Response of *Pistia stratiotes* to Heavy Metals (Cr, Ni, and Zn) and Phosphorous

M. M. Mufarrege · H. R. Hadad · M. A. Maine

Abstract The effects of Cr, Ni, Zn, and P exposure on the root anatomic structure, growth, and chlorophyll *a* concentration of *Pistia stratiotes* L. were studied. Plastic aquaria containing 50 g of wet plants and 5 L of pond water added with the contaminant(s) were disposed. The treatments were: (1) Cr, (2) Ni, (3) Zn, (4) P, (5) Cr + Ni + Zn, (6) Cr + Ni + Zn + P, and (7) control. Contaminant additions were done seven times. In each addition, concentrations of 1 mg of metals or 5 mg of P per liter of water were added. Chlorophyll *a* was an indicator more sensitive to Zn and Cr toxicity than the relative growth rate. Ni and Cr + Ni + Zn treatments were the most toxic ones, in which biomass and the root anatomical parameters (root length, cross-sectional areas [CSAs] of root, stele, and metaxylem vessels) decreased significantly. The addition of P to the treatment with combined metals attenuated the decrease in plant growth and root length, and caused a significant increase in CSAs of total metaxylem vessels, suggesting that P increased the tolerance of *P. stratiotes* to metals. This fact has important implications for the use of this macrophyte in constructed wetlands for industrial wastewater treatment.

M. M. Mufarrege (✉) · H. R. Hadad · M. A. Maine
Química Analítica, Facultad de Ingeniería Química,
Universidad Nacional del Litoral, Santiago del Estero
2829, Santa Fe (3000), Argentina
e-mail: mmufarrege@fiq.unl.edu.ar

M. M. Mufarrege · H. R. Hadad · M. A. Maine
Consejo Nacional de Investigaciones Científicas y Técnicas
(CONICET), Buenos Aires, Argentina

Floating macrophytes such as *Eichhornia crassipes* (Mart.) Solms. (water hyacinths), *Pistia stratiotes* L. (water lettuce), and *Salvinia* sp. (water fern) have been widely studied because of their capability of absorption of contaminants in water and their subsequent use in wetlands constructed for wastewater treatment. In consequence, most studies were aimed at assessing their removal efficiencies (Gersberg et al. 1986; Delgado et al. 1993; Maine et al. 2001, 2004, 2006; Miretzky et al. 2004; Paris et al. 2005). However, studies of contaminant toxic effects would allow us to determine their tolerance and provide basic information related to the potential use of locally available macrophytes in water depuration (Cardwell et al. 2002). In contrast to terrestrial plants, macrophytes have rarely been studied with special regard to specific features of their anatomical structure in polluted water bodies. The toxic effects of contaminants on aquatic vegetation growing in constructed wetlands and wetland microcosms is usually estimated from changes in some population, biological and physiological parameters of plants, such as biomass increment (Ellis et al. 1994; Sen and Bhattacharyya 1994; Maine et al. 2007), chlorophyll concentration (Satyakala and Kaiser 1997; Manios et al. 2003), and content of soluble sugars (Steinbachová-Vojtísková et al. 2006). However, the anatomic structure of roots also can be affected by different concentrations of P (Ciro et al. 1999; Wahl et al. 2001; López-Bucio et al. 2003; Campanella et al. 2005) as well as heavy metals (Kapitonova 2002; Hadad et al. 2008). Variations in root structure and root diameter are closely associated with ecological requirements of plant species, such as water and nutrient absorption, and may affect the ability of plants to absorb contaminants from water. Plants in their natural habitats, aquatic or terrestrial, usually are exposed to low concentrations of both heavy metals and P. The conditions for

plants growing in wetlands constructed for industrial wastewater treatment are completely different: both heavy metal and P concentrations tend to be high. *P. stratiotes* is usually used in constructed wetlands due to its high efficiency in the absorption of phosphorous and metals (Aoi and Hayashi 1996; Hadad and Maine 2001; Maine et al. 2001, 2004; Miretzky et al. 2004; Hadad et al. 2006).

Bahco Argentina S.A. constructed a wetland to treat wastewater at its tool factory in Santo Tomé, Santa Fe (Argentina). The effluent had a high pH and conductivity and contained Cr, Ni, and Zn (Maine et al. 2007). The wetland has been in operation for 6 years. Assemblages of locally common macrophytes were transplanted into the pond system. Floating macrophytes (*P. stratiotes*, *E. crassipes*, etc.) covered most of the surface for almost 1 year, followed by a receding stage that took another 6 months. Since then, only *Typha domingensis* Pers. (Cattail) attained dense stands. The wetland was very efficient in metal retention; metal accumulation was faster in floating than emergent macrophytes, suggesting that metal exposure contributed to the earlier disappearance of the former (Maine et al. 2009).

The aims of this study were: (1) to determine the toxic effects of Cr, Ni, and Zn in single and combined treatments on *P. stratiotes*, (2) to determine the effect of high P concentrations on *P. stratiotes*, and (3) to evaluate the effect of P on *P. stratiotes* exposed to Cr + Ni + Zn treatment. Cr, Ni, and Zn were studied for being contaminants found in the effluents treated at this constructed wetland. Effects were evaluated studying anatomic structure of roots, growth, and chlorophyll *a* concentration. The effect of P on *P. stratiotes* exposed to Cr + Ni + Zn was evaluated, because we hypothesized that P enrichment enhances the metal tolerance of floating macrophytes and would therefore enable the development of floating vegetation in constructed wetlands at metal concentrations which would otherwise inhibit plant growth. This study would allow us to determine the tolerance of *P. stratiotes* and provide basic knowledge to evaluate vegetation management in the wetland.

Methods

Experimental Design

Water and plants were collected from a pond located within an ecological reserve near Santa Fe city, Argentina (31°38′17.08″S; 60°39′53.64″W). Table 1 reports the physicochemical parameters measured in the pond water.

The collected plants were healthy. After collection, they were washed and acclimatized in a greenhouse. For experimental purposes, plants of a uniform size (number of

Table 1 Physicochemical parameters measured in water from the natural environment where water and plant sampling was done

Parameter	
pH	7.40 ± 0.01
Conductivity (μmhos cm^{-1})	900 ± 2
Turbidity (NTU)	9.0 ± 1
Color	30 ± 5
Dissolved oxygen (mg L^{-1})	7.6 ± 0.2
Total solids (mg L^{-1})	598 ± 1
CO$_3^{2-}$ (mg L^{-1})	ND (<2)
HCO$_3^-$ (mg L^{-1})	409.4 ± 0.2
Alkalinity (mg L^{-1} CaCO$_3$)	335.6 ± 0.2
Total hardness (mg L^{-1} CaCO$_3$)	232.8 ± 0.3
Ca (mg L^{-1})	43.2 ± 0.2
Mg (mg L^{-1})	30.3 ± 0.2
Na (mg L^{-1})	119.3 ± 0.3
K (mg L^{-1})	23.4 ± 0.2
Cl$^-$ (mg L^{-1})	46.9 ± 0.3
SO$_4^{2-}$ (mg L^{-1})	119.8 ± 0.5
SRP (mg L^{-1} P)	0.057 ± 0.004
NO$_3^-$ (mg L^{-1})	4.12 ± 0.15
NO$_2^-$ (mg L^{-1})	ND (LD = 0.005)
NH$_4^-$ (mg L^{-1})	0.580 ± 0.010
Fe (mg L^{-1})	0.193 ± 0.009
Cr (mg L^{-1})	ND (LD = 0.0003)
Ni (mg L^{-1})	ND (LD = 0.001)
Zn (mg L^{-1})	ND (LD = 0.025)

Note: *ND* not detected. Values in parentheses are the detection limits of the method

leaves per plant = 11 ± 3; root length = 12.5 ± 2.5 cm) and weight (12.2 ± 2.4 g fresh weight) were selected.

Plastic aquaria containing 50 g of wet plants and 5 L of pond water added with the contaminant(s) to be studied were set up. Contaminants were added as follows.

Treatment 1: Cr
Treatment 2: Ni
Treatment 3: Zn
Treatment 4: P
Treatment 5: Cr + Ni + Zn
Treatment 6: Cr + Ni + Zn + P
Treatment 7: control (without additions)

Contaminant additions were done seven times (daily for the first 5 days and subsequently on days 9 and 13) to avoid plant senescence due to stress caused by a high initial concentration of contaminants. In each addition, 1 mg of metal or 5 mg of P per liter of water was added in each aquarium of treatments 1 to 4. The amount of contaminant added after the seven additions resulted in a nominal concentration of 7 mg L^{-1} of each metal or 35 mg L^{-1} of

P. In treatments 5 and 6, 0.33 mg of each metal by L of water was added in each addition, resulting, after the seven additions, in a nominal concentration of 2.33 mg L^{-1} of each metal. In treatment 6, 5 mg of P by L of water was also added in each addition, resulting, after the seven additions, in a nominal concentration of 35 mg L^{-1} of P. Metals were added as solutions of $CrCl_3 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2Om$ and $ZnCl_2 \cdot 6H_2O$; and P as KH_2PO_4. Aquaria were set up in triplicate.

The experiment lasted 30 days and was carried out in spring (November), in a greenhouse receiving natural light, at an ambient temperature which ranged from 24 to 28°C. Plants were collected after the exposure period. Chlorophyll *a*, metal, and total phosphorus (TP) concentrations were determined in plant tissues at the beginning and the end of the experiment.

Plant Study

At the end of the experiment, sections approximately 30 mm long were cut from the middle of the root and stored in 4% formaldehyde. After 48 h, root sections were immersed in 70% ethanol 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). To distinguish cell walls from the background, the material was stained with aniline blue, which stains cellulose blue. Sections were examined by light microscopy ($\times$ 100 and $\times$ 400). Thirty sections of roots of plants from each treatment (10 per aquarium) 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 the CSAs of the whole root, stele, and metaxylem vessels (Wahl et al. 2001). Also, the number of metaxylem vessels per section was recorded and the total metaxylem CSA was calculated by adding the areas of all the vessels per section.

External morphology of roots was described by measuring root length. To measure dry weight, plants were dried at 105°C until a constant weight was reached (Westlake 1974; APHA 1998). The relative growth rate was calculated according to Hunt's equation (1978):

$$R = \ln(W_2 - \ln W_1)/(T_2 - T_1)$$

where R is the relative growth rate (g g^{-1} day^{-1}), W_1 and W_2 are the initial and final dry weight, respectively, and $(T_2 - T_1)$ is the experiment time.

Chemical Analysis

A physicochemical characterization of the water from the pond where the plants were collected was done according to the APHA (1998). The plants were washed with tap and distilled water, followed by thorough rinsing, and then oven-dried at 60°C for 48 h. Dried plant samples (leaves and roots) were ground, sieved, and digested with a $HClO_4$:HNO_3:HCl (7:5:2) mixture. In the digests, TP was measured as SRP, following Murphy and Riley (1962) (Spectrophotometer UV-VIS Perkin Elmer Lambda 20), and Cr, Ni, and Zn concentrations were determined by atomic absorption spectrometry (Perkin Elmer 5000) (APHA 1998). Certified standard solutions were used. The detection limits were 0.3, 0.6, and 9 µg g^{-1} for Cr, Ni, and Zn, respectively. These determinations were carried out in triplicate. The recovery percentage ranged from 85 to 105 for all metals and tissues.

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 UV-Vis spectrophotometer 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 in chlorophyll *a* concentration, root length, and relative growth rates among the different treatments. Two-way ANOVA (factors: treatments and vegetative organs) was performed to determine whether significant differences existed in TP and metal concentrations in plant tissues. The normality of residuals had been previously tested graphically, and the homocedasticity of variances was checked applying Bartlett's test. Duncan tests were used to differentiate means where appropriate. Since the root anatomic structure parameters (CSA of root, stele, metaxylem vessels and total metaxylem, and number of vessels) did not show a normal distribution, nonparametric tests and box and whisker plots were performed. Kruskal–Wallis analysis was applied to check the differences between the anatomic parameters measured in roots among the different treatments. Wilcoxon's test was used to differentiate medians where appropriate (Walpole and Myers 1992). In all comparisons Bonferroni correction was applied ($p < 0.002$).

Results

The initial metal concentrations in roots of the plants used in the experiment were 0.9, 10.1, and 25.9 µg g^{-1} for Cr, Ni, and Zn, respectively, while concentrations in leaves were 0.4, 1.9, and 10.3 µg g^{-1} for Cr, Ni, and Zn, respectively. In the control, metal concentrations in plant tissues did not present significant differences between the beginning and the end of the experiment ($p > 0.002$). By

the end of the experiment, Cr, Ni, and Zn root concentrations were significantly higher than concentrations in leaves (Fig. 1a–c, respectively) ($p < 0.002$). In Cr + Ni + Zn and Cr + Ni + Zn + P treatments, metal concentrations in roots were significantly lower than those recorded in the treatments with the addition of each metal separately ($p < 0.002$). As expected, the TP concentration in leaves and in roots was significantly higher in the P treatment than in the control ($p < 0.002$). There was a significantly higher concentration in roots than in leaves in the P treatment (Fig. 1d); however the opposite occurred in the control ($p < 0.002$).

At 30 days the chlorophyll *a* concentration was significantly lower in the Zn treatment than in the other treatments ($p < 0.002$) (Fig. 2), followed by the Cr and Ni treatments, which showed a significantly lower chlorophyll *a* concentration than in the Cr + Ni + Zn + P and control treatments. The pigment concentration in Cr + Ni + Zn was significantly lower than in the Cr + Ni + Zn + P treatment but significantly higher than in the Zn and Cr treatments.

Relative growth rates were negative in Ni, Cr + Ni + Zn, and Cr + Ni + Zn + P treatments. However, Cr + Ni + Zn treatments showed a relative growth rate significantly lower than in the Cr + Ni + Zn + P
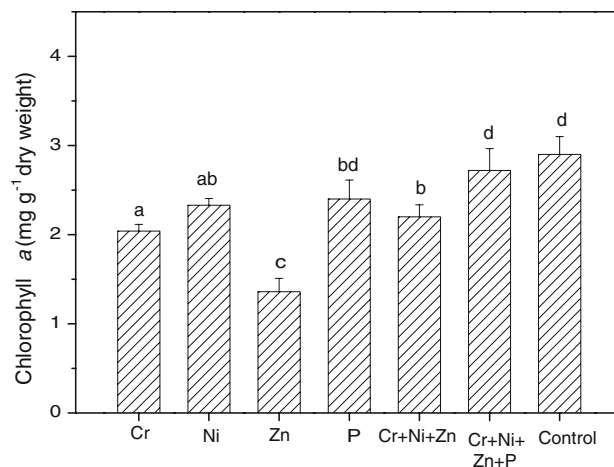

Fig. 2 Chlorophyll *a* concentrations of *P. stratiotes* obtained in each treatment at 30 days. Different letters represent statistically significant differences among treatments, according to ANOVA ($F = 44.82$, $p = 0.00001$; $n = 6$). Error bars represent standard deviation

treatment ($p < 0.002$) (Fig. 3a). Relative growth rate corresponding to Cr, Zn, and P treatments did not show statistically significant differences compared to the control. Ni and Cr + Ni + Zn treatments presented a root length significantly shorter than the control ($p < 0.002$) (Fig. 3b). Root length in Cr + Ni + Zn + P treatment was not
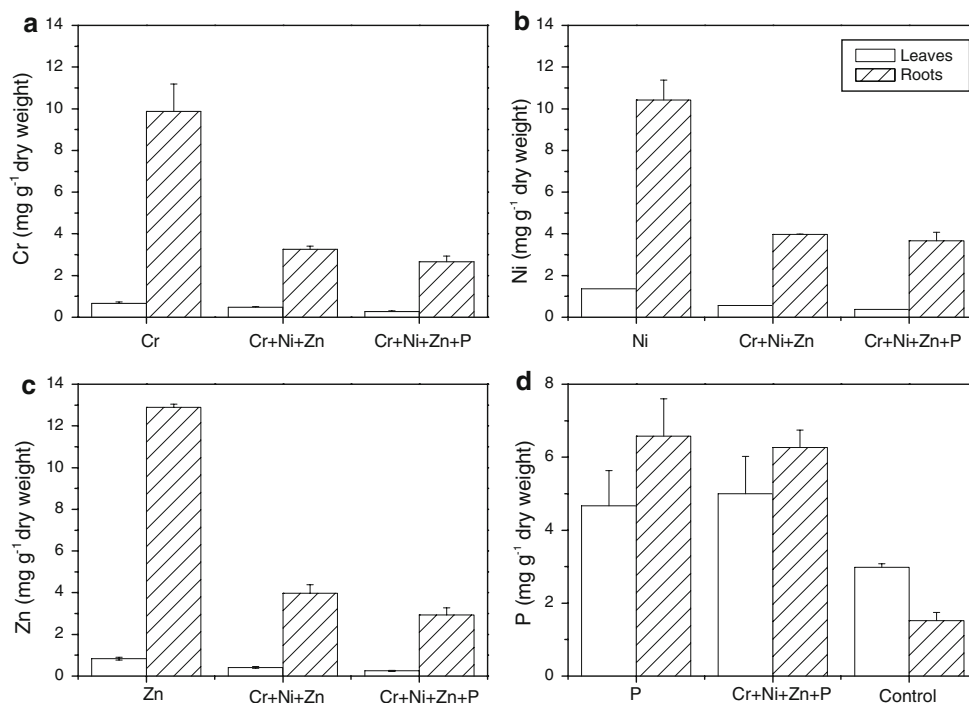

Fig. 1 Concentrations of Cr (**a**), Ni (**b**), Zn (**c**), and total P (**d**) in leaves and roots of *P. stratiotes*, obtained at 30 days, in the different treatments. Error bars represent standard deviation. Data were analyzed by two-factor ANOVA (Cr: $F_{organ} = 221.47$, $p = 0.00001$, $F_{treatment} = 56.06$, $p = 0.0001$, $F_{interaction} = 46.97$, $p = 0.0002$; Ni: $F_{organ} = 405.66$, $p = 0.00001$, $F_{treatment} = 91.77$, $p = 0.00001$,

$F_{interaction} = 52.86$, $p = 0.0002$; Zn: $F_{organ} = 2097.75$, $p = 0.00001$, $F_{treatment} = 626.23$, $p = 0.00001$, $F_{interaction} = 503.03$, $p = 0.00001$; P: $F_{organ} = 15.50$, $p = 0.0018$, $F_{treatment} = 27.77$, $p = 0.0009$, $F_{interaction} = 5.85$, $p = 0.039$; $n = 6$). When interactions were significant, Duncan test was applied to compare means at each level of the factor
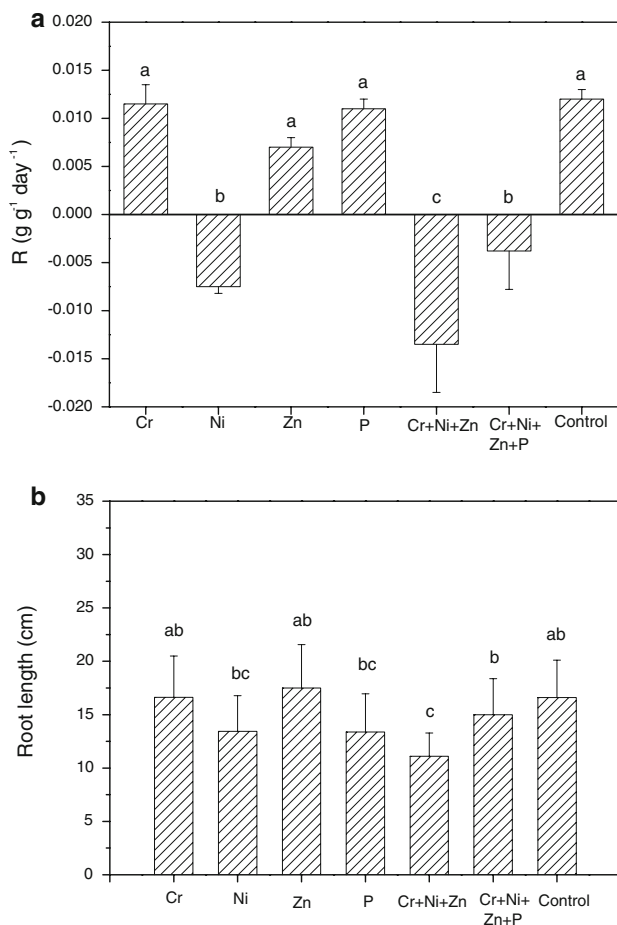
Fig. 3 Relative growth rate (R; **a**) and root length (**b**) of *P. stratiotes* obtained at 30 days in each treatment. Different letters represent statistically significant differences among treatments, according to ANOVA ($F = 25.38$, $p = 0.0001$, $n = 3$, for relative growth rate and $F = 4.06$, $p = 0.0011$, $n = 10$, for root length). Error bars represent standard deviation

Fig. 4 Box-and-whisker plots of cross-sectional areas (CSAs) of root (**a**), stele (**b**), and metaxylematic vessels (**c**) of *P. stratiotes* obtained in the different treatments at 30 days. Different letters represent statistically significant differences among treatments, according to Wilcoxon test ($p < 0.002$; $n = 30$)

significantly different from the control but was significantly higher than in the Cr + Ni + Zn treatment.

Zn and Cr + Ni + Zn + P treatments showed the highest root and stele CSAs, whereas the lowest were found in Ni and Cr + Ni + Zn treatments (Fig. 4a and b, respectively) ($p < 0.002$). Cr + Ni + Zn + P treatment showed the highest metaxylem vessel CSA, whereas Ni and Cr + Ni + Zn treatments showed a metaxylem vessel CSA significantly lower than that in the other treatments (Fig. 4c) ($p < 0.002$). Zn and P showed a significantly higher number of vessels than in the other treatments (Fig. 5a) ($p < 0.002$). Ni treatment showed a total metaxylem vessel CSA significantly lower than that in the other treatments (Fig. 5b) ($p < 0.002$), but significant differences were not found between Cr, Zn, P, and Cr + Ni + Zn treatments and controls. Figure 6 shows images of light microscopy of the root cross sections of *P. stratiotes* subjected to the different treatments.
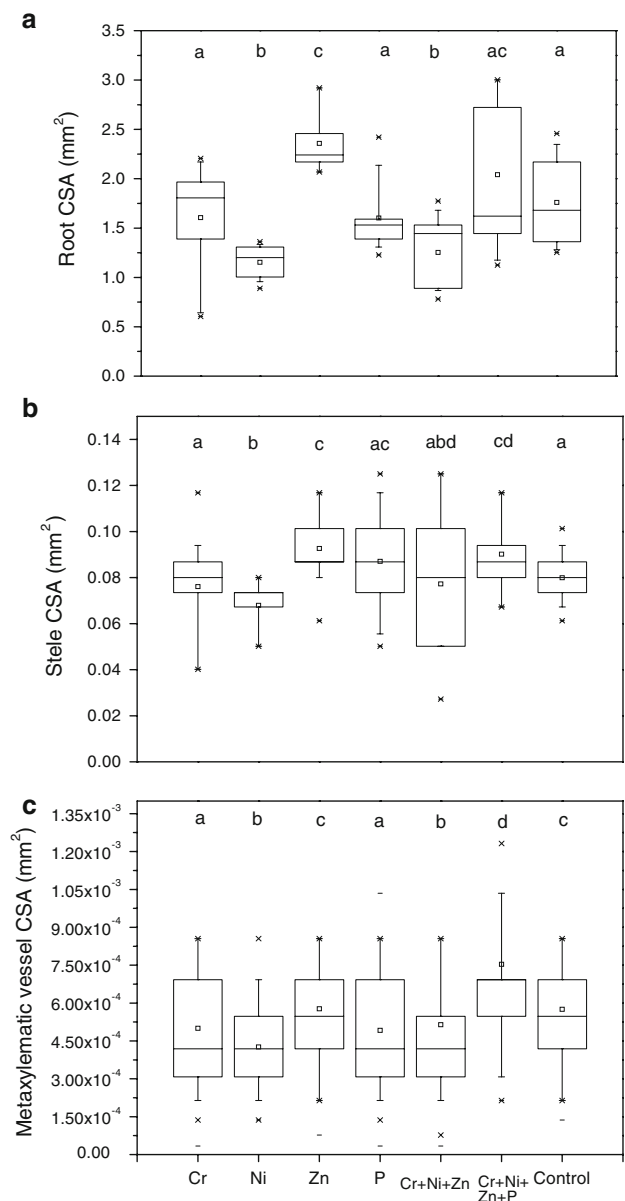
Discussion

Metal concentration was significantly higher in roots than in leaves, in agreement with the results obtained by other authors who studied free-floating macrophytes (Sen et al. 1987; Sen and Bhattacharyya 1994; Banerjee and Sarker 1997; Satyakala and Kaiser 1997; Paris et al. 2005; Hadad et al. 2006). Maine et al. (2001, 2004) and Suñé et al. (2007) reported that metals are poorly traslocated to the aerial parts. As expected, metal concentrations in plant tisuues of
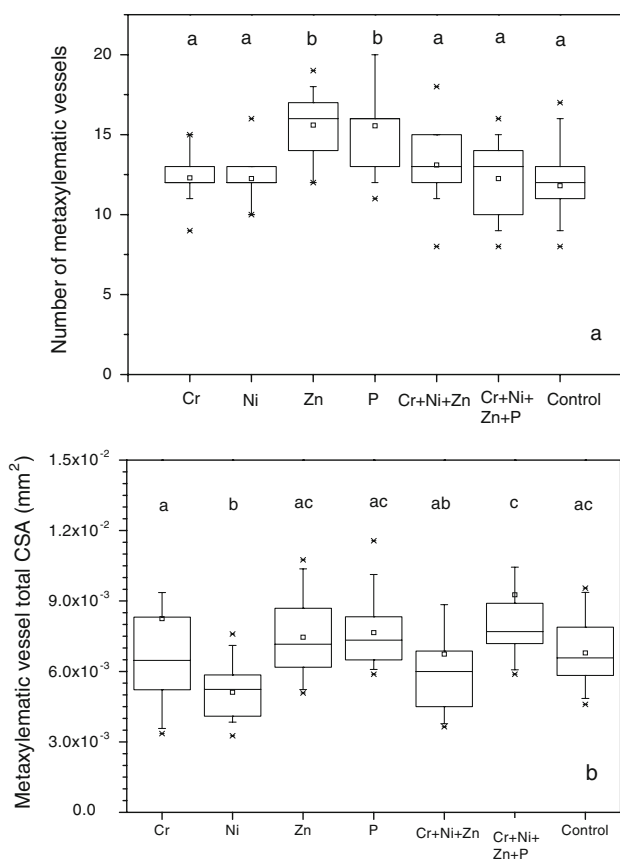
Fig. 5 Box-and-whisker plots of the number of metaxylematic vessels (**a**) and total cross-sectional area (CSA) of metaxylematic vessels (**b**) of *P. stratiotes* obtained in the different treatments at 30 days. Different letters represent statistically significant differences among treatments ($p < 0.002$; $n = 30$)

Cr + Ni + Zn and Cr + Ni + Zn + P treatments were significantly lower than in the single-metal treatments, because the concentration of such metals in the treatment where they were combined was a third of the concentration applied in the treatments where they were added individually. The aforementioned would indicate a dose-dependent accumulation of metals. Maine et al. (2004) reported that Cr concentrations in roots and aerial parts of *P. stratiotes* and *S. herzogii* showed significant linear relationships with the amount of Cr added. A similar type of linear increase in the uptake of Cu and Cd was reported previously in water hyacinth (O'Keefe et al. 1984), in *Azolla filiculoides* (Sela et al. 1989), and in *P. stratiotes* (Satyakala and Kaiser 1997; Maine et al. 2001).

In the treatments in which P was added, there was a higher TP concentration in roots than in leaves, whereas in the control, higher TP concentrations were recorded for leaves in comparison with roots. On the contrary, Hadad and Maine (2001) recorded higher TP concentrations in leaves than in roots of *P. stratiotes* and *S. herzogii* when

working with additions of P of lower concentrations. This might happen because plants, faced with a scarcity of P, absorb this nutrient and translocate it to the leaves, whereas when there is a large availability of P, plants absorb it, translocate it to the leaves up to a certain concentration, and subsequently start to accumulate it in roots. This is probably a growth strategy for further biomass development.

Even though relative growth rates were not affected in the Cr and Zn treatments, chlorophyll synthesis mechanisms were affected by these two metals. Chlorophyll concentration in plants was reported to be a good toxicity indicator for different metals (Burton et al. 2004; Kolotov et al. 2004). However, the response depends on the contaminant and the macrophyte species. Maine et al. (2004) also recorded a decrease in chlorophyll when *P. stratiotes* was exposed to 4 mg L^{-1} Cr, whereas *S. herzogii* did not show a decrease in this pigment up to a Cr concentration of 6 mg L^{-1}. Manios et al. (2003) suggested an increase in chlorophyll *a* hydrolysis due to the accumulation of 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) in *Typha latifolia* L.

The chlorophyll concentration in the Zn treatment was the lowest. Chaney (1993) reported that some plant species became chlorotic when they were exposed to high Zn concentrations. Mishra and Tripathi (2009) found that chlorophyll concentration showed a decrease due to accumulation of Zn in *E. crassipes* after a 7-day incubation period.

In contrast to the single metals, the combined metals did not produce synergic toxic effects on the concentration of this pigment, probably because the concentration of such metals in the treatment where they were combined was a third of the concentration applied in the treatments where they were added individually. Low Cr concentrations can enhance chlorophyll concentrations by improving availability of biologically active Fe in plant tissue (Bonet et al. 1991).

Ni was the most toxic metal for *P. stratiotes*, which was evidenced in the reduction of relative growth rate and chlorophyll *a* concentration, in agreement with what was reported by Hadad et al. (2007) on determining the tolerance of *S. herzogii* regarding Cr, Ni, and Zn. The decrease in CSA of root, stele, and metaxylem vessels and total metaxylem vessels also suggested Ni toxicity.

The combination of metals caused a toxic effect on relative growth rate, root length, and anatomic structure of roots. As Cr and Zn did no produce toxic effects on these parameters, the observed toxic effect in the Cr + Ni + Zn treatments could be due to the presence of Ni or to a synergic effect. The effects of synergism of heavy metals were recorded by Sarkar and Jana (1987), who studied the effects of Hg + As + Pb + Cu + Cd + Cr on the Hill activity of *Azolla pinnata* R. Br., and by Paris et al. (2005),
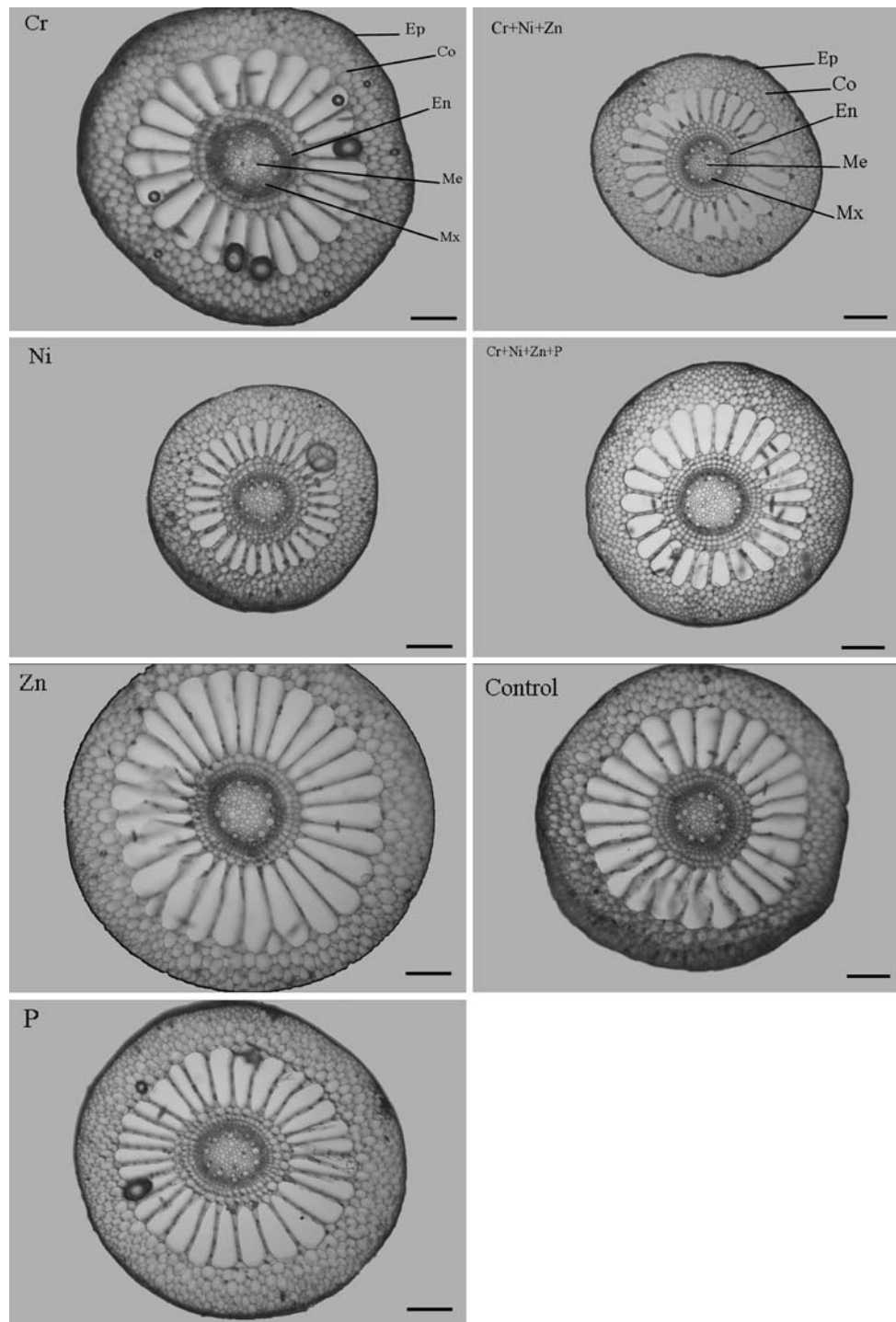
Fig. 6 Optical microscopy images of cross-sectional sections of *P. stratiotes* roots obtained in the different treatments. Ep, epidermis; Co, cortex; En, endodermis; Me, stele; Mx, metaxylematic vessels. Bar = 350 μm

who exposed *P. stratiotes* and *S. herzogii* to Cr + Cd + Pb.

Cr + Ni + Zn + P treatment presented the highest root and stele CSAs, showing a lower toxicity in comparison with the other treatments, suggesting that P induced a higher plant tolerance to Cr + Ni + Zn. Reductions in relative growth rate and root length due to Cr + Ni + Zn exposure were also attenuated by P enrichment, suggesting an improving effect of nutrient enrichment on the metal tolerance of *P. stratiotes*. Nutrient enrichment will improve

metal accumulation by increasing macrophyte production, leading to a higher metal uptake by the macrophyte biomass, and also by enhancing the overall biological activity. The diminution of the toxic effects on *P. stratiotes* can be corroborated by the chlorophyll *a* concentration, agreeing with Hadad et al. (2007), who found that *S. herzogii* increased its tolerance to the same metals in the presence of P and N.

The response of *P. stratiotes* when subjected to P did not show significant changes in root length and root and stele CSAs, contrary to what was observed in the work by Xie and Yu (2003) and Campanella et al. (2005), who studied *E. crassipes.* In these works, root length depended on the concentration of P to which plants were exposed, showing a plastic phenotypic response in the presence of P. The P treatment showed an increase in the number of vessels, in agreement with Campanella et al. (2005). Nutrients such as P act as signals which could be a trigger for molecular mechanisms that modify the processes of cellular division and differentiation in roots (López-Bucio et al. 2003). P caused changes only in certain parameters measured in *P. stratiotes*, which could be due to the fact that a longer exposure to this nutrient is required for the internal metabolic mechanisms to generate modifications. Campanella et al. (2005) recorded changes in the internal and external morphology after *E. crassipes* had been in contact for 3 months with an effluent containing P at a mean concentration of 5 mg L^{-1} P in a constructed wetland. Contrarily, exposure to metals caused changes in a short time, probably because the kinetics of metal sorption of a macrophyte is much faster that that of P (Maine et al. 1998, 2001, 2004; Suñé et al. 2007).

Conclusion

- Ni and Cr + Ni + Zn were the most toxic treatments. Toxic effects were evidenced in relative growth rates, anatomic structure, and root length.
- Chlorophyll *a* was a more sensitive indicator of Zn and Cr toxicity than relative growth rate.
- The addition of P to the treatment with combined metals attenuated the diminution of plant growth and root length, and caused a significant increase in CSAs of total metaxylem vessels, suggesting that P increased the tolerance of *P. stratiotes* to metals. This effect has important implications for the use of constructed wetlands for industrial wastewater treatment. Many processes in metallurgic industries produce wastewaters containing metals. The addition of P might be attained by mixing the sewage from the factory facilities with the industrial wastewater after the appropriate environmental safeguard has been fulfilled.

References

Aoi T, Hayashi T (1996) Nutrient removal by water lettuce (*Pistia stratiotes*). Water Sci Technol 43:407–412. doi:10.1016/S0273-1223(96)00772-X

APHA (1998) Standard methods for the examination of water and wastewater. American Public Health Association, New York, 1268 pp

Banerjee G, Sarker S (1997) The role of *Salvinia rotundifolia* in scavenging aquatic Pb(II) pollution: a case study. Bioprocess Eng 17:295–300

Bonet A, Poschenrieder Ch, Barceló J (1991) ChromiumIII-iron interactions in Fe-deficient and Fe-sufficient bean plants. I. Growth and nutrient content. J Plant Nutr 14:403–414. doi:10.1080/01904169109364211

Burton KW, King JB, Morgan E (2004) Chlorophyll as an indicator of the upper critical tissue concentration of cadmium in plants. Water Air Soil Pollut 27:147–154. doi:10.1007/BF00464777

Campanella MV, Hadad H, Maine MA, Markariani R (2005) Efectos del fósforo de un efluente cloacal sobre la morfología interna y externa de *Eichhornia crassipes* (Mart.) Solms, en un humedal artificial. Limnetica 24(3–4):263–272

Cardwell AJ, Hawker DW, Greenway M (2002) Metal accumulation in aquatic macrophytes from southeast Queensland, Australia. Chemosphere 48:653–663. doi:10.1016/S0045-6535(02)00164-9

Chaney R (1993) Zinc phytotoxicity. In: Robson A (ed) Zinc in soils and plants. Dordrecht, Kluwer, pp 135–150

Ciro AR, Joao PTW, Silvelena V, Valdir JR (1999) The significance of root growth on cotton nutrition in an acidic low-P soil. Plant Soil 212:185–190

D'Ambrogio de Argüeso A (1986) Manual de técnicas en histología vegetal. I–IV. Hemisfero Sur S.A., Buenos Aires

Delgado M, Bigeriego M, Guardiola E (1993) Uptake of Zn, Cr and Cd by water hyacinths. Water Res 27(2):227–269

Ellis JB, Shutes RB, Revitt D, Mand T, Zhang T (1994) Use of macrophytes for pollution treatment in urban wetlands. Res Cons Rec 11:1–12

Gersberg RM, Elkins BV, Lyon SR, Goldman CR (1986) Role of aquatic plants in wastewater treatment by artificial wetlands. Water Res 20:363–368

Hadad H, Maine MA (2001) Efectos del fósforo sobre el crecimiento y competencia de *Salvinia herzogii* de la Sota (Salviniaceae) y *Pistia stratiotes* L. (Araceae). Rev FABICIB 5:49–56

Hadad H, Maine MA, Bonetto C (2006) Macrophyte growth in a pilot-scale constructed wetland for industrial wastewater treatment. Chemosphere 63(10):1744–1753

Hadad H, Maine MA, Natale GS, Bonetto C (2007) The effect of nutrient addition on metal tolerance in *Salvinia herzogii*. Ecol Eng 31:122–131

Hadad HR, Mufarrege MM, Pinciroli M, Di Luca G, del Sastre V, Maine MA (2008) Macrophyte morphological response to the industrial effluent toxicity in a constructed wetland. In: Russo RE (ed) Wetlands: ecology, conservation and restoration. Nova Science, New York, pp 295–323

Hunt R (1978) Plant growth analysis studies in biology. No. 96. Edward Arnold, London

Kapitonova OA (2002) Specific anatomical features of vegetative organs in some macrophyte species under conditions of industrial pollution. Russ J Ecol 33(1):59–61

Kolotov BA, Demidov VV, Volkov SN (2004) Chlorophyll content as a primary indicator of the environment degradation due to contamination with heavy metals. Doklady Biol Sci 393:550–552

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol 6:280–287

Maine MA, Panigatti MC, Pizarro MJ (1998) Role of macrophytes in phosphorus removal in Paraná Medio wetlands. Pol Arch Hydrobiol 45(1):23–34

Maine MA, Duarte M, Suñé N (2001) Cadmium uptake by floating macrophytes. Water Res 35:2629–2634

Maine MA, Suñé N, Lagger SC (2004) Chromium bioaccumulation: comparison of the capacity of two floating aquatic macrophytes. Water Res 38:1494–1501

Maine MA, Suñé N, Hadad H, Sánchez G, Bonetto C (2006) Nutrient and metal removal in a constructed wetland for waste-water treatment from a metallurgic industry. Ecol Eng 26:341–347

Maine MA, Suñé N, Hadad H, Sánchez G, Bonetto C (2007) Removal efficiency of a constructed wetland for wastewater treatment according to vegetation dominance. Chemosphere 68:1105–1113

Maine MA, Suñé N, Hadad H, Sánchez G, Bonetto C (2009) Influence of vegetation on the removal of heavy metals and nutrients in a constructed wetland. J Environ Manage 90:355–363

Manios T, Stentiford E, Millner P (2003) The effect of heavy metals accumulation on the chlorophyll concentration of *Typha latifolia* plants, growing in a substrate containing sewage sludge compost and watered with metaliferus water. Ecol Eng 20:65–74

Miretzky P, Saralegui A, Fernandez Cirelli A (2004) Aquatic macrophytes potential for the simultaneous removal of heavy metals (Buenos Aires, Argentina). Chemosphere 57:997–1005

Mishra VK, Tripathi BD (2009) Accumulation of chromium and zinc from aqueous solutions using water hyacinth (*Eichhornia crassipes*). J Hazard Mater 164:1059–1063

Murphy J, Riley J (1962) A modified single solution method for determination of phosphate in natural water. Anal Chim Acta 27:31–36

O'Keefe DH, Hardy JK, Rao RA (1984) Cadmium uptake by water hyacinth: effects of solution factors. Environ Pollut 34:133–147

Paris C, Hadad H, Maine MA, Suñé N (2005) Eficiencia de dos macrófitas flotantes libres en la absorción de metales pesados. Limnetica 23(3–4):237–244

Sarkar A, Jana S (1987) Effects of combinations of heavy metals on Hill activity of *Azolla pinnata*. Water Air Soil Pollut 35:141–145

Satyakala G, Kaiser J (1997) Chromium induced biochemical changes in *Eichhornia crassipes* (Mart.) Solms. and *Pistia stratiotes* L. B. Environ Contam Toxicol 10:159–169

Sela M, Carty J, Taylor E (1989) The accumulation and the effect of heavy metals on the water fern *Azolla filiculoides*. New Physiol 112:7–12

Sen AK, Bhattacharyya M (1994) Studies of uptake and toxic effects of Ni (II) on *Salvinia natans*. Water Air Soil Pollut 78:141–152

Sen AK, Mondal NG, Mandal S (1987) Studies of uptake and toxic effects of Cr(VI) on *Pistia stratiotes*. Water Sci Technol 19:119–127

Steinbachová-Vojtísková L, Tylová E, Soukup A, Novická H, Votrubová O, Lipavská H, Cizková H (2006) Influence of nutrient supply on growth, carbohydrate, and nitrogen metabolic relations in *Typha angustifolia*. Environ Exp Bot 57:246–257

Suñé N, Sánchez G, Caffaratti S, Maine MA (2007) Cadmium and chromium removal kinetics from solution by two aquatic macrophytes. Environ Pollut 145:467–473

Wahl S, Ryser P, Edwards PJ (2001) Phenotypic plasticity of grass root anatomy in response to light intensity and nutrient supply. Ann Bot 88:1071–1078

Walpole R, Myers R (1992) Probability and statistics for engineers and scientists, 4th edn. Macmillan, New York

Westlake DF (1974) Macrophytes. In: Vollenweider RA (ed) A Manual on methods for measuring primary production in aquatic environments. IBP Handbook No. 12. International Biological Programme. Blackwell Scientific, Oxford, pp 32–42

Xie Y, Yu D (2003) The significance of lateral roots in phosphorus (P) acquisition of water hyacinth (*Eichhornia crassipes*). Aquat Bot 75:311–321