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RESEARCH ARTICLE

The ability of *Typha domingensis* to accumulate and tolerate high concentrations of Cr, Ni, and Zn

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Abstract The tolerance and removal efficiency of *Typha domingensis* exposed to high concentrations of Cr, Ni, and Zn in single and combined treatments were studied. Sediment and two plants were disposed in each plastic reactor. The treatments were 100 and 500 mg L^{-1} of Cr, Ni, and Zn (single solutions); 100 mg L^{-1} Cr+Ni+Zn (multi-metal solutions) and 500 mg L^{-1} Cr+Ni+Zn (multi-metal solutions); and a control. Even though the concentrations studied were extremely high, simulating an accidental metal dump, the three metals were efficiently removed from water. The highest removal was registered for Cr. The presence of other metals favored Cr and did not favor Ni and Zn removal from water. After 25 days, senescence and chlorosis of plants were observed in Ni and Comb500 treatments, while Cr and Zn only caused growth inhibition. *T. domingensis* accumulated high metal concentrations in tissues. The roots showed higher metal concentration than submerged parts of leaves. Cr translocation to aerial parts was enhanced by the presence of Ni and Zn. Our results demonstrate that in the case of an accidental dump of high Cr, Ni, and Zn concentrations, a wetland system dominated by *T. domingensis* is able to retain metals, and the macrophyte is able to tolerate them the time necessary to remove them from water. Thus, the environment will be preserved since the wetland would act as a cushion.

Keywords Macrophyte · Tolerance · Metals · Effluents · Phytoremediation · Wetlands

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Introduction

Macrophytes have a remarkable ability to concentrate metals in their belowground tissues at levels that exceed those of the surrounding sediment. The exclusion of metals from aboveground tissues is a metal tolerance strategy (Taylor and Crowder 1983; Kabata-Pendias 2011; Hechmi et al. 2014). There is a direct relationship between the chemical composition of water and sediment and the morphological and physiological responses of plants (Mench et al. 2009; Hadad et al. 2010; Mufarrege et al. 2010, 2011; Preeti and Triphati 2011).

Aquatic plants in their natural habitats 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. However, studies of bioaccumulation or toxic effects on plants are usually focused on a single contaminant (Sousa et al. 2008; Mangabeira et al. 2011; Todeschini et al. 2011; Lyubenova et al. 2013). For this reason, we compared the macrophyte response when it was exposed to single and combined metal solutions.

Typha spp. is a widespread and dominant plant species 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 (Maine et al. 2009; Chandra and Yadav 2010; Vymazal 2011). The specie *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, 2013). Cr, Ni, and Zn were studied for being contaminants found in the effluents treated 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 study the response of *T. domingensis* exposed to high concentrations of:

- Cr, Ni, and Zn independently (single solutions)
- Cr+Ni+Zn (multi-metal solutions)

determining plant tolerance, metal removal efficiency, and metal accumulation in tissues.

Material and methods

Plant material and experimental design

T. domingensis 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 a uniform size and weight were selected. The plants were pruned for their transport to the greenhouse.

Plastic reactors were disposed outdoors under a semi-transparent plastic roof. Each plastic reactor of 10 L capacity contained two plants and 4 Kg of sediment. After 15 days of acclimation, the plants were pruned again to a height of approximately 20 cm, and contaminant solutions (5 L) were added to the reactors to obtain treatments with water concentrations of:

1. Cr100: 100 mg L^{-1} Cr
2. Ni100: 100 mg L^{-1} Ni
3. Zn100: 100 mg L^{-1} Zn
4. Comb100: 100 mg L^{-1} Cr+100 mg L^{-1} Ni+100 mg L^{-1} Zn
5. Cr500: 500 mg L^{-1} Cr
6. Ni500: 500 mg L^{-1} Ni
7. Zn500: 500 mg L^{-1} Zn
8. Comb500: 500 mg L^{-1} Cr+500 mg L^{-1} Ni+500 mg L^{-1} Zn
9. Control: water without the addition of metal

The solutions were prepared using water from the sampling site and $CrCl_3{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, and $ZnCl_2{\cdot}6H_2O$. Water level in the reactors was maintained by adding water from the sampling site. Temperature ranged from 21.1 to 31.3 °C during the experimental period. The experiment lasted 28 days, and it was carried out in triplicate.

Plant study

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

Chlorophyll concentration was measured at the beginning and at the end of the experiment. Relative growth rate (RGR) (cm cm^{-1} day^{-1}) was calculated in each treatment considering initial and final plant height according to:

$$\mathrm{RGR} = \frac{1nH_2 - 1nH_1}{T_2 - T_1}$$

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).

Chemical analysis

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

In each reactor, water was sampled at 0, 1, 2, 4, 7, 14, and 28 days. According to the treatments, Cr, Ni, and Zn concentrations were determined in water samples by atomic absorption spectrometry (Perkin Elmer AAnalyst 200).

At the beginning and at the end of the experiment, the contaminant concentrations in plants and sediment were determined. Plants were sampled and separated into roots, rhizomes, and submerged and aerial parts of leaves. They 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 $HClO_4/HNO_3/HCl$ (7:5:2) mixture. Sediment was sampled using a 3-cm diameter PVC corer and stored at 4 °C until analysis.

Sediment samples were digested in the same way as plant samples. Plant and sediment digests were analyzed for Cr, Ni, and Zn by atomic absorption spectrometry (Perkin Elmer, AAnalyst 200). These determinations were carried out in triplicate.

Cr, Ni, and Zn amounts (mg) were estimated by multiplying Cr, Ni, or Zn concentration in plant tissues, sediment (mg g^{-1} dry weight), or in water (mg L^{-1}) by mass (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 in order to calculate chlorophyll *a* concentration (Westlake 1974).

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine whether significant differences existed among treatments in relative growth rate and chlorophyll *a* concentrations. Two-way ANOVA (factors: treatments and plant tissues) was performed to determine whether significant differences existed in metal concentrations in water, sediment, and plant tissue and metal tissue amounts (aerial parts, submerged parts of leaves, rhizomes and roots). Duncan's test was used to differentiate means where appropriate. In all comparisons, a level of $p<0.05$ was used.

QA/QC

All glassware was pre-cleaned and washed with 2 N HNO_3 prior to each use. $CrCl_3 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, and $ZnCl_2 \cdot 6H_2O$ used to prepare metal solution 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). Detection limits were 30, 20, and 3 μg g^{-1} for Cr, Ni, and Zn, respectively, for sediment and plant tissues.

Results and discussion

The chemical composition of the water from the sampling site used in the experiment is showed in Table 1. The chemical composition of the plant tissues at the beginning of the experiment is shown in Table 2. The chemical composition of the sediment used in the experiment was organic matter (OM)=8 %; pH=7.67; Eh=280 mV (Ag/AgCl).

Studied metal concentrations in water decreased in all treatments along time (Fig. 1). Cr was the metal that showed the fastest removal from water (Fig. 1). Cr(III) may precipitate as $Cr(OH)_3$ to sediment (Guo et al. 1997). Cr(III) may also form soluble complexes with organic compounds suspended

Table 1 Physicochemical parameters measured in water from the sampling site used in the experiment (mean±standard deviation)

Parameter	
pH	6.7
Conductivity (μS cm^{-1})	210±1
Alkalinity (mg L^{-1})	103.2±1.2
Cl^- (mg L^{-1})	9.6±1.0
SO_4^{2-} (mg L^{-1})	7.2±1.0
$Ca2^+$ (mg L^{-1})	9.7±0.1
$Mg2^+$(mg L^{-1})	2.5±0.2
Na^+ (mg L^{-1})	32.1±0.5
K^+ (mg L^{-1})	12.1±0.5
Fe (mg L^{-1})	0.392±0.005
Cr (mg L^{-1})	ND (5 μg L^{-1})
Ni (mg L^{-1})	ND (5 μg L^{-1})
Zn (mg L^{-1})	ND (5 μg L^{-1})
SRP (mg L^{-1})	0.015±0.002
NO_2^- (mg L^{-1})	ND (5 μg L^{-1})
NO_3^- (mg L^-1)	0.580±0.005
NH_4^+ (mg L^{-1})	0.773±0.005
DO (mg L^{-1})	6.72±0.10

The values in parentheses correspond to the detection limits of the method

ND not detected

Table 2 Metal and TP concentrations measured in sediment and plant tissues (roots, leaves, and rhizomes) at the beginning of the experiment

Contaminants	Sediment	Root	Rhizome	Leaves
Cr (mg g^{-1})	0.015	0.008	0.005	0.004
Ni (mg g^{-1})	0.006	ND	ND	ND
Zn (mg g^{-1})	0.132	0.071	0.030	0.049
TP (mg g^{-1})	0.512	0.781	0.533	1.984

ND not detected

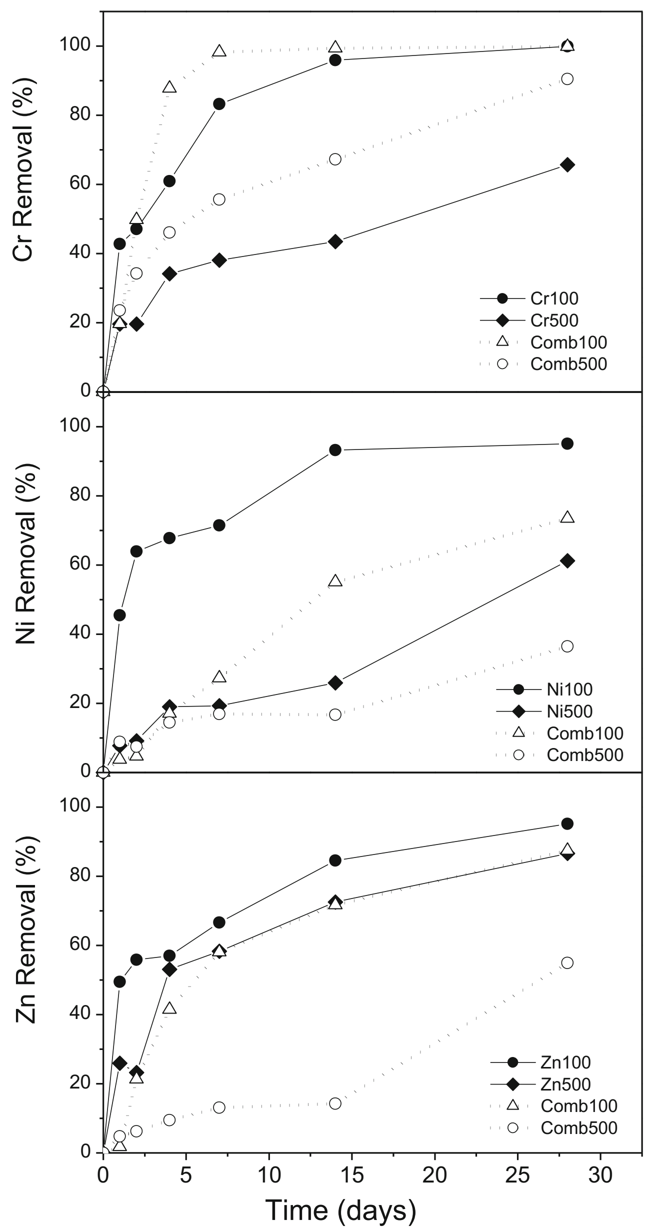

Fig. 1 Cr, Ni, and Zn removal percent from water along time

in water. In turn, these soluble complexes can flocculate and be sorbed to the sediment, removing Cr(III) from the solution. Cr removal was almost complete in Comb100 and Cr100 (99.9 % in both cases) at the end of the experiment. However, it can be seen that Cr removal percentages were significantly higher in Comb100 than in Cr100 treatments during all samplings. Cr removal in Comb500 was higher than Cr500 treatments in all samplings, being at the end of the experiment 90.5 and 65.7 %, respectively. These results indicate that the presence of Ni and Zn favored Cr removal.

For Ni and Zn, the highest contaminant removal percentages were registered in the treatment of 100 mg L^{-1} Ni or Zn throughout the experiment. For these metals, the lowest removal was observed in Comb500 treatment. The final Ni removal percentages were 95.1 and 73.5 % for Ni100 and Comb100 treatments and 61.2 and 36.5 % for Ni500 and Comb500 treatments, respectively. The final Zn removal percentages were 95.1 and 87.5 % for Zn100 and Comb100 treatments, respectively, and 86.6 and 55 % for Zn500 and Comb500 treatment, respectively. Contrarily to Cr, the presence of the other metals is unfavorable for Ni and Zn removal from water. At the end of the experiment, the pH values were Cr100=7.46, Cr500=7.71, Ni100=6.88, Ni500=7.03, Zn100=6.05, Zn500=6.19, Comb100=6.5, and Comb500=3.48. Figure 2 showed that metal concentrations in sediment were significantly lower than in plant tissues. Ni presented the lowest concentration in plant tissues, probably due to its toxicity that was also reflected in chlorophyll concentration and RGR. It is important to highlight that Cr transport to aerial parts was enhanced by the presence of Ni and Zn in all cases. In the Cr500 and Comb500 treatments, the root was the organ which showed the highest metal concentration, followed by the submerged parts of leaves. It was widely reported that metals are accumulated in root tissues as a tolerance strategy (Taylor and Crowder 1983; Stoltz and Greger 2002; Sinha and Gupta 2005; Hadad et al. 2007, 2011; Chandra and Yadav 2010; Mufarrege et al. 2010; Vymazal 2011; Hechmi et al. 2014). However, the highest Ni and Zn concentrations in tissues were found in the submerged parts of the leaves in all treatments, probably because these tissues were in direct contact with the experimental solution. The same was observed in Cr100 and Comb100 treatments. Zn presented higher concentrations in submerged parts of the leaves than the other metals, probably due to its function in plant physiology. Maine et al. (2001, 2004) and Suñé et al. (2007) reported that floating macrophytes can take up metals by roots or by leaves that are in direct contact with water. Table 3 shows the translocation factors (concentrations of metals in aerial parts of leaves/metal concentrations in roots) calculated at the end of the experiment. The study of metal accumulation in different tissues of leaves of emergent macrophytes was not found in the literature.

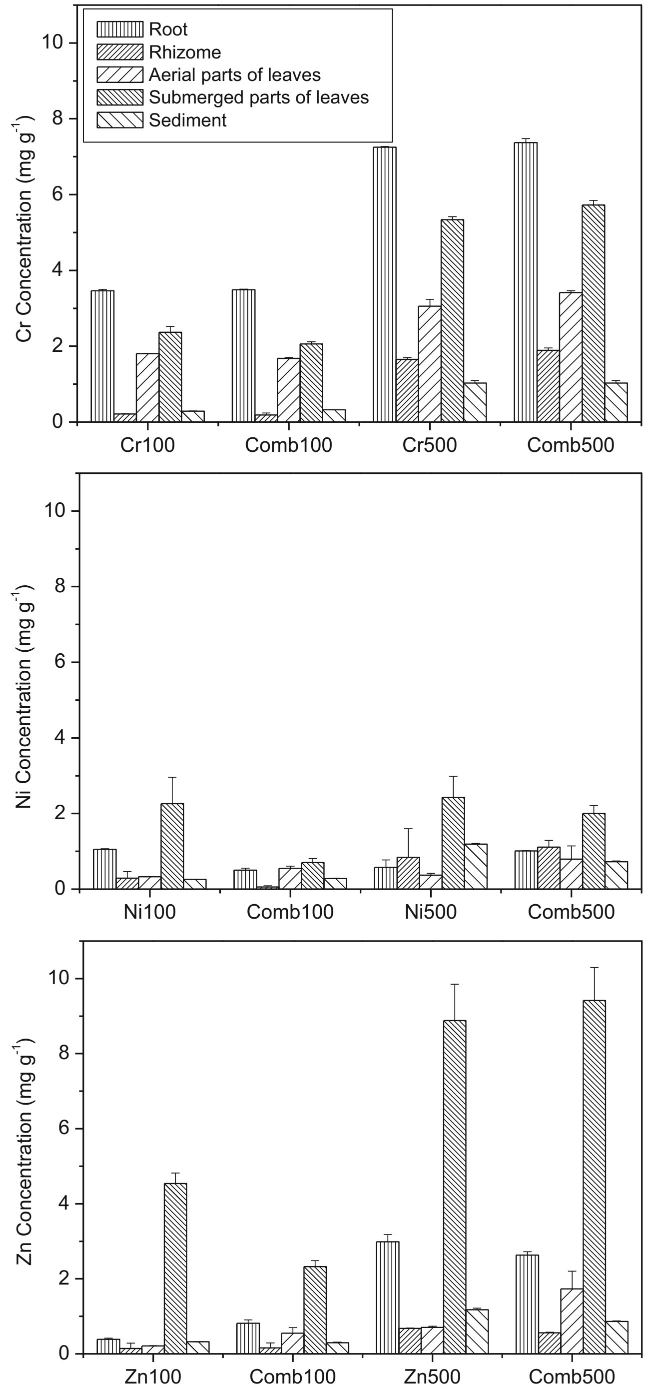

Fig. 2 Final concentrations of Cr, Ni, and Zn (mg g^{-1}) in roots, rhizomes, aerial parts of leaves, submerged parts of leaves of *T. domingensis*, and sediment. *Bars* represent standard deviations

To estimate the extent of metals accumulated in each compartment (sediment and different plant tissues), Cr, Ni, and Zn amounts (mg) were estimated. Despite their high concentrations, plant tissues were not an efficient compartment for metal accumulation in comparison with sediment (Fig. 3). Previous works showed that the sediment is the main accumulation compartment of metals

Table 3 Translocation factors (concentrations of metals in aerial parts of leaves/metal concentration in roots) calculated at the end of the experiment

Treatment	Translocation factor
Cr100	0.521
Cr500	0.421
Cr (Comb100)	0.481
Cr (Comb500)	0.436
Ni100	0.309
Ni500	0.648
Ni (Comb100)	0.985
Ni (Comb500)	0.784
Zn100	0.546
Zn500	0.235
Zn (Comb100)	0.675
Zn (Comb500)	0.658

(Maine et al. 2009). Nevertheless, the advantage of macrophytes is the possibility of being harvested, which leads to important removal rates of contaminants in short periods of time. Besides, macrophytes are involved in the sediment biogeochemistry through oxygen transport from the aerial parts to the rhizosphere, enhancing metal accumulation in sediment. Cr presented the highest removal from water and the highest accumulation in sediment in Cr100 and Comb100 treatments (Fig. 3). The metal treatments of 500 mg L^{-1}, added individually and combined, showed the lowest metal removal from water. In agreement with the results mentioned above, Cr sorption to sediment and plant tissues was favored by the presence of the other metals, while the opposite was observed in Ni and Zn.

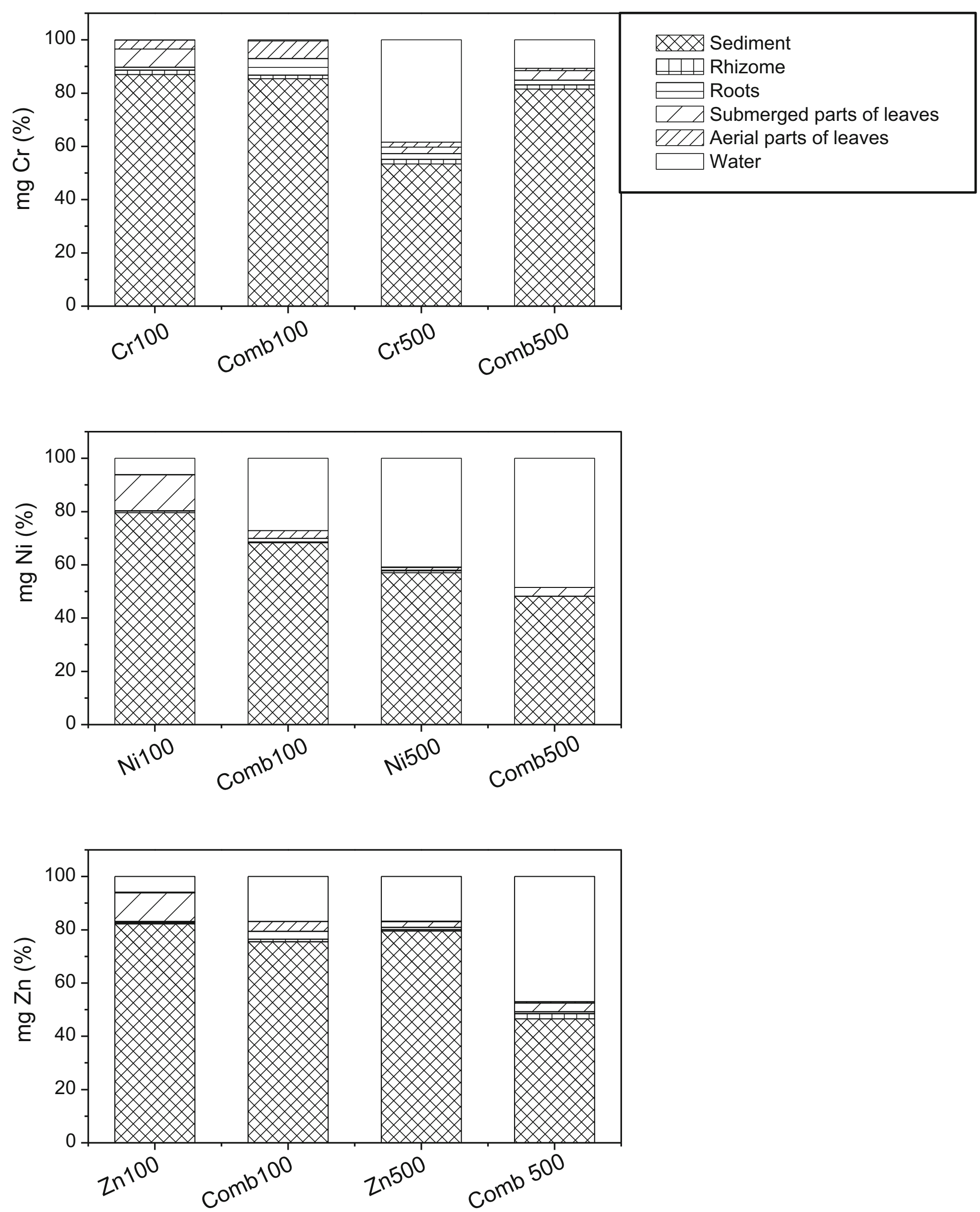

Fig. 3 Cr, Ni, and Zn total amounts (expressed in %) in water, sediment, and tissues of *T. domingensis* along time

The relative growth rate was positive for the treatments of Cr, Zn, and Comb100, being significantly lower than that obtained in the control (Fig. 4a). Ni treatments and Comb500 treatment showed negative relative growth rates. After 25 days, the external appearance of plants evidenced injury symptoms and chlorosis in Ni and Comb500 treatments. For this reason, the experiment ended on day 28. Ni100 treatment showed a negative relative growth rate, but it was significantly higher than that obtained in Ni500 and Comb500 treatments, demonstrating the Ni and metal combination toxicity in comparison with the other treatments. Cr, Ni, and Zn are involved in a variety of critical functions related to gene control, oxygen transport, and metabolism enzymes (Bonilla 2008). However, when metal concentrations reach a threshold value, they first become inhibitory and then toxic. Compared to floating macrophytes, these results are consistent with those reported by Hadad et al. (2007) and Mufarrege et al. (2010) who reported that *Salvinia herzogii* and *Pistia stratiotes* were more tolerant to Cr and Zn than Ni. Delgado et al. (1993) observed that *Eichhornia crassipes* also tolerated Cr and Zn. However, it is important to highlight that these floating macrophytes were exposed to significantly lower metal concentrations than those used in this work, being in the order of 1–10 mg L^{-1}. Since floating macrophytes are in direct contact with the experimental solution, they show lower tolerance than rooted macrophytes. Sediment acts as a barrier and balances the system ionically, enhancing the tolerance of emergent species.

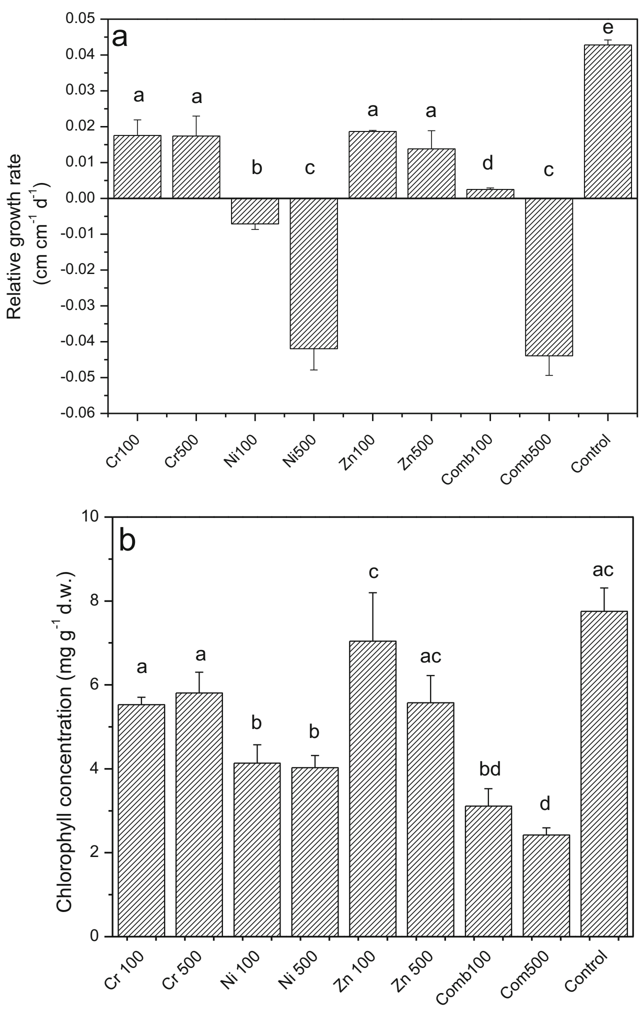

Fig. 4 **a** Relative growth rates and **b** final chlorophyll *a* concentrations (mg g^{-1} d.w.). *Different letters* represent statistically significant differences among the treatments. *Bars* represent standard deviations

No significant differences were observed in chlorophyll concentrations among Cr and Zn treatments and control (Fig. 4b). Despite the high metal concentrations, Cr and Zn did not produce toxicity on this parameter. Cr increases the availability of Fe biologically active in plant tissues (Bonet et al. 1991) and Zn is involved in the photosynthetic processes (Bonilla 2008). Ni and combined metal treatments showed a significant lower chlorophyll concentration than that obtained in the control. This is in agreement with a lower Ni concentration in tissues than the other studied metals. Manios et al. (2003) suggested an increase in chlorophyll *a* hydrolysis in *Typha latifolia* when it was exposed to the combined metals (4 mg L^{-1} Cd, 80 mg L^{-1} Cu, 40 mg L^{-1} Ni, 40 mg L^{-1} Pb, and 80 mg L^{-1} Zn). Regarding studies of floating species, Hadad et al. (2011) observed a toxic effect on chlorophyll concentration by exposing *E. crassipes* at a Ni concentration of 1 mg L^{-1}.

Despite its growth inhibition, *T. domingensis* was tolerant to Cr and Zn. Chandra and Yadav (2010) evaluated the potential of *Typha angustifolia* to be used in phytoremediation of heavy metals from aqueous solution containing 296.32 mg L^{-1} Fe, 2.43 mg L^{-1} Cr, 33.92 mg L^{-1} Pb, 48.0 mg L^{-1} Cu, and 8.0 mg L^{-1} Cd, reporting that this species was tolerant. Arduini et al. (2006) observed that the growth of the terrestrial species *Miscanthus sinensis* was inhibited when it was exposed to an experimental solution of 150 mg L^{-1} Cr.

Phytotoxicity results in chlorosis, leaf necrosis, weak plant growth, yield depression, and may be accompanied by disorders in plant metabolism such as reduction of the meristematic zone (Maleci et al. 2001), plasmolysis, and reduced chlorophyll and carotenoids production (Corradi et al. 1993). *T. domingensis* was affected by Ni and metal combination after 25 days. Notwithstanding, it was efficient in metal removal from water and tissue accumulation in all treatments.

Conclusions

The highest removal was registered for Cr. The presence of other studied metals favored Cr and did not favor Ni and Zn removal from water. Metals were efficiently accumulated in roots and submerged parts of leaves that were in direct contact with the experimental solution. Cr transport to aerial parts was enhanced by the presence of Ni and Zn. Cr and Zn caused

T. domingensis growth inhibition while Ni and metal combination produced plant senescence after 25 days.

Our results demonstrate that in the case of an accidental dump of high Cr, Ni, and Zn concentrations, *T. domingensis* is able to tolerate them the necessary time for their efficient removal by the wetland system. Thus, the environment will be preserved since the wetland would act as a cushion.

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