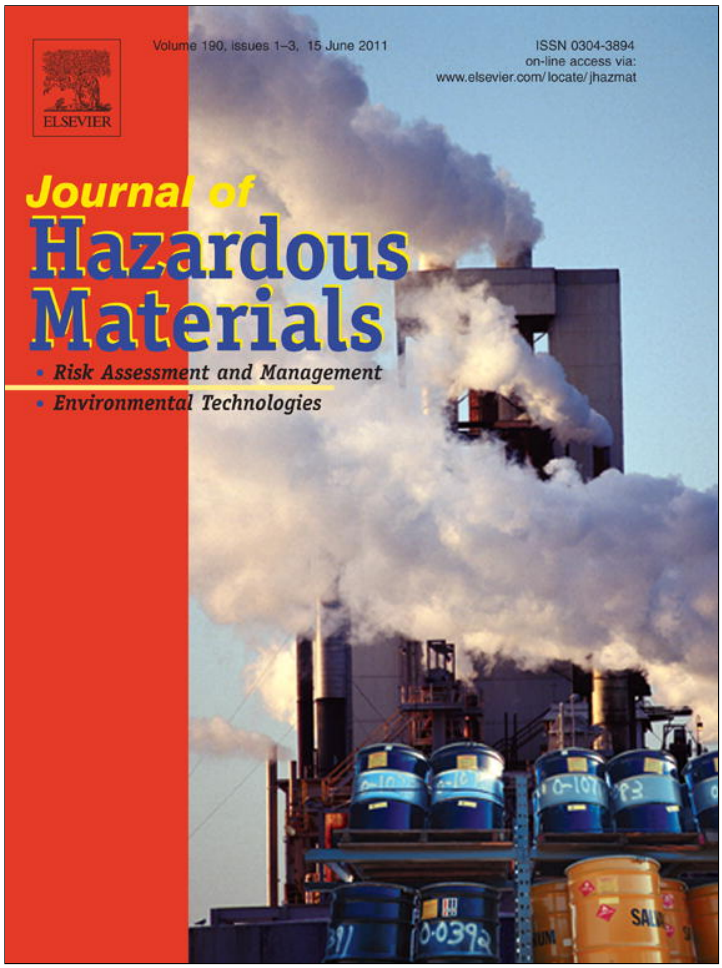

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Bioaccumulation kinetics and toxic effects of Cr, Ni and Zn on *Eichhornia crassipes*

H.R. Hadad[a,b,*], M.A. Maine[a,b], M.M. Mufarrege[a,b], M.V. Del Sastre[a], G.A. Di Luca[a,b]

[a] Química Analítica, Facultad de Ingeniería Química, Universidad Nacional del Litoral. Santiago del Estero 2829 (3000), Santa Fe, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Santa Fe, Argentina

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ABSTRACT

The aim of this work was to assess the uptake efficiencies, the uptake and bioaccumulation kinetics and the toxic effects of Cr, Ni and Zn on *Eichhornia crassipes*. Plants were exposed to 1 mg L^{-1} of each metal and sampled during 30 days. *E. crassipes* removed 81%, 95% and 70% of Cr, Ni and Zn, respectively. Metal removal from water involved a fast and a slow component. Metals were accumulated fundamentally by roots. Cr was scarcely translocated to aerial parts. In these tissues, Ni showed the highest accumulation amount while Zn presented the highest accumulation rate. Metal toxicity on the biomass was different among treatments. However, biomass did not decrease in any case. All the studied metals produced chlorophyll decrease. The root cross-sectional area (CSA) and vessel number increased and the root length decreased when plants were exposed to Zn. Despite the toxic effects, *E. crassipes* accumulated Cr, Ni and Zn efficiently.

1. Introduction

Pollutants reach water bodies via numerous pathways, including industrial and sewage effluent discharges, urban and agricultural run-off, etc. The ability of aquatic plants to retain contaminants from the surrounding environment is a widely recognized phenomenon with a number of important implications [1–3]. Floating macrophytes such as *Eichhornia crassipes* (Mart.) Solms., *Pistia stratiotes* L., and *Salvinia herzogii* de la Sota, have been studied because of their metal removal capacity from water and their subsequent use in wetlands constructed for wastewater treatment [1–11]. 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 [2].

Regarding metal bioaccumulation kinetics, the most important processes of Cd uptake were biological in *S. herzogii*, while adsorption, chelation and ionic exchange were observed in *P. stratiotes* [7,12,13]. 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* [7]. Hadad et al. [9] compared the uptake kinetics of a metal and a nutrient, and reported that *E. crassipes* removed the metal faster than the nutrient, suggesting that adsorption to the cell walls of roots was probably the process responsible for the high bioaccumulation rate of the metal.

The ability of plants to absorb contaminants from water can depend on their morphological adaptive capacity. Macrophytes can modify the internal morphology of their roots in order to grow in polluted water bodies [11,14]. 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. [11]. Plants exposed to Ni and combined metals (Cr + Ni + Zn) showed toxicity through a decrease in the cross-sectional areas (CSA) of roots, stele, metaxylem vessels and total metaxylem vessels. Plants exposed 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 [15,16].

The aim of this research was to assess Cr, Ni and Zn uptake efficiencies, tissue accumulation kinetics and their toxic effects on *E. crassipes*. This species was chosen since it was the dominant floating macrophyte in a wetland constructed for the treatment of effluents of the metallurgic industry Bahco Argentina S.A. [17]. Cr, Ni and Zn were studied for being contaminants found in the effluents treated at this constructed wetland. Studies of metal bioaccumulation kinetics and the toxic effects on *E. crassipes* would allow us to determine its tolerance and provide basic knowledge to evaluate vegetation management in the wetland.

* Corresponding author at: Química Analítica, Facultad de Ingeniería Química, Universidad Nacional del Litoral. Santiago del Estero 2829 (3000) Santa Fe, Argentina. Tel.: +54 0342 4571164x2514.
E-mail address: hhadad@fiq.unl.edu.ar (H.R. Hadad).

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2. Materials and methods

2.1. Experimental design

E. crassipes plants and water were collected from an unpolluted pond of the Middle Paraná River floodplain. Only healthy plants of a uniform size (aerial part height = 8.1 ± 2.4 cm; root length = 10.5 ± 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. Cr (as $CrCl_3{\cdot}6H_2O$), Ni (as $NiCl_2{\cdot}6H_2O$) or Zn (as $ZnCl_2{\cdot}6H_2O$) were added initially to reach 1 mg l^{-1}. This concentration was chosen due to the fact that it was the maximum concentration registered in the effluents treated in the studied constructed wetland [17]. Prior to metal addition, water pH was adjusted to 5.4–5.8 to avoid metal precipitation. Controls without metal 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 d. Samplings were done initially, at 30 min and at 2, 8 and 24 h and at 2, 7, 10, 15 and 30 d. In each sampling all the water and the total plant biomass of three replicate aquaria was collected. Cr, Ni and Zn were determined in water. Plant samples were separated into aerial parts (foliar sheets, petioles and stems) and roots. Aerial parts and roots were dried at 105 °C until constant weight was reached [18,19]. Cr, Ni and Zn concentrations were determined in plant tissues.

Leaf chlorophyll *a* concentrations were determined at the beginning and at the end of the experiment. External morphology was described measuring root length. 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 [20]. 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 (X100 and X400). Sixty sections of roots from each treatment were analyzed. The diameters of roots were measured using a micrometric ocular. The values of CSA of the whole root were obtained calculating the area of a circle [21]. Also, the number of metaxylem vessels per section was recorded.

2.2. Chemical analysis

The physicochemical characterization of water used in the experiment was done according to APHA [19]. The chemical composition of the water used in the experiment was (mean ± standard deviation): conductivity = 170 ± 1 μS cm^{-1}; dissolved oxygen (DO) = 7.6 ± 0.10 mg l^{-1}; Soluble reactive phosphorous (SRP) = 0.024 ± 0.006 mg l^{-1}; NH_4^+ = 0.360 ± 0.019 mg l^{-1}; NO_3^- = 0.028 ± 0.012 mg l^{-1}; NO_2^- = 0.009 ± 0.002 mg l^{-1}; Ca^{2+} = 13.6 ± 0.8 mg l^{-1}; Mg^{2+} = 6.9 ± 0.5 mg l^{-1}; Na^+ = 15.7 ± 1.0 mg l^{-1}; K^+ = 3.50 ± 0.5 mg l^{-1}; Fe = 0.09 ± 0.05 mg l^{-1}; Cl^- = 10.6 ± 1.3 mg l^{-1}; SO_4^{2-} = 11.4 ± 1.8 mg l^{-1}; Total alkalinity = 68.2 ± 1.2 mg l^{-1}; Cr = non detected (Detection limit = 5 μg l^{-1}); Ni = non detected (Detection limit = 5 μg l^{-1}), Zn = non detected (Detection limit = 5 μg l^{-1}).

Chlorophyll was extracted with acetone for 48 h in cold darkness (3–5 °C) [19]. The percentage of transmittance of the extracts at 645 and 665 nm was recorded with a spectrophotometer UV–vis [18].

Dried plant tissues were ground and digested with a $HClO_4$:HNO_3:HCl (7:5:2) mixture [6]. Cr, Ni and Zn concentrations were determined in water samples and in digests of plant tissues by atomic absorption spectrometry (Perkin Elmer 5000) [19]. Cr, Ni and Zn amounts (mg) were estimated by multiplying Cr, Ni or Zn concentration in plant tissues or in water (mg g^{-1} dry weight or mg l^{-1}) by biomass or volume (g dry weight or l).

The bioconcentration factor (BCF) was calculated for aerial parts and roots using the following formula [22]:

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

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

Data from water concentrations and metal amounts in tissues were adjusted, leading to the following equation, which is the best adjusted to data [7]:

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

In which A_0 = initial amount or water concentration of Cr, Ni or Zn, A = amount or water concentration of Cr, Ni or Zn at time *t*, *t* = time. The other parameters (A_1, A_2, r and s) are empirical constants.

2.3. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine whether significant differences existed in biomass, chlorophyll *a* concentration and root length among the different treatments, and metal tissue amounts (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 roots 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%) 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 [23]. In all comparisons a level of $p < 0.05$ was used.

3. Results and discussion

3.1. Uptake and bioaccumulation kinetics

Fig. 1 shows the Cr, Ni and Zn removal percentages from water over time. After 2 h, 24 h and 7 d the Cr removal was 8%, 32% and 56%, respectively. The final Cr removal was 81%. Ni removal occurred fundamentally during the first hours of contact, obtaining a 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. Zn removal was 12% at 2 h and 28% at 24 h. The final removal of Zn was 70%. Studied metal removal percentages from water did not change significantly after the first 15 d. Cr removal by other free-floating macrophytes with lower biomass, such as *P. stratiotes* and *S. herzogii*, demonstrated efficiencies of 98–99% after 30 d of experimentation using concentrations of 1, 2, 4 and 6 mg l^{-1}, regardless the initial concentration [7]. These authors proposed that the obtained removal was due to the fact that the sorption of Cr(III) is probably a competitive-consecutive mechanism of reversible reaction steps. Hadad et al. [8] reported a Cr removal percentage of 99% using *S. herzogii.* However, Ni and Zn removal percentages were similar to those found in this work. Maine et al. [7] proposed that *P. stratiotes* and *S. herzogii*

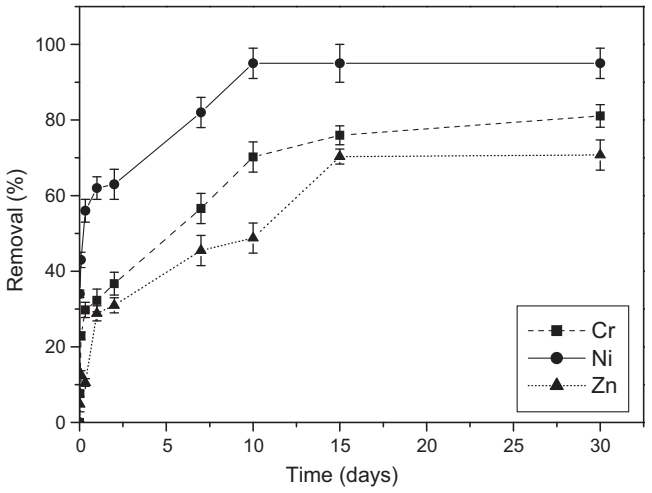

Fig. 1. Cr, Ni and Zn removal from water along time. Bars represent standard deviations.

can uptake Cr from water through adsorption to the leaf surface. We can see that adsorption to the aerial parts of *E. crassipes* is an almost negligible process. The foliar morphology of *E. crassipes* is different from those macrophytes, avoiding the Cr adsorption by aerial parts. For this reason, translocation from roots to aerial parts was the only responsible process for the Cr accumulation in aerial parts.

Fig. 2 shows Cr, Ni and Zn total amounts (expressed in %) in water, roots and aerial parts of *E. crassipes* along time. Total amounts (mg) were estimated by multiplying the values of volume (L) or biomass (g dry weight) by the values of Cr, Ni or Zn total concentration in water ($mg\,l^{-1}$) or in plant tissues ($mg\,g^{-1}$ dry weight). After 8 h, the Cr accumulation was 31% and 1% in roots and aerial parts, respectively (Fig. 2a). At 15 d, 73% of the added Cr was accumulated in roots and 5% in aerial parts. The accumulation of Cr in aerial parts along time was significantly lower than that obtained for Ni and Zn. Ni accumulation in roots increased 21% and 40% in the first 30 min and 24 h of contact, respectively (Fig. 2b). The amount of Ni in roots continued increasing during the first 10 d, accumulating 72% of the added Ni in this period. Then, Ni did not increase significantly in roots. In aerial parts, a continuous increase of 10%, 16% and 22% were observed at 24 h, 10 d and 15 d, respectively. Zn amount increased 32% and 7% after 8 h of contact in roots and aerial parts, respectively (Fig. 2c). At the end of the experiment, metal accumulation in roots was 75%, 73% and 61% for Cr, Ni and Zn, respectively, while metal accumulation in aerial parts was 6%, 23% and 10% for Cr, Ni and Zn, respectively.

The BCFs calculated for aerial parts and roots along the experiment are shown in Table 1. The BCFs obtained in roots were significatively higher than those obtained in aerial parts. Both, the aerial parts and root BCFs increased along the experiment. Ni presented significantly higher BCFs than the other studied metals.

For each metal, both terms of the Eq. (2) were represented versus time in Fig. 3, showing the metal amount removed from water or accumulated in tissues along time. The values of the parameters of Eq. (2) are shown in Table 2. According to Fig. 3, it can be proposed that metal uptake from water by *E. crassipes* involved a fast and a slow component. The slow component was the main responsible for the removal of Cr and Zn. For Ni, there was no significant difference in water removal between the two components at the end of the experiment, demonstrating faster water removal kinetics than Cr and Zn.

In aerial and root tissues, the same components as in water were present. The fast metal root uptake suggests that adsorption to the cell walls is probably the process responsible for the fast component of root accumulation. The efficiency of the metal adsorption processes was also corroborated using non-living roots [12,13,24,25]. This fast stage might also include the processes of chemical sorption (absorption) as chelation [12,24] and ion exchange [26]. Root-mediated precipitation and biological processes as intracellular uptake (transported through the plasmalemma into the cells) were probably responsible for the slower stage of metal removal from the solution.

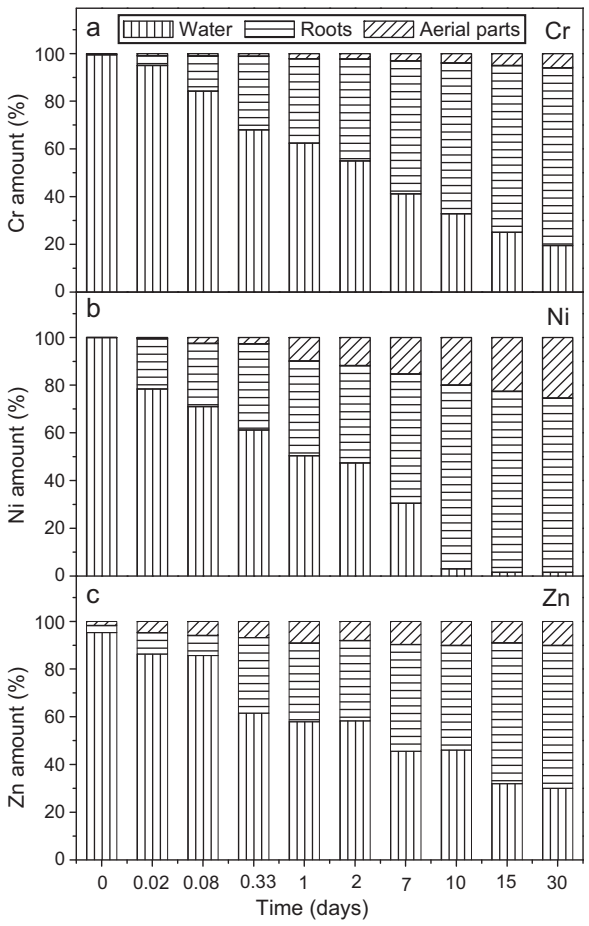

Fig. 2. Cr (a), Ni (b) and Zn (c) total amounts (expressed in %) in water, roots and aerial parts of *E. crassipes* along time. Total amounts in water (mg) were estimated by multiplying the values of volume (l) by the values of Cr, Ni or Zn total concentration in water ($mg\,l^{-1}$). Total amounts in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

Both fast and slow processes were responsible for Ni bioaccumulation in roots and aerial parts. Contrarily, root Cr accumulation was produced mainly by the slow process while Zn by the fast process. In aerial parts, Cr accumulation was the lowest, whereas Ni presented the highest amount, probably due to the root damage with biomass reduction. In the case of Zn, no significant differences were recorded between the bioaccumulation rate in aerial parts between the two terms of Eq. (2) in the first hours of contact, suggesting a high translocation rate. Zn acts on the photosynthetic process in foliar tissues [27]. This fact could justify the higher rate of translocation to the aerial parts, in comparison with the other metals. Cr and Ni translocations were slower but continuous processes along the experiment.

3.2. Plant study

For the purpose of comparison, biomass and chlorophyll *a* increase were expressed in %. Biomass increased in all treatments. At 30 d, total biomass increase percentage was significantly lower

Table 1
Bioconcentration factors (BCFs) vs. time of the studied metals obtained along the experiment.

Sampling Time	Cr		Ni		Zn	
	Leaf/water	Root/water	Leaf/water	Root/water	Leaf/water	Root/water
0.5 h	0.039 ± 0.012	0.225 ± 0.027	0.013 ± 0.004	1.345 ± 0.015	0.041 ± 0.008	0.317 ± 0.001
2 h	0.015 ± 0.003	0.561 ± 0.066	0.104 ± 0.008	2.132 ± 0.072	0.060 ± 0.007	0.347 ± 0.003
8 h	0.015 ± 0.006	1.144 ± 0.404	0.147 ± 0.048	2.961 ± 0.393	0.107 ± 0.046	2.041 ± 0.233
24 h	0.035 ± 0.003	0.997 ± 0.126	0.585 ± 0.073	4.576 ± 0.122	0.179 ± 0.067	2.375 ± 0.036
48 h	0.034 ± 0.016	1.517 ± 0.037	0.528 ± 0.002	4.633 ± 0.540	0.161 ± 0.039	2.094 ± 0.328
7 d	0.055 ± 0.007	1.986 ± 0.014	0.835 ± 0.037	7.868 ± 1.052	0.275 ± 0.154	4.059 ± 1.376
10 d	0.145 ± 0.020	2.774 ± 0.152	4.564 ± 0.911	37.484 ± 5.636	0.340 ± 0.045	4.589 ± 0.903
15 d	0.160 ± 0.041	5.603 ± 1.652	3.570 ± 0.260	37.909 ± 8.492	0.598 ± 0.222	5.143 ± 1.613
30 d	0.205 ± 0.007	5.964 ± 1.639	5.359 ± 0.708	35.830 ± 1.708	0.350 ± 0.117	4.859 ± 0.572

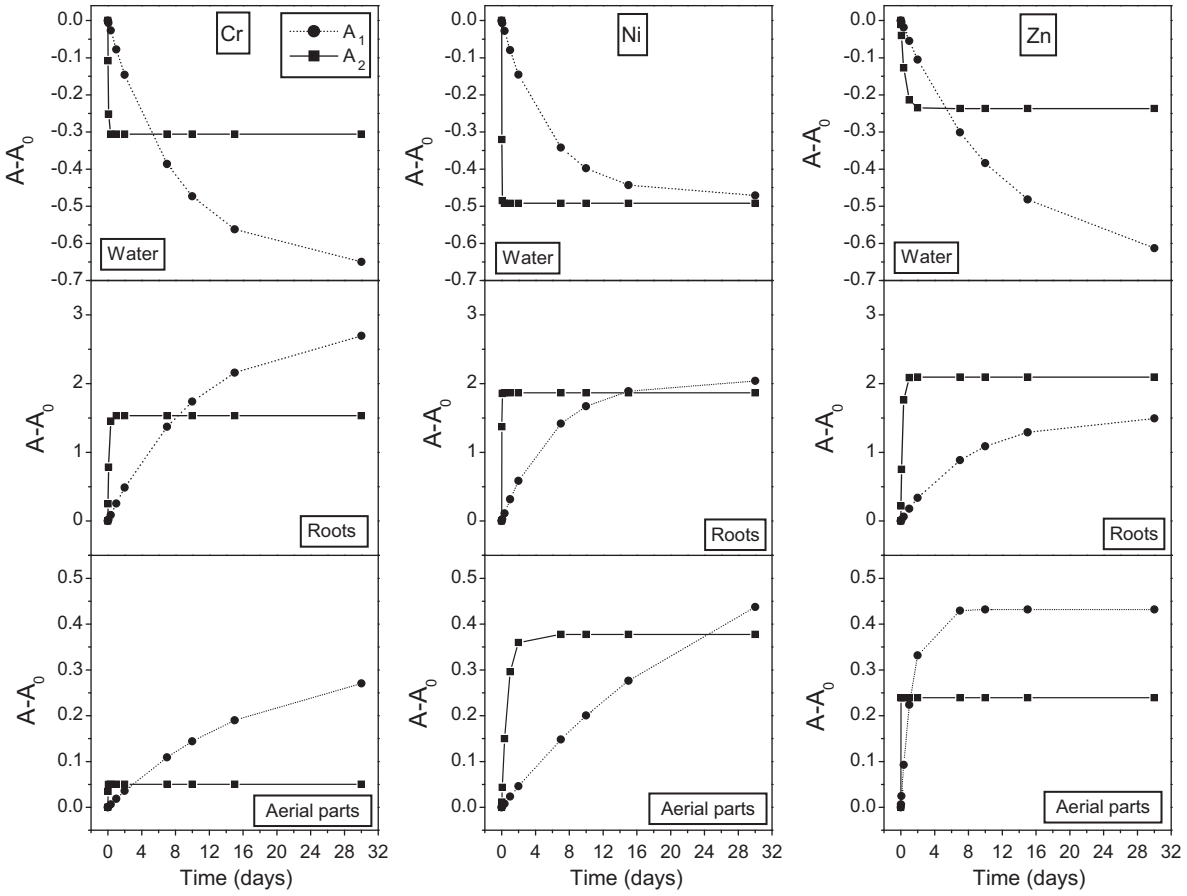

Fig. 3. Metal concentration decrease from water and metal amount increase in tissues vs. time, according to Eq. (2). A_0 = initial amount of Cr, Ni or Zn, A = amount of Cr, Ni or Zn at time t.

in the Ni treatment than that of the control and the other treatments (Fig. 4a). The treatment of Cr did not present significant differences in biomass increase regarding the control. In the Ni and Zn treatments, the aerial biomass increase was significantly lower than that of the obtained in the Cr treatment and in the control (Fig. 4b). The aerial biomass increase did not show statistical differences between the Ni and Zn treatments. The lowest root biomass increase was observed in the Ni treatment (Fig. 4c). The root biomass increase in the Zn treatment was also significantly lower than that obtained in the Cr treatment and in the control.

Cr, Ni and Zn are essential to the metabolism of plants. They are involved in a variety of critical functions including gene control,

Table 2
Empirical constants obtained in Eq. (2) for Cr, Ni and Zn concentrations in water and amounts in aerial parts and roots.

Empirical constants	Water concentration			Aerial part amount			Root amount		
	Cr	Ni	Zn	Cr	Ni	Zn	Cr	Ni	Zn
A_1	−0.666	−0.473	−0.662	0.329	0.659	0.432	2.873	2.053	1.530
A_2	−0.306	−0.492	−0.237	0.050	0.377	0.239	1.534	1.865	2.095
r	8.070	5.44	11.54	17.397	27.539	1.368	10.776	5.958	8.072
s	0.0.46	0.019	0.428	0.017	0.652	0.0002	0.112	0.015	0.180

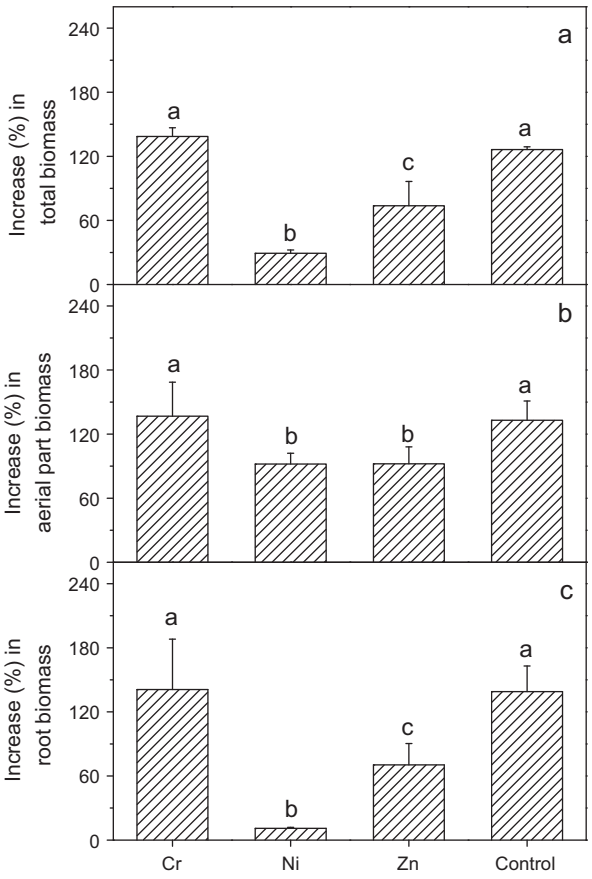

Fig. 4. Increase (in %) of the total biomass (a), aerial part biomass (b) and root biomass (c) of *E. crassipes* obtained at 30 d compared with control. Different letters represent statistically significant differences among the treatments. Bars represent standard deviations.

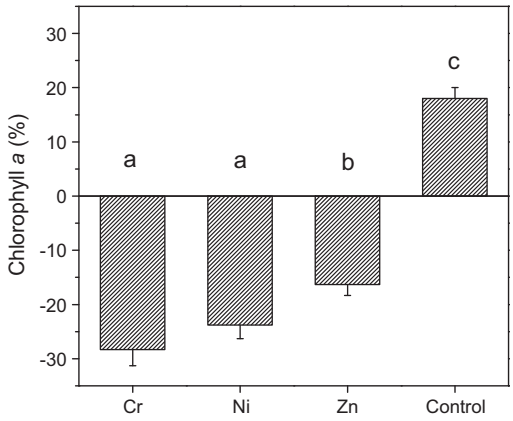

Fig. 5. Increase (in %) of the chlorophyll *a* concentrations in the metal treatments compared with control. Different letters represent statistically significant differences among the treatments. Bars represent standard deviations.

oxygen transport and active centres in enzymes [27,28]. However, when their concentrations reach a threshold value, they become first inhibitory and afterwards toxic. Comparing with the control, biomass showed an inhibitory effect for Ni and Zn while Cr did not affect the total biomass increase. Nevertheless, biomass increased in all treatments. These results are in agreement with Hadad et al. [8] on studying *S. herzogii* tolerance to Cr, Ni, and Zn, and Mufarrege et al. [11] and Odjegba and Fasidi [15] on studying *P. stratiotes* response to the same metals. Delgado et al. [4] reported that *E. crassipes* did not show weight reduction when exposed to concentrations up to 2 mg l^{-1} Cr or Zn.

In the control, the increase in chlorophyll *a* concentration was significantly higher in comparison with that obtained in the metal treatments, which showed a decrease in this parameter (Fig. 5). The Cr and Ni treatments showed a decrease in the chlorophyll *a* concentration significantly higher than the obtained in the Zn treatment.

Chlorophyll concentration in plants is a good toxicity indicator for different metals [8,29,30]. However, the plant responses depend on the contaminant and the macrophyte species. The studied metals were toxic for chlorophyll *a* production in *E. crassipes.* Although low Cr concentrations can enhance chlorophyll concentration by improving availability of biologically active Fe in plant tissue [31], and Zn is involved in photosynthetic processess [27], these metals were toxic in the concentration used for chlorophyll production. Delgado et al. [4] observed chlorosis in *E. crassipes* during exposure to several increasing concentrations of Cr and Zn, being more intense in the case of Zn. Khellaf and Zerdaoui [16] found a toxic threshold value of 0.5 mg l^{-1} Ni for *Lemna gibba*. In our work we used a concentration of 1 mg l^{-1} Ni and observed that this metal exhibited the most noxious effects on *E. crassipes*, represented by a toxic effect on chlorophyll concentration. Maine et al. [7] 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. [32] 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.

Even though chlorophyll concentration was sensitive to the studied metals, *E. crassipes* adaptability was represented by the biomass increase, particularly in the Cr treatment, which did not showed significant differences with the control. These responses allowed this species to survive and mantain a high metal accumulation.

In all treatments a root length decrease was observed. Ni treatment showed the highest decrease, while the control showed an increase (Fig. 6a). In the Zn treatment, the root CSA was significantly higher than the values obtained in the other treatments and in the control (Fig. 6b). Root CSA was not significantly different between the Ni treatment and the control. The Zn treatment showed the highest number of metaxylematic vessels (Fig. 6c). No significant differences were observed among the Cr and Ni treatments and the control.

Plants exposed to Zn decreased their root length and significantly increased root CSA and number of vessels. Mufarrege et al. [11] reported a similar response in the internal root morphology of *P. stratiotes* when exposed to Zn. Coarse roots are an adaptive response to an increase in nutrient concentrations in the environment [33]. Zn produced changes in the root morphology similar to the changes that the nutrients produced. Variations in 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. [21] demonstrated that an increase in root CSA has a positive influence on hydraulic conductance of roots. Therefore, a higher root CSA and number of vessels, promote higher uptake, accumulation and transport of the metals to aerial parts. Root morphological plasticity of *E. crassipes* is an important mechanism to improve the uptake and tolerance of metals. The observed morphological changes enhanced the Zn transport to aerial parts during the first days of the experiment.

Despite the toxic effects observed, the three metals were efficiently accumulated in *E. crassipes* tissues at the same time they were removed from water. Ni presented the lowest initial concentration in tissues and the highest removal from water and it exhibited the most noxious effects on the plant. Zn was the metal

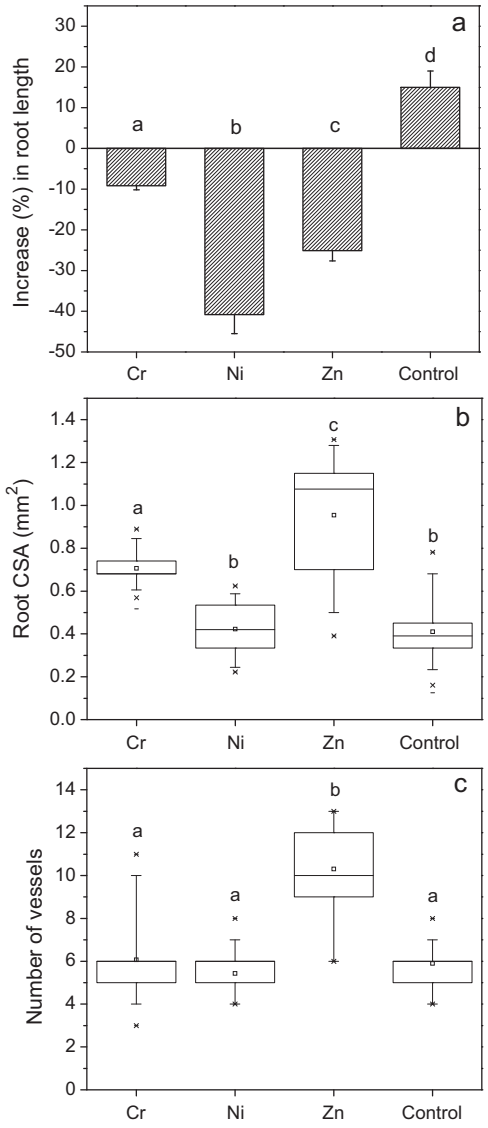

Fig. 6. Increase (in %) of the root length (a), box and whisker plots of root CSA, and number of vessels (c) of *E. crassipes* at the end of the experiment. Different letters represent statistically significant differences among treatments.

with the highest initial root concentration (Fig. 2c), being the metal that showed the lowest water removal. Because at the end of the experiment Zn remained in water, probably the root CSA and number of metaxilematic vessels increased in order to maintain its uptake. Probably, in a longer experiment, Zn would be removed completely from water.

4. Conclusions

Metal uptake involves a fast and a slow component. The slow process was the main responsible for Cr and Zn removal, while both components carried out Ni removal. Ni showed the highest accumulation rate in roots. In aerial parts, Zn presented the fastest accumulation rate while Cr and Ni presented a slower but continuous accumulation process. Ni and Zn produced growth inhibition and the three metals produced a chlorophyll decrease. Despite the sublethal effects registered, *E. crassipes* demonstrated that it could uptake Cr, Ni and Zn efficiently and survive in polluted water bodies, such as constructed wetlands.

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