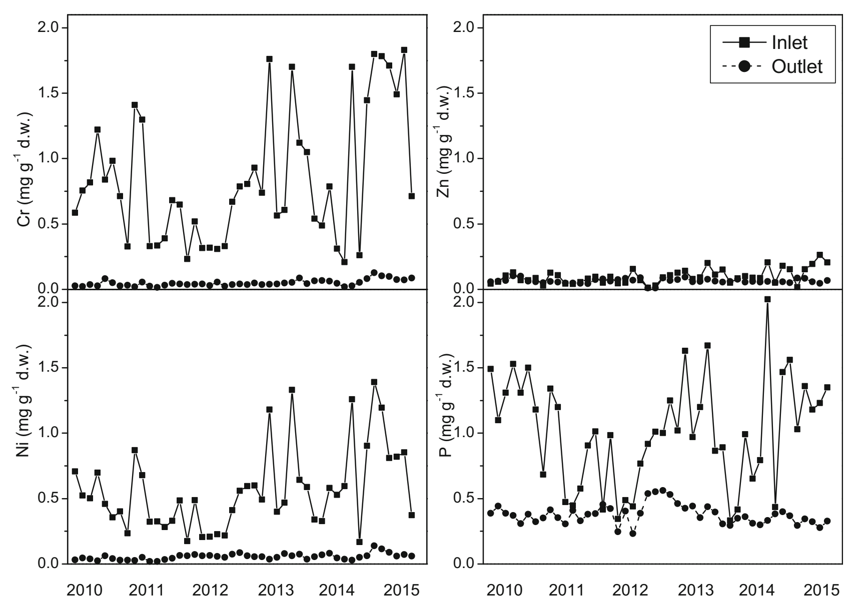

Fig. 1 Cr, Ni, Zn, and P concentrations measured along the study in the sediment in the inlet and outlet zones of the CW

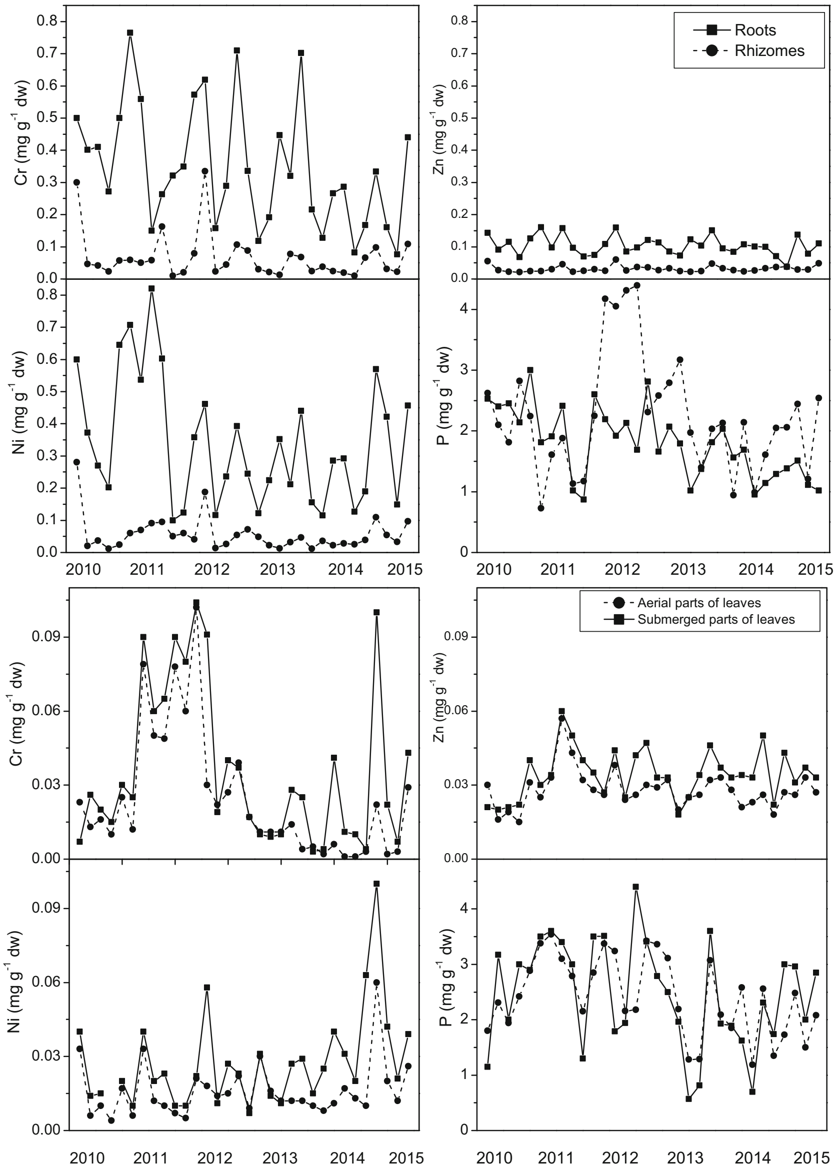

Fig. 2 Cr, Ni, Zn, and P concentrations measured along the study in the roots, rhizomes, and submerged and aerial parts of leaves of *T. domingensis*. Note the different concentration scale in the case of P

synthesis, needed for other physiologically active molecules, or incorporated in membranes or other cellular components (Rengel 1999).

Regarding belowground tissues, metal concentrations in the roots were significantly higher than those of rhizomes (ANOVA, $p < 0.05$) (Fig. 2). Metals are retained by roots, while P is mainly accumulated in rhizomes. Since P is a plant nutrient, it is stored to carry out photosynthesis and develop biomass. The same result was reported for *T. domingensis* plants growing in natural wetlands from the Middle Paraná River floodplain (Argentina) as a strategy for biomass increase (Hadad and Maine 2007). The high translocation of P to aboveground organs in *T. domingensis* makes this species suitable for its phytoextraction from the system, while the low metal translocation makes *T. domingensis* suitable for phytostabilization. In the case of metals, Eid and Shaltout (2014) concluded that higher translocation of Cd in the aboveground organs of *P. australis* allows its phytoextraction, while the lower translocation for Cu, Fe, and Zn enhances their phytostabilization.

It was widely reported that metal concentrations in macrophytes growing in constructed and natural wetlands are usually higher in belowground than in aboveground parts (Bonanno 2011; Hadad et al. 2006, 2007, 2011; Larsen and Schierup 1981; Maddison et al. 2009; Peverly et al. 1995; Nuñez et al., 2011; Vymazal, 2011, 2013; Windham et al. 2003; Zhang et al. 2014). Most studies only consider above and belowground parts. In our work, we consider the submerged parts of leaves which are a rarely studied plant compartment. It is remarkable that such plant compartment showed significantly higher metal concentrations than the aerial parts of leaves. The tissues of the

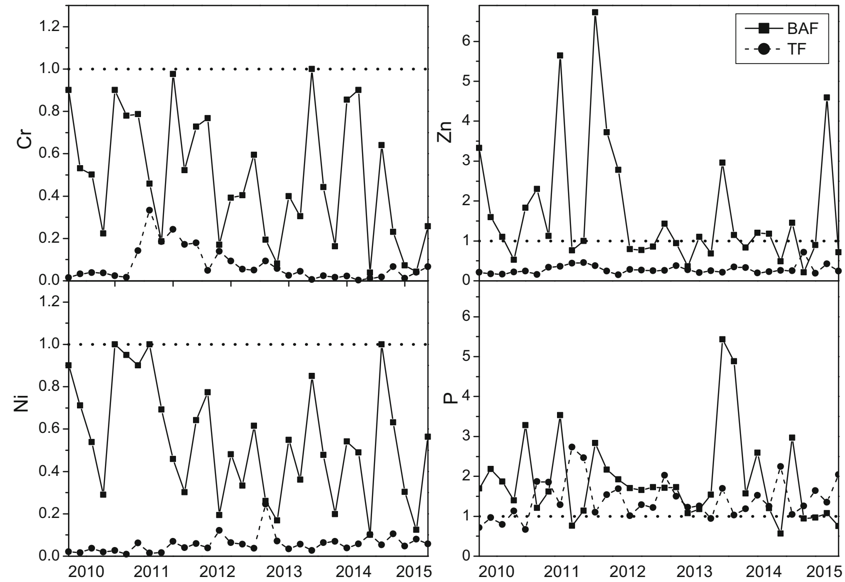

Fig. 3 BAF and TF values of Cr, Ni, Zn, and P calculated along the study. Note the different scale in the case of Zn and P

submerged parts of leaves were in direct contact with the wastewater. For this reason, not only translocation but also adsorption from water were probably the mechanisms responsible for metal uptake in these tissues. Mufarrege et al. (2015) reported a similar result studying *T. domingensis* responses when exposed to Cr, Ni, and Zn during greenhouse experiments. This result acquires importance because the submerged parts of leaves are not considered in studies on contaminant bioaccumulation by macrophytes. In comparison with the other plant compartments, metal bioaccumulation in the aerial parts of leaves was negligible (ANOVA, $p < 0.05$). However, leaves transport oxygen to the belowground tissues and sediment, enhancing the contaminant removal processes.

In comparison with submerged macrophytes (Harguinteguy et al. 2014; Nyquist and Greger 2007; Yabanli et al. 2014), *T. domingensis* has a significantly higher biomass, which enhances efficiency in contaminant removal and extraction from the system. In CW, contaminant accumulation in the biomass of emergent macrophytes could allow phytoextraction from the system by harvesting, and phytostabilization by accumulation in the belowground tissues (Brezinová and Vymazal 2015). Another advantage of *T. domingensis* is that it is found worldwide in remarkable abundance, being available in natural wetlands close to the sites where treatment wetlands may be constructed. Guittonny-Philippe et al. (2015) highlight the use of native macrophytes growing at short distances from industrial discharges to be treated.

Figure 4 shows the estimated mean contaminant retention by macrophyte tissues and sediment in the inlet area of CW (%). Metals and P were retained by plants and sediment; however, sediment was the main accumulation compartment (ANOVA, $p < 0.05$). As it can be seen, macrophytes are not important sinks for metal removal, which is in agreement with the findings of Mays and Edward (2001) and Lee and Scholz (2007). This is an advantage since metals were phytostabilized in wetland sediment. Plants certainly contribute to metal trapping into the substrate via rhizodeposition (Kidd et al. 2009).

Among plant compartments, the submerged parts of leaves were the main contaminant compartment due to a higher biomass and contaminant concentration in comparison with the other plant compartments (ANOVA, $p < 0.05$). The bioaccumulation of Zn and P in the aerial parts of leaves was significantly higher than that of Cr and Ni due to higher translocation. The results obtained could be used to know in more detail about the CW functioning and the assessment of harvesting in order to remove contaminants from the system, definitely.

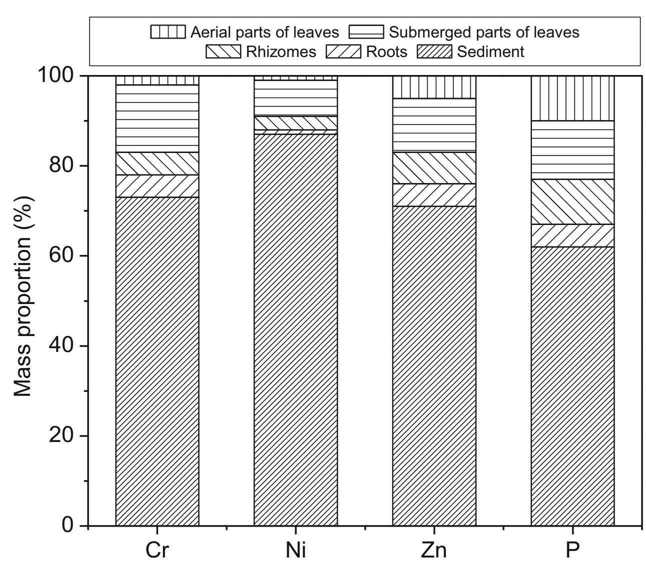

Fig. 4 Estimated mean contaminant retention by macrophyte tissues and sediment in the inlet zone of the CW (%)

Conclusions

- Contaminant removal efficiencies were satisfactory in the studied CW and *T. domingensis* showed tolerance to the wastewater.
- The concentrations of Zn and P were significantly higher in the roots than in sediment, indicating that root plants are a compartment to be considered during the effluent treatment using CWs.
- Cr, Ni, and Zn were scarcely translocated from the roots to the aerial parts of leaves, while P showed a high translocation. This implies that Cr, Ni, and Zn were phytostabilized in the belowground tissues and sediment, while P could be phytoextracted from the system by harvesting the aboveground parts due to the higher accumulation of this element in the aboveground tissues.
- The results obtained could be used to predict the accumulation pattern of contaminants in different compartments to carry out a suitable management in a CW.

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