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Nickel and phosphorous sorption efficiencies, tissue accumulation kinetics and morphological effects on *Eichhornia crassipes*

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Abstract The aim of the research was to assess the uptake efficiencies of Ni and P, their distribution in tissues along time and their toxic effects on the internal and external morphologies of *Eichhornia crassipes*. Aquaria with plants exposed to 1 mg Ni l^{-1} or 5 mg P l^{-1} and control were arranged in triplicate. Water and plants (aerial parts and roots) were sampled along 30 days. Ni uptake and tissue bioaccumulation kinetics was significantly faster than that of P. Mean root length, number of leaves, biomass and chlorophyll concentration were negatively affected by Ni, while these parameters were significantly increased by P in comparison with the control. Stele and metaxylem vessel cross-sectional areas (CSA) in the P treatment were significantly lower in comparison with that obtained in the Ni treatment and in control. Metaxylem vessels CSA in plants exposed to Ni were significantly higher while the number of vessels was significantly lower than those obtained in the control. Despite the toxic effects, *E. crassipes* efficiently accumulated Ni, probably due to the morphological plasticity of its root system.

Keywords *Eichhornia crassipes* · Nickel (Ni) · Phosphorous (P) · Morphology

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Introduction

Pollutants reach water bodies via numerous pathways, including industrial and sewage effluent discharges, urban and agricultural run-off, etc. The ability of the aquatic organisms to retain contaminants from the surrounding environment is a widely recognized phenomenon with a number of important implications (Banerjee and Sarker 1997; Cardwell et al. 2002). Floating macrophytes such as *Eichhornia crassipes* (Mart.) Solms., *Pistia stratiotes* L., and *Salvinia herzogii* de la Sota, have been studied because of their contaminant removal capacity from water and their subsequent use in wetlands constructed for wastewater treatment (Delgado et al. 1993; Sen and Bhattacharyya 1994; Banerjee and Sarker 1997; Maine et al. 2001; Maine et al. 2004; Hadad et al. 2006). In most cases, the research goal was assessing contaminant removal efficiencies. However, studies of bioaccumulation process by macrophytes and 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).

P and Ni are ubiquitous contaminants in effluents. Both play an important role as nutrients and micronutrients in plants. However, they produce toxic effects at high concentrations (Kabata-Pendias and Pendias 1984; Stumm and Morgan 1996). The presence of high P (López-Bucio et al. 2003) or heavy metals concentrations (Mufarrege et al. 2006) can affect the internal morphology of roots. Variations in root anatomy and root diameter are closely associated with ecological requirements of plant species, and may affect the ability of plants to absorb contaminants and water. Wahl et al. (2001) described the phenotypic plasticity of grass root anatomy as a response to the increase of nutrients exposure. In a wetland constructed for

treating sewage, Campanella et al. (2005) compared the morphology of *E. crassipes* plants found at the inlet and outlet of a constructed wetland, reporting that the plants growing at the wetland inlet, where the water was rich in nutrients, developed the aerial part mainly, in comparison with the plants growing at the outlet. On the other hand, those growing at the outlet developed the longest system of roots. The same study reported an increase in the number of vessels and in the cross-sectional area (CSA) of roots and metaxylem vessels of the plants growing at the inlet. The effects of P, Cr, Ni, and Zn on the internal root and external plant morphologies of *P. stratiotes* were evaluated by Mufarrege et al. (2006). The exposure to Ni and combined metals (Cr + Ni + Zn) showed toxicity through a decrease in CSA of roots, stele, metaxylem vessels and total metaxylem vessels. Exposure to Cr + Ni + Zn + P showed the highest root CSA, demonstrating a lower toxicity. The mechanisms regulating metal tolerance in macrophytes are not completely identified and they could consist of different mechanisms operating simultaneously.

Regarding heavy metal kinetics, the most important processes of Cd uptake were biological in *S. herzogii*, while adsorption, chelation and ionic exchange were observed in *P. stratiotes* (Maine et al. 2001; Suñé et al. 2007). The main processes of Cr uptake kinetics in both macrophytes were adsorption, chelation and ion exchange. Cr precipitation induced by roots also occurred in *P. stratiotes* and Cr uptake through aerial parts was probably the main cause of the increase of Cr in the aerial parts of *S. herzogii* (Maine et al. 2004).

The aim of the research was to assess Ni and P sorption efficiencies, their tissue accumulation kinetics and their effects on morphological characteristics of *E. crassipes*. This species was chosen due to it was the dominant floating macrophyte in a wetland constructed for effluent treatment of the metallurgic industry Bahco Argentina S. A. (Maine et al. 2006). P and Ni were studied for being contaminants found in the effluents treated at this constructed wetland. Studies of the bioaccumulation kinetics of Ni and P in *E. crassipes*, and their effects on the internal and external morphologies would allow us to determine its tolerance and provide basic knowledge to evaluate vegetation management in the wetland.

Materials and methods

Experimental design

Eichhornia crassipes plants and water were collected from an unpolluted pond of the Middle Paraná River floodplain. Only mature plants of a uniform size (height = 12 ± 3 cm; root length = 14.5 ± 2.5 cm) and weight (25 ± 5 g fresh weight) were selected.

Sixty aquaria were placed outdoors under a semi-transparent plastic roof. During the experimental period (spring) temperature ranged from 24 to 28°C. Fresh biomass of plants (50 g) and 5 l of pond water were used in each aquarium. Ni (as $NiCl_2 \cdot 6H_2O$) or P (as PO_4H_2K) were added initially to reach 1 mg l^{-1} Ni or 5 mg l^{-1} P. These concentrations were chosen due to the fact they were the maximum concentrations registered in the effluents of the studied constructed wetland (Maine et al. 2006). Samplings were done initially, at 30 min and at 2, 8 and 24 h and at 2, 7, 10, 15 and 30 days. In each sampling all the water and the total plant biomass of three replicate aquaria was collected. Controls without Ni or P addition were used. Pond water was added daily to compensate water losses through plant transpiration and evaporation, maintaining the initial volume of 5 l. The experiment lasted 30 days.

Water was homogenized and sampled for Ni and soluble reactive phosphorous (SRP) analysis. Plant samples were dried and separated into aerial parts (foliar sheets and petioles) and roots. Dried plants were ground, digested and Ni and P concentrations and dry weight were determined.

At the end of the experiment, sections approximately 30 mm long were cut from the middle of the root and stored in formaldehyde 4%. After 48 h, root sections were immersed in ethanol 70% 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. The sections were examined by light microscopy (×100 and ×400). Sixty sections of roots from each treatment 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 CSA of the whole root, stele and metaxylem vessels (Wahl et al. 2001). The total metaxylem CSA was calculated adding the areas of all the vessels per section. Also, the number of metaxylem vessels per section was recorded.

External morphology was described measuring root length and number of leaves per plant. In addition, dry weights of aerial and submerged parts were dried at 105°C until constant weight was reached (Westlake 1974; APHA 1998). *E. crassipes* relative growth rate was calculated in each treatment according to Hunt's equation (1978):

$$R = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

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

Chemical analysis

The physicochemical characterization of water from the site where the plants were collected was done according to APHA (1998). The chemical composition of the water used in the experiment was (mean ± standard deviation): pH = 6.5 ± 0.2; conductivity = 120 ± 12 μS cm^{-1}; dissolved oxygen (DO) = 2.6 ± 0.30 mg l^{-1}; SRP = 0.034 ± 0.006 mg l^{-1}; NH_4^+-N = 0.390 ± 0.099 mg l^{-1}; NO_3^--N = 0.028 ± 0.012 mg l^{-1}; NO_2^--N = 0.009 ± 0.002 mg l^{-1}; Ca^{2+} = 10.1 ± 1.8 mg l^{-1}; Mg^{2+} = 13.9 ± 0.5 mg l^{-1}; Na^+ = 15.7 ± 2.0 mg l^{-1}; K^+ = 3.50 ± 0.4 mg l^{-1}; Cl^- = 10.6 ± 1.3 mg l^{-1}; SO_4^{2-} = 11.4 ± 1.8 mg l^{-1}; HCO^{3-} = 83.2 ± 1.2 mg l^{-1}; Ni = non detected (detection limit = 5 μg l^{-1}).

Plant tissues (aerial parts and roots) were digested with a $HClO_4$:HNO_3:HCl (7:5:2) mixture (Maine et al. 2001). Ni concentrations were determined in water samples and in digests of plant tissue by atomic absorption spectrometry (Perkin Elmer 5000; APHA 1998). SRP was determined in water by the colorimetric molybdenum blue method (Murphy and Riley 1962; UV–VIS Perkin Elmer Lambda 20). Total phosphorous (TP) concentration in water was determined as SRP, after digestion with sulphuric and nitric acid (APHA 1998). Total phosphorous (TP) concentration was measured in the plant tissue digests as SRP. Ni and P amounts (mg) were estimated by multiplying the values of biomass or volume (g dry weight or l) by the values of Ni or P concentration in plant tissues or in water (mg g^{-1} dry weight or mg l^{-1}).

The bioconcentration factor (BCF) was estimated using the following formula (Gobas and Morrison 2000):

$$\mathrm{BCF} = (C_e - C_i)/C_w \quad (1)$$

where C_e = contaminant concentration in tissue (μg g^{-1} dw) during contaminant exposure, C_i = initial contaminant concentration in tissue (μg g^{-1} dw) before contaminant exposure, and C_w = contaminant concentration in water (μg l^{-1}).

Data from water and tissue amounts were adjusted, leading to the following equation, which is the best adjusted to data (Maine et al. 2004):

$$A - A_0 = A_1(1 - e^{-t/r}) + A_2(1 - e^{-t/s}) \quad (2)$$

In which, A_0 = initial amount of Ni or P, A = amount of Ni or P at time t, t = time.

During the experiment, chlorophyll was extracted with acetone for 48 h in cold darkness (3–5°C; APHA 1998). The percentage of transmittance of the extracts at 645 and 665 nm was recorded with a spectrophotometer UV–Vis (Westlake 1974).

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine whether significant differences existed in chlorophyll concentration, root length, number of leaves per plant and TP and Ni tissue concentrations (aerial parts and roots) among the different exposure times. The normality of residuals was tested graphically, and the homocedasticity of variances was checked applying Bartlett's test. Duncan's test was used to differentiate means where appropriate. A level of $p < 0.05$ was used in all comparisons.

Since root morphology parameters (CSA of root, stele, metaxylem vessels and total metaxylem, and number of vessels) did not show a normal distribution, non-parametric tests and box and whisker plots were performed using median as central trend measure and interquartile range (25 and 75%) as its variability measure. Kruskal–Wallis analysis was applied to check the differences between the morphometric 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 a level of $p < 0.05$ was used.

Results

Bioaccumulation and removal kinetics of Ni and P

Figure 1 shows the SRP and Ni percent removal from water over time. A 13 and 21% decrease of SRP was observed at 24 and 48 h, respectively. The SRP removal was 58% at 30 days of experiment. Contrarily, Ni removal occurred fundamentally during the first 24 h, obtaining a

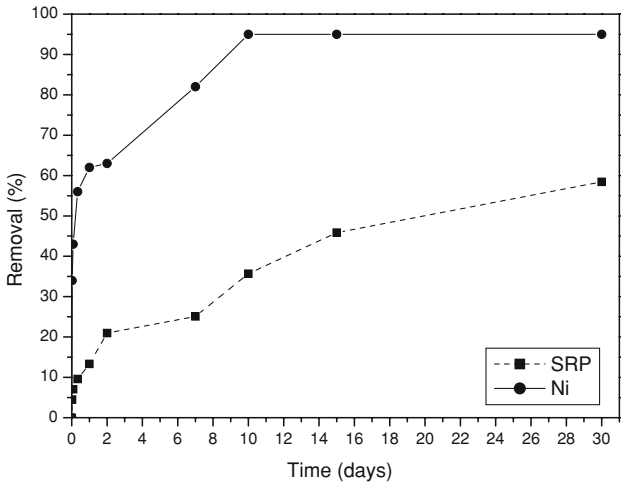

Fig. 1 SRP and Ni percent removal from water during time

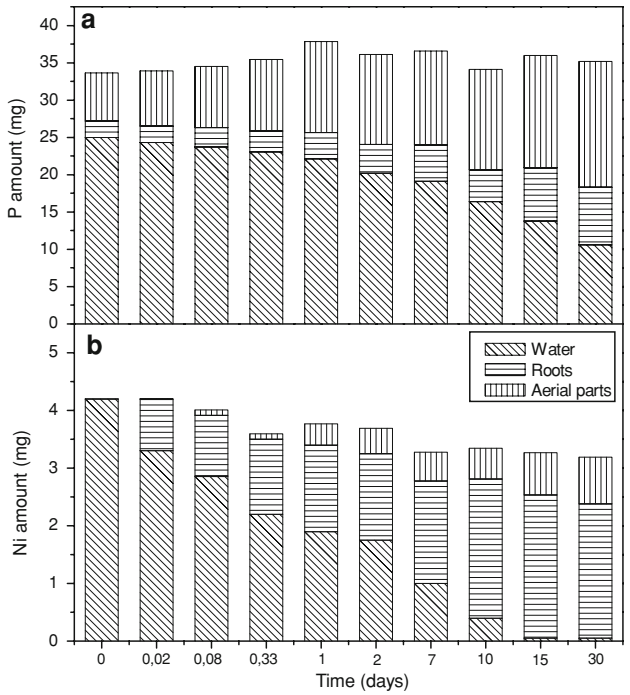

Fig. 2 P (**a**) and Ni (**b**) amounts in water, roots and aerial parts of *E. crassipes* along time. Total Ni and P amounts (mg) were estimated by multiplying the values of biomass or volume (g dry weight or l) by the values of Ni or P total concentration in plant tissues or in water (mg g^{-1} dry weight or mg l^{-1})

removal of 43% at 2 h and a removal of 62% at 24 h. At the end of the experiment, a 95% removal was observed.

Ni and P amounts were compared among water, roots and aerial parts (Fig. 2a, b). P was accumulated fundamentally by aerial parts reaching a final amount significantly higher than the initial value. At the end of the experiment a 48% of the added P to water was accumulated in aerial parts. P began to accumulate in roots after 10 days of experiment, accumulating at the end of the experiment a 22% of the added P (Fig. 2a). On the other hand, Ni bioaccumulation was observed fundamentally in roots reaching a final amount significantly higher than the initial root amount and final aerial part amount. Ni accumulation in roots was fast, increasing a 21 and 40% in the first 30 min and 24 h of contact, respectively. The amount of Ni in roots continued increasing during the first 10 days, accumulating 72% of the added Ni at this period. Then, it did not increase significantly in roots. In aerial parts increases of 10% and 16% were observed at 24 h and 10 days, respectively (Fig. 2b). BCF values (Table 1) also indicate that Ni was fundamentally retained by roots, reaching the steady state after 4 days, while in leaves BCF continued increasing.

Representing by separate graphs both terms of the Eq. 2 versus time for each contaminant (Fig. 3; Table 2), we can see the contaminant amount removed from water or accumulated in tissues along time. For Ni, it can be proposed that removal from water involved a fast and a slow component. There was no significant difference in Ni water removal by the two components at the end of the experiment. In the case of P, a component was slower than the other, but the slower process carried out the main P removal. In aerial and root tissues, the same components were present. In the case of Ni, the accumulation in roots had a fast component that was produced during the first minutes of contact. However, there was no significant difference between the amount of Ni accumulated in roots by the fast and slow processes at 30 days of the experiment. In the case of P, the slow process was mainly responsible for the accumulation in roots. P accumulation in aerial parts by the fast process is produced in the first 2 days of contact. However, there was no significant difference between the Ni or P amounts accumulated in aerial tissues by the slow and fast processes at the end of the experiment.

Plant study

Mean relative growth rates of *E. crassipes* measured in the Ni and P treatments and the control were 0.0048, 0.023 and 0.027 g g^{-1} day^{-1}, respectively. For the purpose of comparison, biomass increase was expressed in

Table 1 Root and leaf Ni concentrations (μg g^{-1}) vs. time. Ni BCF of leaves, roots and whole plants obtained along the experiment

Sampling time	Root concentration	Leaf concentration	BCF leaf/water	BCF root/water	BCF plant/water
0.5 h	902 ± 10	13 ± 2	0.019 ± 0.008	1.366 ± 0.265	0.502 ± 0.053
2 h	1.260 ± 89	67 ± 12	0.118 ± 0.009	2.211 ± 0.166	0.755 ± 0.068
8 h	1.320 ± 173	83 ± 14	0.189 ± 0.008	2.993 ± 0.108	0.984 ± 0.084
24 h	1.750 ± 46	176 ± 18	0.464 ± 0.028	4.612 ± 0.224	1.680 ± 0.092
48 h	1.730 ± 199	199 ± 20	0.569 ± 0.088	4.938 ± 0.168	1.572 ± 0.077
4 days	1.820 ± 242	299 ± 17	0.906 ± 0.033	5.526 ± 0.137	1.782 ± 0.103
10 days	1.890 ± 281	259 ± 33	5.180 ± 0.122	37.764 ± 1.123	14.991 ± 1.224
15 days	1.910 ± 424	283 ± 13	5.660 ± 0.207	38.189 ± 0.998	13.426 ± 1.008
30 days	1.810 ± 85	272 ± 16	5.439 ± 0.150	37.110 ± 1.011	13.955 ± 0.953

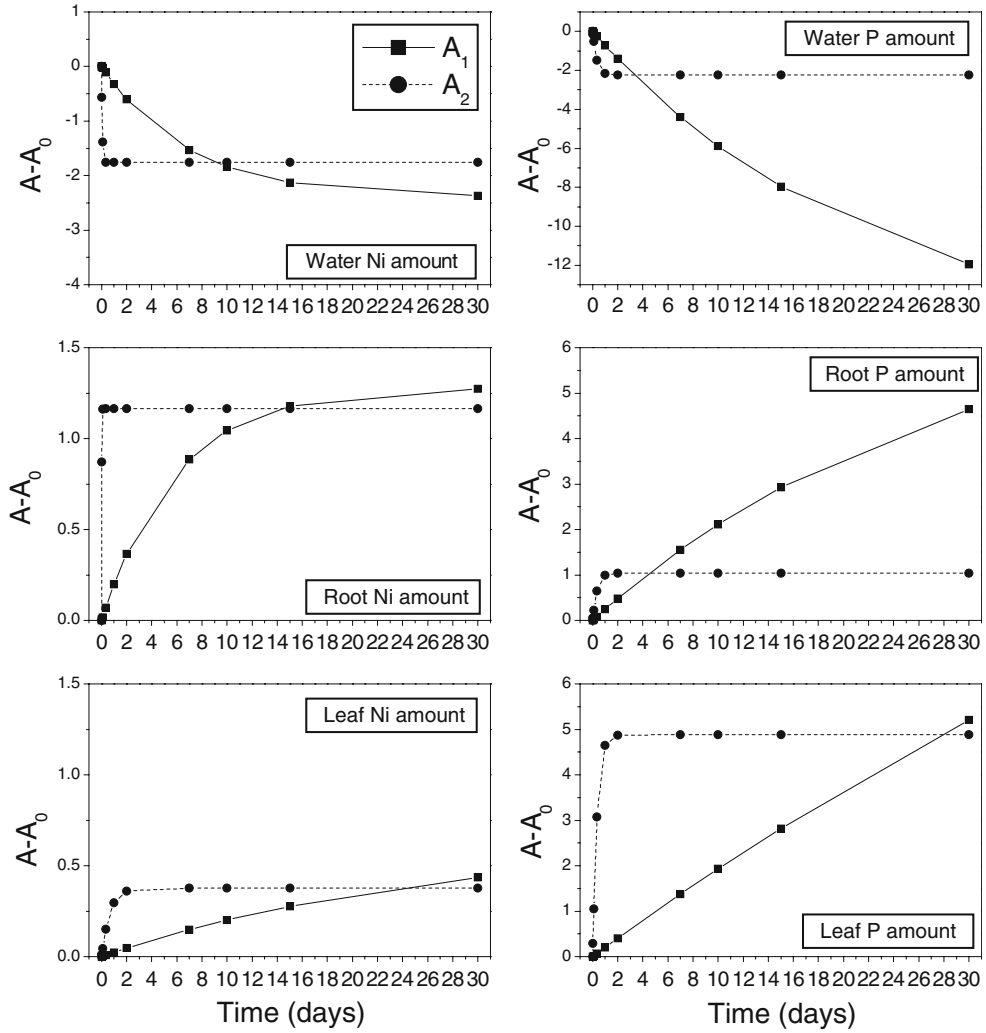

Fig. 3 Contaminant amount decrease from water and increase in tissues vs. time, according to Eq. 2. A_0 = initial amount of Ni or P, A = amount of Ni or P at time t

Table 2 Empirical constants obtained in Eq. 2 for Ni and P amounts in water, aerial parts and roots

	Water amount		Aerial part amount		Root amount	
	Ni	P	Ni	P	Ni	P
A_1	−2.404	−16.023	0.659	18.539	1.283	7.135
A_2	−1.757	−2.224	0.377	4.885	5.958	28.458
r	6.904	21.843	27.539	91.013	1.166	1.042
s	0.051	0.301	0.652	0.332	0.015	0.335

%. At 30 days, total biomass increase was significantly lower in the Ni treatment than that of the control (Fig. 4a). At 30 days, in the P treatment, both the leaf and root biomass increase was higher than that obtained in the control (Fig. 4b, c, respectively). In the Ni treatment, the aerial biomass increase recorded at 30 days was significantly lower than that of the control (Fig. 4b). The root biomasses increase observed at 15 and 30 days in the Ni treatment were significantly lower than those of controls (Fig. 4c).

Chlorophyll concentrations in the P treatment were significantly higher than that obtained in the Ni treatment (Fig. 5a). At 30 days, chlorophyll concentration (2.3 mg g^{-1}) in the Ni treatment was significantly lower than its initial value (3.0 mg g^{-1}), while the concentration in the P treatment at 30 days was not significantly different from the initial concentration (Fig. 5a). At 30 days, plants exposed to Ni showed chlorosis (75% of leaves of the aquaria), while the plants exposed to P were healthy. At 30 days, a significant increase in the number of leaves per plant was observed in the P treatment (Fig. 5b), while in the Ni treatment, these values were negative (−12.5%) (Fig. 5b). In the P treatment root length increase was significantly lower in the control in comparison with that obtained at 15 and 30 days (Fig. 5c), while in the Ni

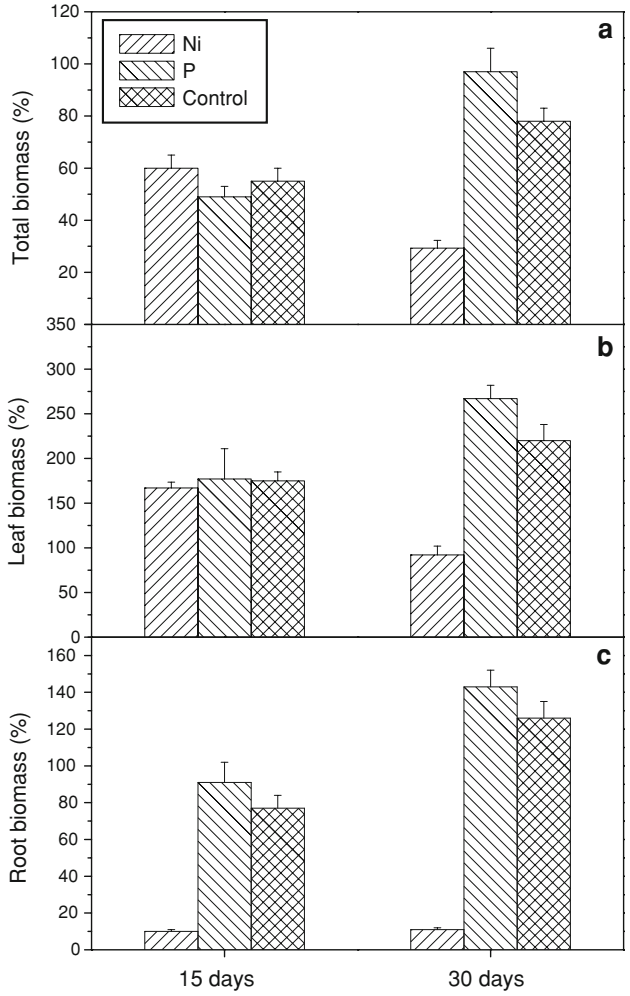

Fig. 4 Increase (in %) of the total biomass (**a**), aerial part biomass (**b**) and root biomass (**c**) of *E. crassipes* obtained at 15 and 30 days compared with controls

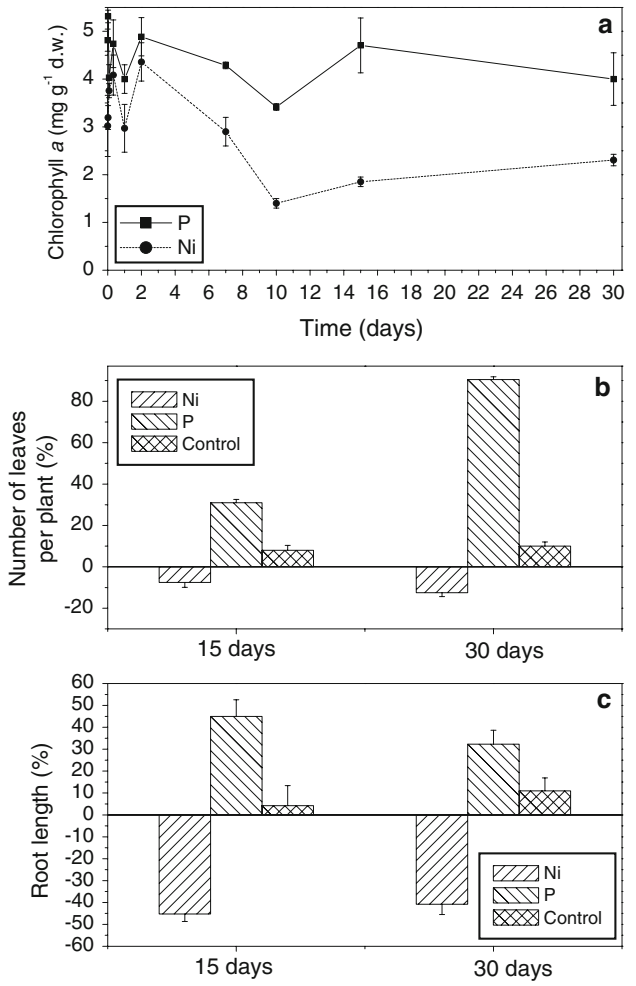

Fig. 5 Chlorophyll *a* concentrations (**a**), increase (in %) of root lenght (**b**) and number of leaves per plant (**c**) in *E. crassipes* obtained at 15 and 30 days compared with controls

treatment, these values were negative (−45.2 and −40.8% for 15 and 30 days, respectively).

In the Ni treatment, root, stele, metaxylem vessels and total metaxylem CSA were significantly higher than the values obtained in the P treatment (Fig. 6). However, root and total metaxylem CSA were not significantly different between each treatment and the control (Fig. 6a, d, respectively). Stele CSA in the P treatment was significantly lower in comparison with that obtained in the Ni treatment and in control (Fig. 6b), while the Ni treatment was not significantly different from control. Metaxylem vessel CSA in the Ni treatment was significantly higher than that of P treatment and control (Fig. 6c). The number of vessels obtained in the Ni treatment was significantly lower than those of P treatment and control (Fig. 6e), being the control not significantly different to P treatment. Figure 7 shows light microscopy images of the root cross sections.

Discussion

Ni affected the growth of *E. crassipes*, in agreement with Hadad et al. (2007) on studying *S. herzogii* tolerance to Ni, Cr and Zn. Monni et al. (2000) reported growth inhibition in *Empetrum nigrum* L., an ericaceous shrub, with increasing Ni concentration in solution. Ni was also toxic for chlorophyll production. Chlorophyll concentration in plants is 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 (Manios et al. 2003; Maine et al. 2004; Hadad et al. 2007).

Metaxylem vessel CSA in plants exposed to Ni were significantly higher while the number of vessels was significantly lower than those obtained in the control and the P treatment. The other morphological parameters were significantly different from the P treatment but not from the control. Mufarrege et al. (2006) reported Ni toxicity on the

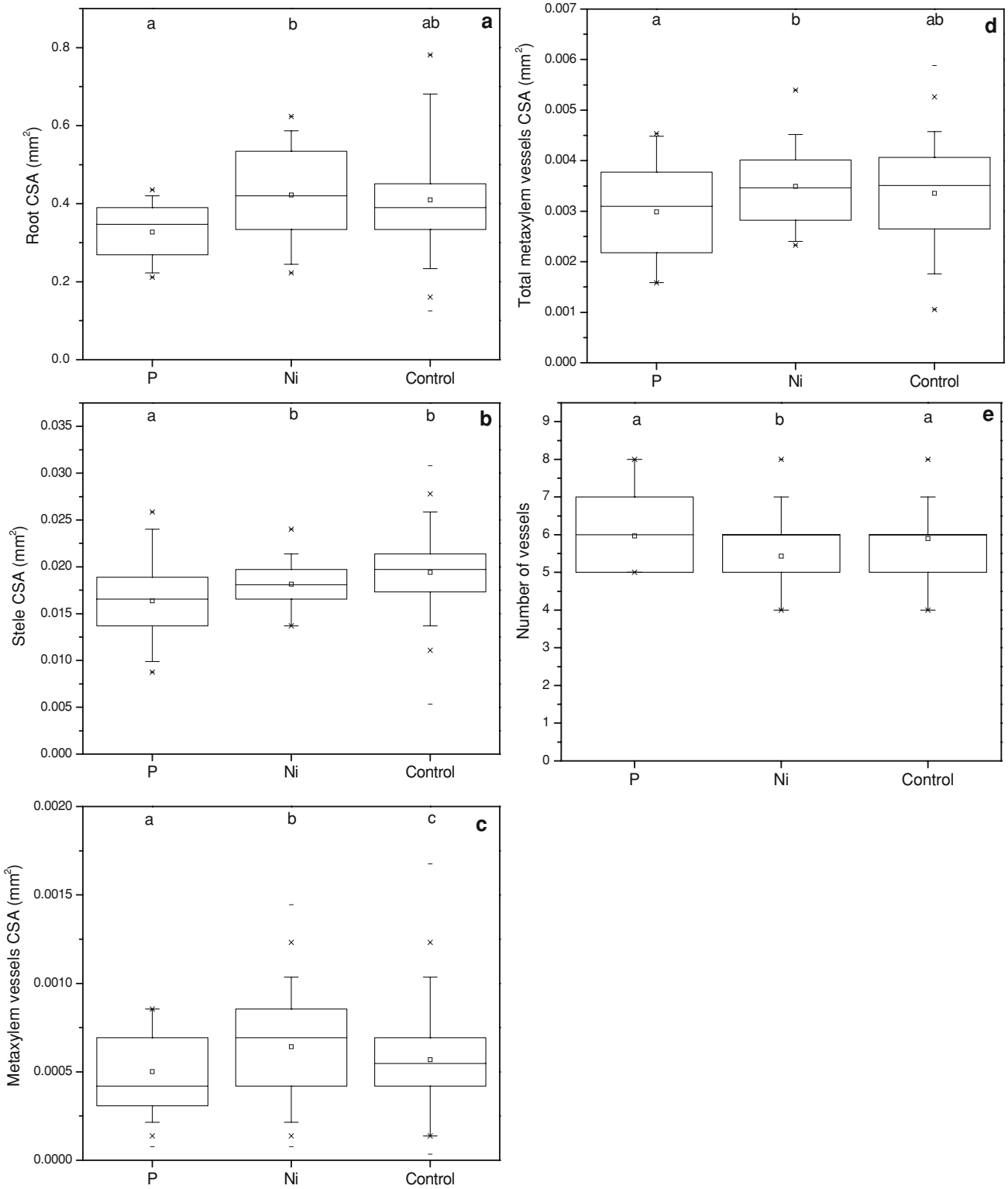

Fig. 6 *Box* and whisker *plots* of root (**a**), stele (**b**), metaxylem (**c**) and total metaxylem (**d**) vessels CSA, and number of vessels (**e**) of *E. crassipes* roots at the end of the experiment. *Different letters* represent statistically significant differences among the treatments

internal root morphology of *P. stratiotes*, indicating a higher Ni tolerance of *E. crassipes* than *P. stratiotes*. In the P treatment, *E. crassipes* did not show significant differences with the control in root CSA, total metaxylem vessel CSA and number of vessels. Contrarily, Xie and Yu (2003) observed an increase in these variables in

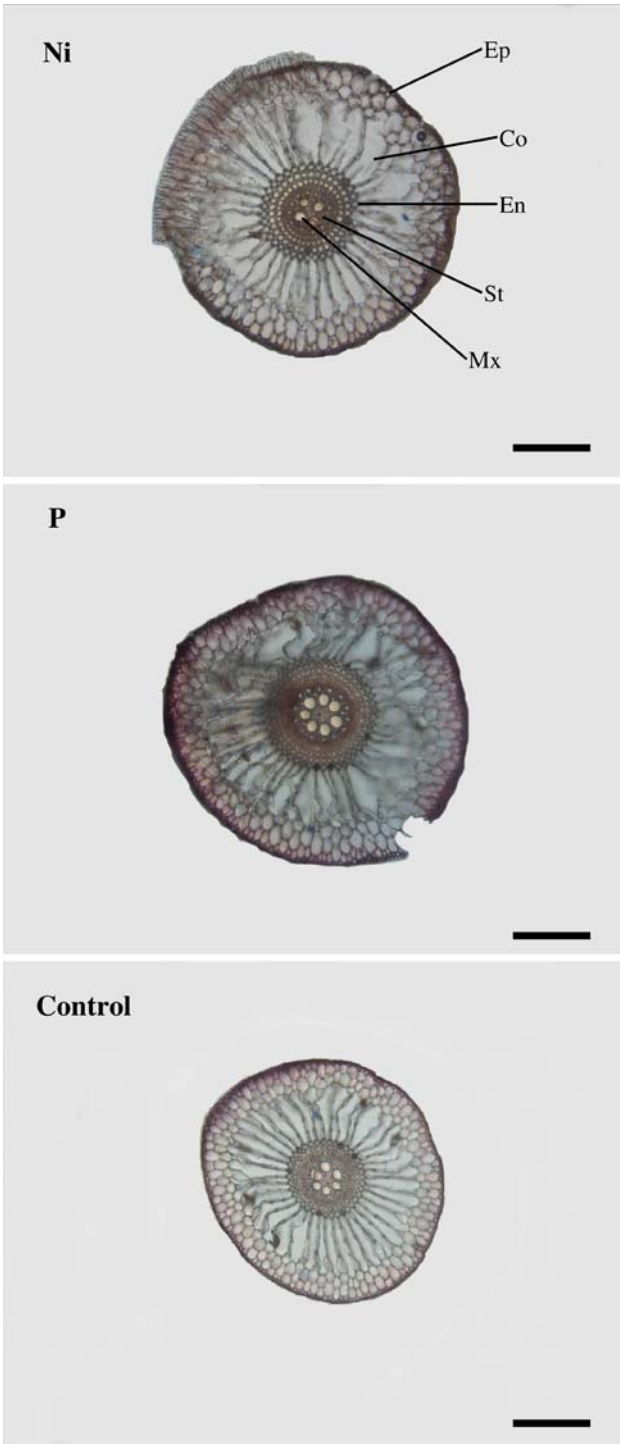

Fig. 7 Optical microscopy image of cross-sectional *E. crassipes* roots from the Ni and P treatments and Control. Ep, epidermis; Co, cortex; En, endodermis; St, stele and Mx, metaxylem vessels. *Bar* 350 μm

E. crassipes, reporting a high morphological plasticity faced with varying availability of P. Nutrients 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). Campanella et al. (2005) also recorded morphological changes after *E. crassipes* had been in contact for 3 months with an effluent containing 5 mg P l^{-1}. Probably, a longer exposure to P would be required to produce morphological changes.

Despite the toxic effects, Ni was efficiently accumulated in *E. crassipes* tissues at the same time it was removed from water, as can be seen in the BCF values. By the end of the experiment, Ni concentration was significantly higher in roots than in aerial parts (1,810 and 272 μg g^{-1}, respectively). Contrarily, in the P treatment, there was a higher TP concentration in aerial parts than in roots, in agreement with other studies of *E. crassipes* growing in natural wetlands (Hadad and Maine 2007) and in constructed wetlands (Campanella et al. 2005; Maine et al. 2005). Macrophytes possess efficient apoplastic nutrient transfer routes to leaf photosynthetic cells (Barnabas 1988).

Ni demonstrated faster removal kinetics from water than P. However, it was slower in comparison with Cd and Cr which accumulated fundamentally during the first minutes of contact (Maine et al. 2001, 2004). In the case of Ni, the rapidity of the root uptake suggests that adsorption to the cell walls of roots is probably the process responsible for the fast process of root accumulation. The efficiency of the metal adsorption processes was also corroborated using non-living roots (Dushenkov et al. 1995; Schneider and Rubio 1999; Miretzky et al. 2006; Suñé et al. 2007). Biological processes (metal transport through plasmalemma into cells) and subsequent translocation to the leaves were probably responsible for the slower stage of root accumulation. However, both processes were responsible for Ni bioaccumulation since there was no significant difference between the amount of Ni accumulated in roots by the fast and slow processes at 30 days of the experiment.

In the case of P, the slow process carried out the main P accumulation in roots, suggesting that biological processes were limited by the nutrient transport velocity through plasmalemma. Protein membrane carriers mobilize P as fast as they can, but, when there is an excess of phosphate, the transport velocity is limited by the saturation of their capabilities of transport (Salisbury and Ross 1994). Some macrophytes such as *P. stratiotes*, *S. herzogii* and *Paspalum repens* Bergius can uptake contaminants from water through the leaf surface (Panigatti and Maine 2002; Maine et al. 2004). The foliar morphology of *E. crassipes* is different from these macrophytes, avoiding the contaminant sorption by leaves. For this reason, translocation from roots to aerial parts was responsible for the fast process for the P accumulation in aerial parts during the first days. The slow process in the aerial parts was probably due to growth, registered in the biomass increase.

In roots, P accumulation was a slower process than Ni accumulation. Roots sorbed Ni fast, but the high affinity for

Ni of the root adsorption sites limited translocation. Probably, Ni transport to aerial parts began when root adsorption sites were saturated. Contrarily, P was quickly transported to aerial parts from roots, indicating that translocation velocity was similar to root uptake velocity.

Conclusion

- Due to the fact that P was accumulated fundamentally in aerial parts and its root accumulation kinetics was slow, a longer exposure to P would be required for the internal metabolic mechanisms to generate root modifications.
- Ni uptake dynamics proved to be a process significantly faster than that of P. However, Ni was scarcely accumulated in aerial parts, which prevented biomagnification through food chain by herbivorous organisms.
- *Eichhornia crassipes* faced with a scarcity of P, absorbed it and then translocated it to the aerial parts. When there was a large availability of P, plants absorbed it, translocated it to the aerial parts up to a certain concentration, and subsequently started to store it in roots. This is probably a growth strategy for further biomass development.
- The morphological plasticity of the root system of *E. crassipes* allows it to survive in polluted water bodies, such as constructed wetlands for the treatment of effluents, modifying the uptake of nutrients and metals.

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