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Metal dynamics and tolerance of *Typha domingensis* exposed to high concentrations of Cr, Ni and Zn



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ABSTRACT

Typha domingensis was exposed to a 100 mg L⁻¹ Cr+100 mg L⁻¹ Ni+100 mg L⁻¹ Zn solution. Metal tolerance and metal accumulation in plant tissues and sediment were studied over time. Although removal rates were different, the three metals were efficiently removed from water. Leaf and root tissues showed high metal concentration. However, the sediment showed the highest accumulation. During the first hours of contact, metals were not only accumulated by sediment and roots but they were also taken up by the leaves in direct contact with the solution. Over time, metals were translocated from roots to leaves and vice versa. Metals caused growth inhibition and a decrease in chlorophyll concentration and affected anatomical parameters. Despite these sub-lethal effects, *T. domingensis* demonstrated that it could accumulate Cr, Ni and Zn efficiently and survive an accidental dump of high concentrations of contaminants in systems such as natural and constructed wetlands.

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1. Introduction

Macrophytes have been studied because of their ability for contaminant removal from water and their subsequent use in wetlands constructed for wastewater treatment (Bharti and Bernajee, 2012; Kadlec and Wallace, 2009; Vymazal, 2011). Wetland plants generally accumulate metals in different percentages both, in below and above-ground parts (Fitzgerald et al., 2003; Kabata-Pendias and Pendias, 2011; Matthews et al., 2004; Vymazal, 2011). The processes performed by plants to uptake metals are sorption by plant tissues (including adsorption, chelation, ionic exchange and chemical precipitation), and biological processes including translocation to aerial parts, accumulation in the apoplast or in cell cytoplasm and precipitation induced by root exudates or by micro-organisms (Dushenkov et al., 1995; Hu et al., 2014; Lyon et al., 1969; Maine et al., 2004; Salt and Kramer, 1999; Skeffington et al., 1976; Suñe et al., 2007). According to literature, root tissues present significantly higher metal concentrations than leaves, indicating that the exclusion of metals from above-ground tissues is a metal tolerance strategy (Sawidis et al., 1995; Taylor and Crowder, 1983). These results were reported for rooted species (Chandra and Yadav, 2010; Sinha and Gupta, 2005; Vymazal, 2011) and free floating

macrophytes (Hadad et al., 2007; Kadlec and Wallace, 2009; Maine et al., 2004, 2013; Miretzky et al., 2010; Mufarrege et al., 2010).

Aquatic plants in their natural habitat are usually exposed to low concentrations of different contaminants. The conditions for plants growing in wetlands constructed for industrial wastewater treatment are completely different since they must have the ability to tolerate high concentrations of several contaminants at the same time. In most cases, the research goal as to wetland systems was to assess contaminant removal efficiencies during short period experiments. However, it is important to understand the mechanisms by which macrophytes or sediments of a wetland system can retain contaminants along time. The ability of plants to accumulate and tolerate contaminants from water may depend on their morphological adaptive capacity. Macrophytes can modify the internal morphology of their roots in order to grow in polluted water bodies (Hadad et al., 2009, 2010, 2011; Kapitonova, 2002; Mufarrege et al., 2010, 2011; Nilratnisakorn et al., 2007).

Typha spp. is a widespread and dominant macrophyte in many aquatic natural systems. It was widely studied due to its high productivity, high tolerance and ability for contaminant removal. For these reasons, it has been largely used in constructed wetlands for the treatment of different effluents (Chandra and Yadav, 2010; Maine et al., 2013; Vymazal, 2011).

Typha domingensis was chosen for this study since it was the dominant macrophyte in a wetland constructed for the treatment of effluents of a metallurgic industry (Maine et al., 2009). Cr, Ni and Zn were studied for being contaminants found in treated

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effluents at this constructed wetland. The concentrations studied were higher than the concentrations commonly found in constructed wetlands simulating extreme events, as an accidental dump.

The aim of this research was to assess metal dynamics in a wetland system (water–plant tissues–sediment), morphological response and tolerance of *T. domingensis* exposed to a combined solution of high concentrations of Cr(III)+Ni(II)+Zn(II). Studies of metal bioaccumulation and the toxic effects on *T. domingensis* would allow us to determine its tolerance and provide us with basic knowledge to evaluate vegetation management in the wetland.

2. Materials and methods

2.1. Experimental design

T. domingensis, sediment and water were collected from an unpolluted pond of the Middle Paraná River floodplain near Santa Fe city, Argentina (31° 32' 45"S; 60° 29' 37"W). Only healthy plants of a uniform size and weight were selected. The plants were pruned for their transport to the greenhouse.

Thirty plastic reactors were disposed outdoors under a semi-transparent plastic roof. Each plastic reactor of 10 L capacity (20 cm diameter and 30 cm height) contained two plants and 4 kg of wet sediment (10 cm depth, approximately). After fifteen days of acclimation, the plants were pruned again to a height of approximately 20 cm and 5 L of a metal solution, containing 100 mg L⁻¹ Cr+100 mg L⁻¹ Ni+100 mg L⁻¹ Zn, was added to the reactors. Plants were almost completely submerged in the solution. Metal solution was prepared using water from the sampling site and CrCl₃·6H₂O, NiCl₂·6H₂O and ZnCl₂. HCl was added to prevent metal precipitation. Water level in the reactors was maintained by adding water from the sampling site. Temperature ranged from 26.1 to 31.3 °C during the experiment. A control (without contaminants) was used. The experiment lasted 90 days and was carried out in triplicate.

Samples were collected initially, at 2, 8 and 24 h and at 2, 7, 14, 21, 28, 60 and 90 days. Water, sediment and the total plant biomass of the reactor were collected in each sampling and the reactor was discarded. Plant samples were separated into leaves, rhizomes and roots. Sediment was sampled using a 3-cm diameter PVC corer. Sediment was sliced in different layers (0–3 cm, 3–7 cm and 7–10 cm). Cr, Ni and Zn were determined in water, sediment and plant tissues.

2.2. Plant study

Leaf chlorophyll *a* concentrations were determined during the experiment. Relative growth rate (RGR) (cm cm⁻¹ day⁻¹) was calculated in each treatment considering plant height, according to

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

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

At the end of the experiment, sections of approximately 30 mm long were cut from the middle of the root and stored in formaldehyde 4 percent. After 48 h, root sections were immersed in ethanol 70 percent for their conservation. For anatomical measurements, the main roots were taken at random and cross-sectioned by hand applying the technique proposed by D'Ambrogio de Argüeso (1986). In order to distinguish cell walls from the background, the material was stained with aniline blue, which stains cellulose blue. Sections were examined by light microscopy (100× and 400×). Sixty sections of roots from the metal treatment and control were analyzed. The diameters of root, stele and metaxylem vessels were measured using a micrometric ocular. The formula to calculate the area of a circle was applied to obtain the values of cross-sectional areas (CSA) of the whole root; stele and metaxylem vessels (Wahl et al., 2001). Also, the number of metaxylem vessels per section was recorded.

Scanning electron microscopy (SEM) was performed at the end of the experiment in the cross-sectional area of roots. Samples of roots of about 1 cm were cut and dried in an oven at 20 °C for ten days so as not to damage the tissues (Suñé et al., 2007). Samples were examined with a Scanning electron microscope JEOL, model JSM-35C.

2.3. Chemical analysis

The physicochemical characterization of water used in the experiment was done according to APHA (1998).

The plants sampled were washed with tap and distilled water, and subsequently oven dried at 60 °C for 48 h. Dried plant samples were ground and digested with a HClO₄:HNO₃:HCl (7:5:2) mixture (Maine et al., 2001). Sediment samples

were oven-dried at 60 °C until constant weight and digested in the same way as plant samples. Cr, Ni and Zn were determined in triplicate by atomic absorption spectrometry (Perkin Elmer, AAnalyst 200) (APHA, 1998) in water and the digests of plant tissues and sediment.

Cr, Ni and Zn amounts (mg) were estimated by multiplying Cr, Ni or Zn concentration in plant tissue, sediment (mg g⁻¹ dry weight) or in water (mg L⁻¹) by biomass (g dry weight) or volume (L).

Chlorophyll was extracted with acetone for 48 h in cold darkness (3–5 °C). The percentage of transmittance of the extracts at 645 and 665 nm was recorded with a spectrophotometer UV–vis (Westlake, 1974).

2.4. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine whether significant differences existed in relative growth rate and chlorophyll *a* concentrations among samples. Two-way ANOVA was performed to determine whether significant differences existed in metal concentrations and metal amounts in plants tissues (leaves, rhizomes and roots) among samples along time. Duncan's test was used to differentiate means where appropriate. Since root morphology parameters (CSA of roots, stele and metaxylem vessels, and number of vessels) did not show a normal distribution, non-parametric tests and box and whisker plots were performed using the median as central trend measure and interquartile range (25 and 75 percent) as its variability measure. Kruskal–Wallis analysis was applied to check the differences in the morphometric parameters measured in roots among the different treatments. Wilcoxon's test was used to differentiate medians where appropriate. In all comparisons a level of $p < 0.05$ was used.

2.5. QA/QC

All glassware were pre-cleaned and washed with 2 N HNO₃ prior to each use. CrCl₃·6H₂O, NiCl₂·6H₂O and ZnCl₂·6H₂O used to prepare experimental metal solutions were of analytical grade.

Certified standard solutions were used in analytical determination. Blank solutions were run. Replicate analyses (at least ten times) of the samples showed a precision of typically less than 4 percent (coefficient of variation). Detection limits were 30, 20, and 3 µg g⁻¹ for Cr, Ni, and Zn, respectively for sediment and plant tissues.

3. Results

3.1. Metal removal from water

The chemical composition of the water used in the experiment was (mean ± standard deviation): pH=7.31; conductivity=210 ± 2 µS cm⁻¹; dissolved oxygen (DO)=6.72 ± 0.10 mg L⁻¹; soluble reactive phosphorous (SRP)=0.015 ± 0.006 mg L⁻¹; NH₄⁺=1.27 ± 0.019 mg L⁻¹; NO₃⁻=0.580 ± 0.012 mg L⁻¹; NO₂⁻=non detected (detection limit=5 µg L⁻¹); Ca²⁺=9.70 ± 0.8 mg L⁻¹; Mg²⁺=2.5 ± 0.5 mg L⁻¹; Na⁺=32.1 ± 1.0 mg L⁻¹; K⁺=12.1 ± 0.5 mg L⁻¹; Fe=0.392 ± 0.05 mg L⁻¹; Cl⁻=9.6 ± 1.3 mg L⁻¹; SO₄²⁻=7.2 ± 1.8 mg L⁻¹; total alkalinity=103.2 ± 1.2 mg L⁻¹; Cr=non detected (detection limit=5 µg L⁻¹); Ni=non detected (detection limit=5 µg L⁻¹), and Zn=non detected (Detection limit=5 µg L⁻¹).

Fig. 1 shows Cr, Ni and Zn removal percentages from water over time. Cr removal rate was the highest while Ni showed the lowest one. After two days of experimentation, metal removal percentages were 60 percent, 5 percent and 21 percent for Cr, Ni and Zn, respectively. On day 14, metals were removed at 98 percent, 27 percent and 58 percent for Cr, Ni and Zn, respectively. Although removal rates were different, the three metals were efficiently removed from water after 60 days, being final metal removal 99.9 percent, 99.0 percent and 99.5 percent for Cr, Ni and Zn, respectively.

3.2. Metal concentration in sediment and plant tissue over time

The chemical composition of the sediment used in the experiment was: organic matter (OM)=8 percent; pH=7.67; Eh=280 mV (Ag/AgCl); Cr=0.015 mg g⁻¹; Ni=0.006 mg g⁻¹; and Zn=0.130 mg g⁻¹. Metal concentrations on the surface layer of

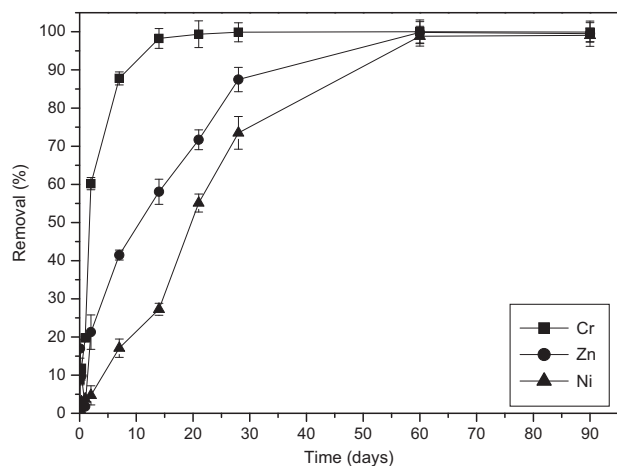


Fig. 1. Cr, Ni and Zn removal percent from water along time.

sediment (0–3 cm) were presented due to metal concentrations in the two deepest layers along time and were not significantly different to the initial ones (data not shown).

Metal concentration in roots and leaves was higher than in sediment over time in all cases. Metal concentration in sediments increased significantly during the first 21 days of the experiment (Fig. 2), remaining almost constant afterwards.

After 2 h of contact, concentration of the studied metals in leaves was significantly higher than in the roots. Then, at 8 h and at 24 h, metal concentration was significantly higher in roots than in the leaves.

On days 7 and 21, Cr concentration in leaves was significantly higher than in roots. Ni concentration in roots and leaves was not significantly different along the experiment. During the experiment, Zn concentration in roots was significantly higher than that of Ni and Cr. Metal concentration in rhizomes was significantly lower than in leaves and roots, indicating that rhizomes acted as a metal transport organ.

3.3. Metal mass balance

In order to compare the accumulation rates in sediment and plants, metal concentrations were converted into metal amount (mg). Thus, not only concentration but also each compartment mass was considered. Fig. 3 shows the metal amount accumulated in water, roots, rhizomes, leaves and sediment over time.

A decrease in metal amounts in water was in coincidence with an increase in metal amounts in plant tissue and sediment. At the end of the experiment, removal from water was not significantly different for the three studied metals. Metal removal from water was faster for Cr compared to that of Zn and Ni. On day 28, all Cr accumulated in the sediment and plant tissues while 20 percent Zn and 30 percent Ni still remained in water (Fig. 3). The amount of Cr in roots increased until day 60, but decreased significantly by day 90. Metal amount in rhizomes was significantly lower than in roots and leaves.

It is important to highlight that the sediment compartment showed the highest metal accumulation.

3.4. Plant tolerance and root morphology

Plant height increased during the experiment showing positive RGR (Fig. 4a). Although RGR were positive in all treatments, they were significantly lower than those for the control, demonstrating growth inhibition.

Chlorophyll concentration decreased significantly after 21 days of exposure (Fig. 4b). Although chlorophyll concentration decreased at

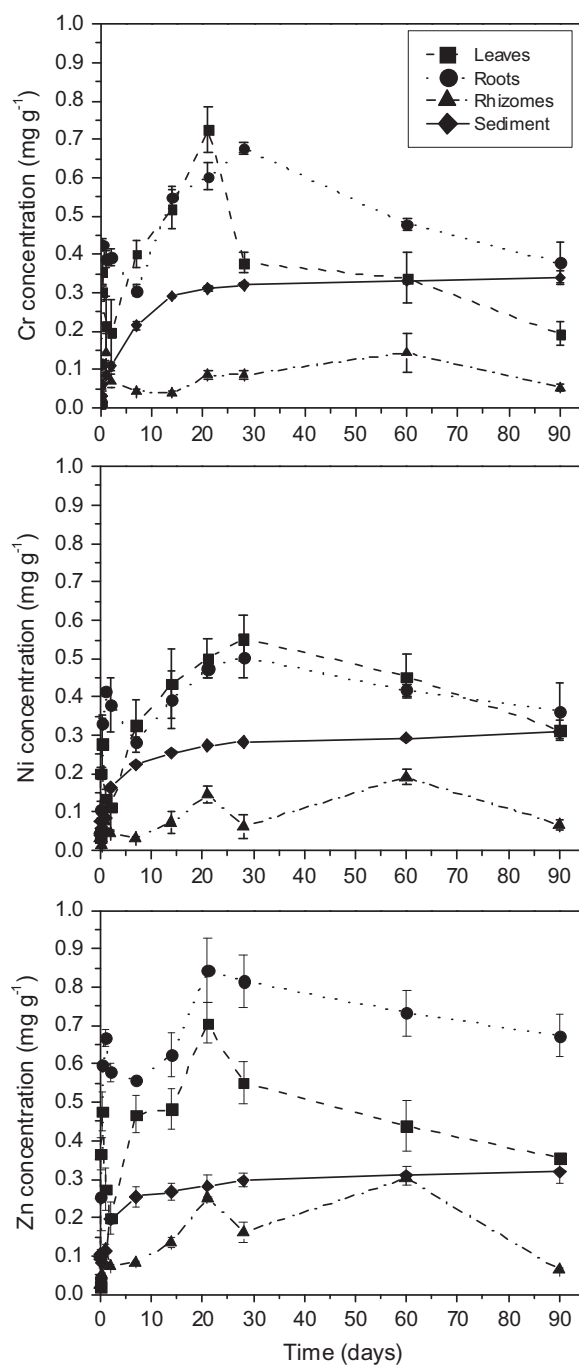


Fig. 2. Cr, Ni and Zn concentrations in plant tissues (leaves, roots and rhizomes) determined along the experiment. Bars represent standard deviations.

the end of the experiment, dead plants were not observed. Chlorosis, leaf necrosis, a lower width of leaves and plant height in comparison with plants from control reactor were observed.

At the end of the experiment, root morphology parameters were significantly lower than those obtained in the control, with the exception of CSA of metaxylematic vessels, which did not show significant differences compared with the control (Fig. 5). These results are shown in the light microscope and SEM images (Fig. 6).

4. Discussion

Although removal rates were different, the three metals were efficiently removed from water. Cr water removal rate was the

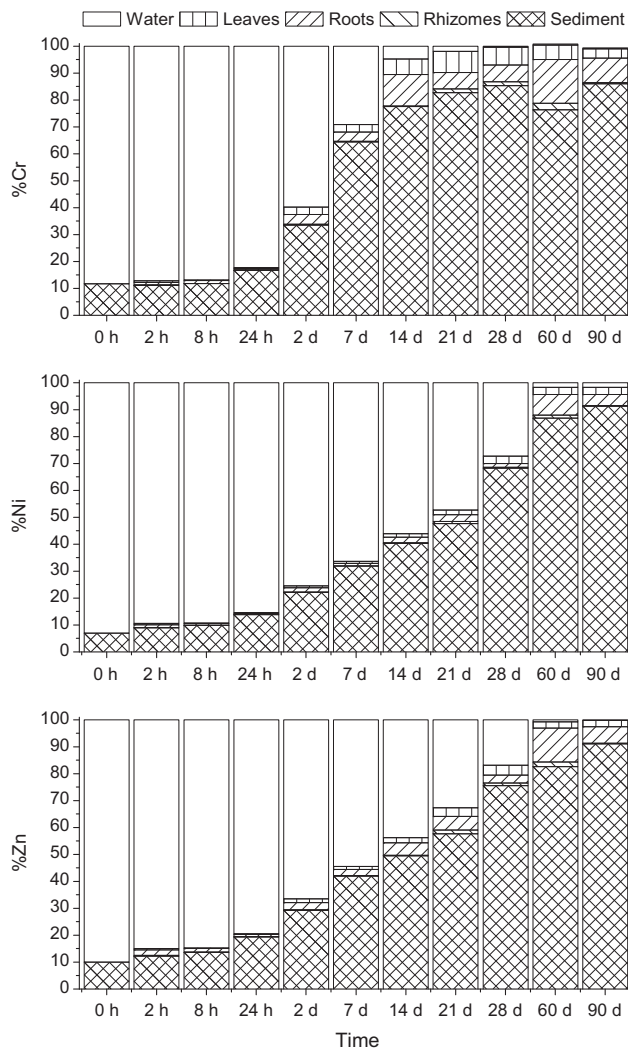


Fig. 3. Cr, Ni and Zn total amounts (expressed in percent) in water, sediment and tissues of *T. domingensis* along time.

highest, in agreement with the results reported for the floating macrophytes *Pistia stratiotes* and *Salvinia herzogii*, which showed efficiencies of 98–99 percent after 30 days of experimentation using different Cr concentrations (Maine et al., 2004; Suñé et al., 2007). These authors proposed that the removal obtained was due to the fact that the sorption of Cr(III) is probably a competitive-consecutive mechanism of reversible reaction steps. Cr(III) may precipitate as $\text{Cr}(\text{OH})_3$ (Guo et al., 1997). However, Cr(III) may also form soluble complexes with organic compounds. In turn, these complexes can also be sorbed to sediment, removing Cr(III) from the solution. Then, plant roots can uptake Cr from sediment, as it can be seen on the first days of experimentation.

The amount of metals in leaves on day 2 can be explained not only by translocation but also by sorption on the submerged parts of leaves in direct contact with water. Maine et al. (2004) reported that Cr can be sorbed by the roots or leaves of floating macrophytes in direct contact with water.

In the case of emergent macrophytes, metals can be taken up from the sediment by plant roots or by the submerged parts of leaves in direct contact with water (Mufarrege et al., 2012). Most researchers have reported that metal concentration in roots is higher than in leaves for macrophytes (Hadad et al., 2007, 2011; Kadlec and Wallace, 2009; Mufarrege et al., 2010; Nilratnisakorn et al., 2007; Vymazal, 2011). However, in our experiment,

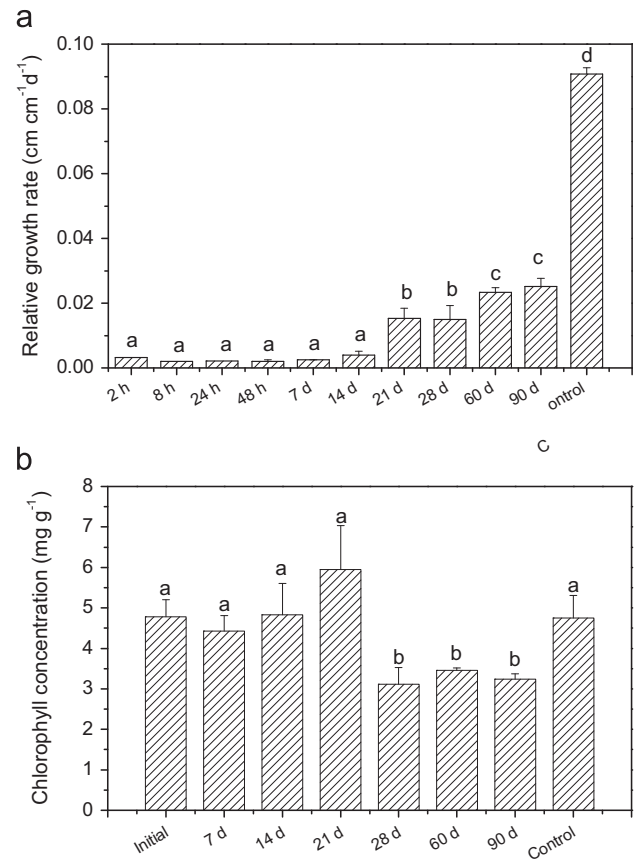


Fig. 4. Relative growth rates (a) and chlorophyll *a* concentrations (b) obtained along the experiment compared with control. Different letters represent statistically significant differences among the treatments. Bars represent standard deviations.

T. domingensis was exposed to high metal concentrations, showing remarkable high concentration in leaves in some samplings. Probably, pruning at the beginning of the experiment activated the plant physiology promoting metal translocation. Greger (1999) observed that metal translocation can occur in the phloem, via the apoplast, and via the xylem, acropetally.

Metal concentration was higher in leaves than in roots at 2 h of contact. This fact can be explained since most part of this organ was submerged in direct contact with the experimental solution. For this reason, sorption from water was the main metal uptake mechanism. After two hours of experimentation, the unexpected responses in the metal accumulation patterns in tissues of *T. domingensis* (increase and decrease metal concentrations) were probably due to the high metal concentration used in this experiment, which are not commonly observed in natural or constructed wetlands for industrial effluent treatment. It can be hypothesized according to the results that plants translocate metals alternatively as a protection mechanism of the active organs (leaves and roots). Arduini et al. (2006) observed that *Miscanthus sinensis* showed higher Cr concentrations in dead leaves in comparison with green leaves during exposure to 150 mg L^{-1} Cr, demonstrating metal mobility inside the plant. Roots took up metals from sediment during the first 60 days of the experiment. After 60 days, some roots died due to metal toxicity becoming part of the sediment. This fact explains the increase of metal amount in sediment after 90 days.

The sediment was the main compartment for contaminant accumulation. This was previously reported by Maine et al. (2013) and Panigatti and Maine (2003). These authors reported that in systems without macrophytes, sediment replaces plants in

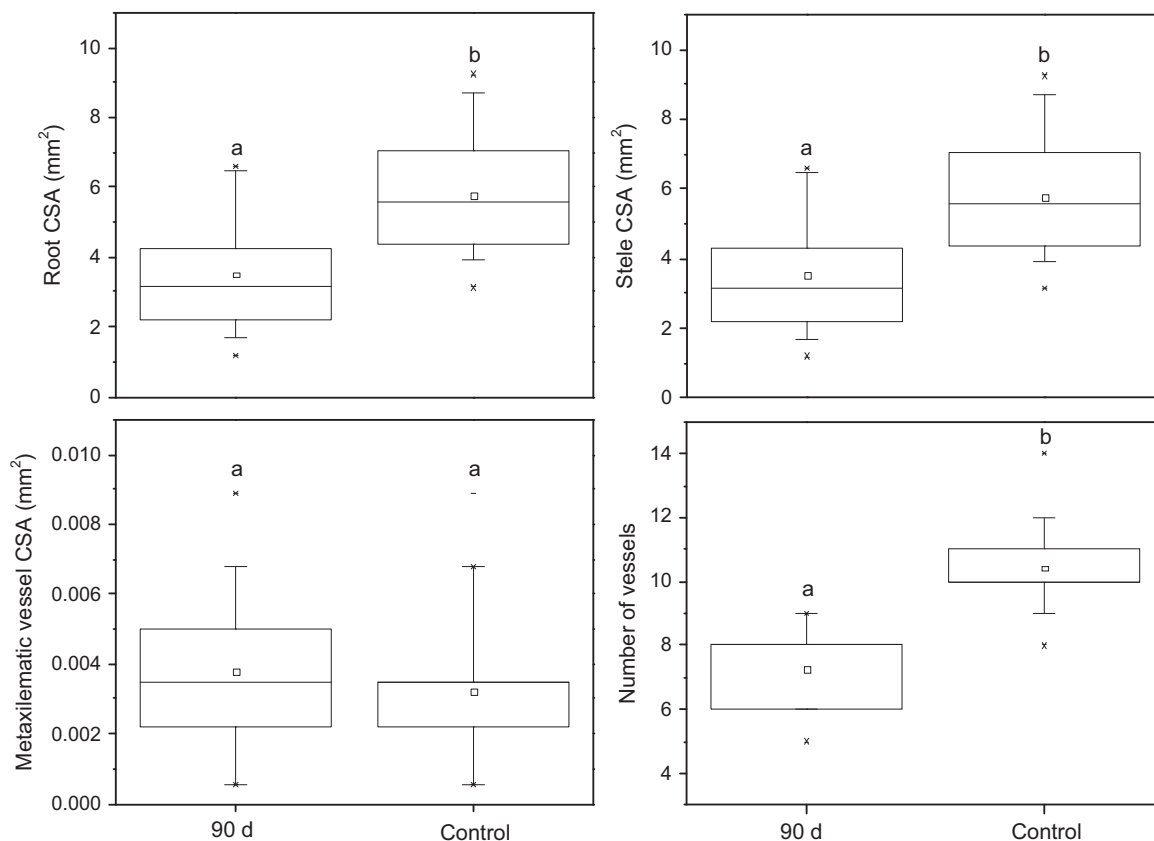


Fig. 5. Box and whisker plots of root, stele and metaxylem vessel CSA, and number of vessels of *T. domingensis* at the end of the experiment in the metal treatment and control. Different letters represent statistically significant differences.

removing contaminants, thus maintaining the system removal efficiency. Nevertheless, the advantage of macrophytes is that they are involved in the sediment biogeochemistry through the oxygen transport from aerial parts to the rhizosphere, enhancing metal accumulation in sediment (Clothier and Green, 1997; Sundby et al., 2005). The ability of a plant to generate this relatively oxidized microzone is largely dependent on the formation of aerenchyma (air-space) tissue, although rhizosphere oxidation may also occur enzymatically (Armstrong, 1967), which allows for the passive diffusion of oxygen from the atmosphere through the plant and into the soil immediately adjacent to the roots.

Cr, Ni and Zn are involved in a variety of critical functions related to gene control, oxygen transport and metabolism enzymes (Bonilla, 2008). However, metal becomes first inhibitory and then toxic when its concentration reaches a threshold value. The *T. domingensis* growth inhibition observed in this experiment was consistent with Mufarrege et al. (2010) and Hadad et al. (2007) who studied the tolerance of *S. herzogii* and *P. stratiotes* to Cr, Ni and Zn. Hadad et al. (2010, 2011) and Delgado et al. (1993) also observed that *Eichhornia crassipes* was tolerant to Cr, Ni and Zn. Since floating macrophytes are in direct contact with the experimental solution, they show lower tolerance than rooted macrophytes. The high tolerance demonstrated by *T. domingensis* was probably enhanced by the sediment. Sediment acts as a barrier and balances the system ionically enhancing the tolerance of emergent species. Chandra and Yadav (2010) evaluated the potential of *Typha angustifolia* to be used in the phytoremediation of Cu, Pb, Ni, Fe, Mn and Zn. These authors observed that this species was tolerant to the exposure to Fe, Cr, Pb, Cu and Cd at low concentrations. Arduini et al. (2006) observed that the growth of the rooted species *M. sinensis* was inhibited when exposed to an experimental solution of 150 mg L⁻¹ Cr. Metal tolerance by plants,

and heavy metal detoxification may be achieved through metal complexation with ligands such as organic acids, amino acids and certain members of the mugineic acids occurring in plant tissues, and also by compartmentalization (Hall, 2002; Carrier et al., 2003).

Metal translocation to leaves decreased chlorophyll concentration, as it can be seen after 28 days. Mangabeira et al. (2011) studied compartmentalization and ultra-structural alterations induced by 25 and 50 mg L⁻¹ Cr in aquatic macrophytes. These authors observed that the presence of 50 mg L⁻¹ Cr induced the most severe modifications, which included changes in nuclear shapes together with modifications in the shape of leaf chloroplasts, resulting in the structural disarrangement of thylakoids and stroma in comparison with the control plants. Manios et al. (2003) observed an increase in chlorophyll *a* hydrolysis in *Typha latifolia* when exposed to combined metals (40 mg L⁻¹ Ni, 80 mg L⁻¹ Zn, 4 mg L⁻¹ Cd, 80 mg L⁻¹ Cu and 40 mg L⁻¹ Pb). In our work, Zn concentrations in roots were significantly higher than those of Ni and Cr. Symptoms of Zn toxicity include growth inhibition of roots and stems (Barceló and Poschenrieder, 1990), modifications of leaf morphology, chlorosis, necrosis (Hermle et al., 2007; Todeschini et al., 2011) and impairment of photosynthesis (Cherif et al., 2010; Hermle et al., 2007).

Metal accumulation in macrophytes is frequently accompanied by cell modifications that may contribute to metal tolerance (Prasad and Freitas, 2003). In our work, *T. domingensis* decreased the root morphology parameters due to extremely high metal concentrations. However, the metaxylem vessels CSA did not decrease to enhance metal transport to leaves. A higher metaxylem vessel CSA represents a higher efficiency in the uptake and accumulation of contaminants in roots, which could increase the efficiency of a constructed wetland in the retention of contaminants. Variations in root diameter are closely associated with

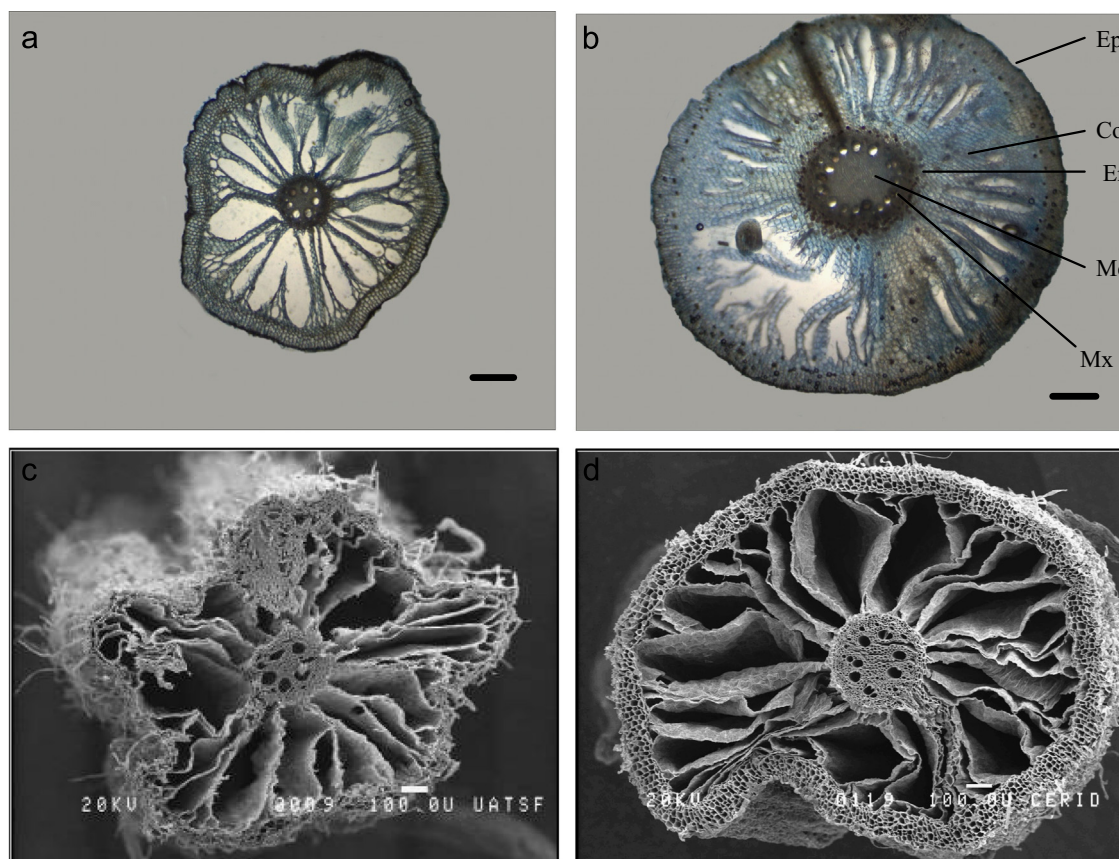


Fig. 6. *T. domingensis* light microscopy images of cross-sectional roots (a and b) and scanning electronic microscopy (SEM) images of cross-sectional roots (c and d) obtained at the end of the experiment in the metal treatment (left column) and in the control (right column) (Ep=epidermis, Co=cortex, En=endodermis, Me=stele, Mx=metaxylem vessels and Bar=350 μm).

ecological requirements of plant species and may affect the ability of plants to absorb contaminants and water. Wahl et al. (2001) demonstrated that an increase in root CSA has a positive influence on the hydraulic conductance of roots. Arduini et al. (2006) observed that the exposure to a concentration of 100 mg L⁻¹ Cr increased root length and decreased the root CSA of *M. sinensis*. Morphological plasticity of *T. domingensis* roots is an important mechanism to improve metal tolerance.

5. Conclusions

Cr removal rate was the highest while Ni showed the lowest one. Although removal rates were different, the three metals were efficiently removed from water after 60 days. Sediment showed the highest accumulation of metals. However, *T. domingensis* leaves and roots showed high metal concentrations. The unexpected responses in the metal accumulation patterns in tissues of *T. domingensis* (increase and decrease metal concentrations) were probably due to the high metal concentration used in this experiment.

Metals caused growth inhibition, a decrease in chlorophyll concentration and affected anatomical parameters. Despite the sublethal effects registered, *T. domingensis* demonstrated that it could uptake Cr, Ni and Zn efficiently and survive in polluted water bodies due to the morphological plasticity of the root system. Due to the fact that the metal concentrations studied are remarkably higher than the concentrations found in natural and constructed wetlands for the treatment of industrial effluents, our results demonstrate the ability of *T. domingensis* to survive an accidental dump of high concentrations of contaminants in an aquatic system.

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