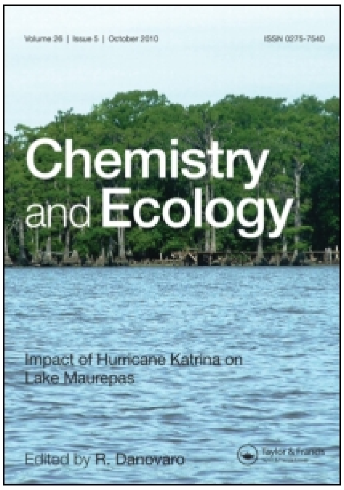

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RESEARCH ARTICLE

Phytoextraction potential of six plant species grown in multimetal contaminated soil

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A pot experiment was conducted to compare the phytoextraction potential of six plant species (*Arabidopsis thaliana*, *Brassica juncea*, *Crotalaria juncea*, *Cynodon dactylon*, *Parthenium integrifolium* and *Phragmitis communis*) for Zn, Ni, Cr and Cd removal by growing on multimetal contaminated soil. The results revealed that metal accumulation was plant specific. *A. thaliana* accumulated maximum Zn and Cd, whereas *B. juncea* and *C. dactylon* accumulated Ni and Cr in their shoots, respectively. *B. juncea* exceeded the Ni hyperaccumulation limit and accumulated 3916 $mgkg^{-1}$ dry wt. The remediation factor for Zn and Cd was higher in *A. thaliana, B. juncea* and *C. juncea*, whereas it was higher for Ni in *B. juncea*. *A. thaliana* exhibited the maximum bioconcentration factor (BCF) for Zn and Cd whereas *B. juncea* showed for Ni and *C. dactylon* for Cr. The value of the translocation factor (TF) was > 1 for all metals in all plant species, except *C. dactylon* for Zn. The maximum TF value for Cd and Zn was recorded in *B. juncea*, for Ni in *A. thaliana* and for Cr in *C. dactylon*. Relatively higher metal content was observed in a $CaCl_2$ extract than a natural soil solution. However, the available metal content in the soil solution tended to decrease at successive suction times.

Keywords: phytoextraction; heavy metals; remediation factor; bioconcentration factor; translocation factor

1. Introduction

Heavy metal pollution has now become a major environmental concern because it adversely affects soil structure, its fertility and crop productivity [1–3]. To remediate heavy metal contaminated sites, application of conventional methods such as landfilling or excavation and extraction have little scope, owing to high energy input and engineering costs [4,5]. Phytoextraction is one of the strategies of phytoremediation, where plants are used to uptake and accumulate heavy metals in above ground biomass, which can be harvested and removed from the site. Depending upon the trace element accumulation capability, plants have been identified as excluders (with avoidance mechanisms for element uptake), highly sensitive indicators (lacking protection mechanisms) and accumulators (with mechanisms of metal tolerance and accumulation

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capability in above ground biomass). Further, hyperaccumulator plants, a sub group of accumulators, have the mechanism of metal tolerance and are able to survive on metalliferous soils without showing any metal toxicity symptoms [6]. Over 400 plants have been reported as metal hyperaccumulators which accumulate more heavy metals in their aboveground biomass than the threshold limits [6,7]. For instance, *Thlaspi caerulescens* or *Alyssum bertolonii* accumulate high amounts of one or more metals in their aboveground tissue but their use in the field is limited because of their slow growth and low biomass production [8]. Recently, few wild plant species such as *Cynodon dactylon*, *Hirsfeldia incana*, *Malva nicaeensis*, *Sylibum marianum* and *Conyza discoridies* have been reported the most promising species for Pb and/or Zn phytoextraction [9,10], and *Valisneria spiralis* for Zn, Pb and Cd [11]. Phytoextraction potential of plants is also influenced by the mobility and availability of heavy metals in soil and plants, and thus the bioconcentration factor (BCF, the ratio of metal concentration in the roots to that in soil) and translocation factor (TF, ratio of metal concentration in the shoots to the roots) could better adjudge phytoextraction potential [12]. Plants exhibiting TF and BCF values greater than one are considered promising phytoextractor [13]. Recently, Yoon et al. [12] reported *Phyla nodiflora* having TF values of 12 and 6.3 for Cu and Zn as suitable phytoextractors, and *Gentiana pennelliana* having BCF values of 11, 22 and 2.6 for Pb, Cu and Zn as a phytostabiliser, and emphasised detailed investigations on the phytoremediation potential of plants.

Rapid industrialisation in western Uttar Pradesh (India) has exposed the soil to various effluent inputs, including heavy metals. This has resulted in the region having contaminated soils [14] which need to be improved for agricultural purposes. In this context, the present study was undertaken with the objectives: (1) to determine the concentrations of Zn, Ni, Cr and Cd in plant biomass growing on a metal contaminated soil; (2) to compare metal concentrations in the aboveground biomass to those in roots and in soils, and (3) to assess the exploitation feasibility of these plants for phytoremediation purposes.

2. Materials and methods

2.1. *Site characterisation*

The soil used in this study was collected from an electroplating industry effluent fed site located in a suburban area of Moradabad, India (latitude 28°28′N and longitude 78°44′E). The discharge of untreated and/or partially treated effluent of metallurgical industries in this region led to the enhancement of Cd, Ni, Cr and Zn content in the soil. The surface soil (0–20 cm) was collected in plastic bags, air-dried, gently ground to pass through a 2-mm sieve and analysed. The soil was sandy loam (sand 60–80%, silt 10–24%, clay 8–16%) having pH 5.7, organic matter 2.4%, CEC 21.6 meq 100 g^{-1}, Zn 584 mg kg^{-1}, Ni 254 mg kg^{-1}, Cr 91 mg kg^{-1} and Cd 9.8 mg kg^{-1}. The total heavy metal content was determined using an atomic absorption spectrophotometer (GBC Avanta Ver. 1.33, Australia) after digesting 0.5 g of dried soil samples with 15 ml of HNO_3, H_2SO_4 and $HClO_4$ in 5:1:1 ratio at 80°C [15] and filtered through Whatman No. 42 filter paper followed by dilution up to 50 ml with triple distilled water.

2.2. *Pot experiment*

A pot experiment study was undertaken to assess Zn, Cr, Ni and Cd phytoextraction potential and remediation effectiveness of locally available and dominant plant species (i.e. *Arabidopsis thaliana*, *Brassica juncea*, *Cynodon dactylon*, *Parthenium integrifolium*, *Phragmitis communis* and *Crotalaria juncea*) grown on multimetal contaminated soil collected from the effluent fed region. Five kg of air-dried soil fertilised with 0.5 g N, 0.16 g P and 0.4 g K was placed into each plastic pot having 25 cm diameter and 30 cm height. Pots were placed in the greenhouse under day/night temperature of 27/18 °C and a relative humidity of 50/70% (day/night). Three plants per pot were grown and fertilised once with limiting nutrients during vegetative growth. As such, there were 18 pots, representing six plant species in triplicate along with three additional pots without plants, as a control. After 60 days of experiment, roots and shoots of tested plant species were harvested separately and plant biomass was computed on a per plant and per pot basis.

Soil solution was collected from the pots after 30 days of experiment to investigate actual concentration of available heavy metals in the soil and $CaCl_2$ extract. The changes in available metal content in the soil solution of different plant species at different suction periods (i.e. after 20, 40 and 60 days) were also determined. The pots installed with specialised suction cups (D.I. Gottfried Wieshammer, Wien, Austria) in triplicate were filled with deionised water up to 100% water holding capacity one day before suction and left for 24 h to reach equilibrium. A total of 10 ml of soil solution from each pot was taken and analysed for Zn, Ni, Cr and Cd concentration by atomic absorption spectrophotometry (AAS).

2.3. *Laboratory analysis*

After harvest, the roots and shoots were washed with deionised water, dried at 65 °C and weighed for plant biomass estimation. Then 0.5 g of milled plant material was digested with a mixture of concentrated HCl/HNO_3 (4:1 v/v) [16] and the solution was analysed for heavy metals using AAS. The availability of heavy metals in soil solution was determined by adding distilled water in a ratio of 1:1 (w/v). Metal availability in the $CaCl_2$ extract was estimated by extracting with 0.01 mol/l $CaCl_2$ in a 1:10 ratio (w/v) [17]. The filtrate was analysed for Zn, Ni, Cr and Cd using AAS. Bioconcentration factor (BCF) for each metal was determined as the ratio of metal concentration in plant roots to that of soil and the translocation factor (TF) was calculated as the ratio of metal concentration in shoot to roots [12]. Remediation capacity of each plant species was determined by calculating the remediation factor (Rf) as the percent of metal removed from a fixed amount of soil with respect to plant metal concentration and plant yield [18,19]. Data are presented as mean values ± standard deviation. To verify the statistical significance of difference among treatments and species, experimental data were analysed using SPSS computer software (Statgraphics Plus v. 5.0) with ANOVA tests ($p < 0.05$).

3. Results and discussion

3.1. *Biomass yield and metal accumulation in plant*

All six plant species grown on metal contaminated soil showed no visible symptoms of toxicity. Plant biomass varied with respect to species and ranged from 4.6–31.3 g dry weight per plant. The maximum biomass yield was observed in *C. juncea* and the minimum in *C. dactylon*. The metal accumulation in tissues also differed among the six plant species grown on the same soils, indicating their varied capabilities for metal extraction (Table 1). The total metal content in plant tissues ranged from 1034–6097 mg kg^{-1} for Zn, 570.8–3916.8 mg kg^{-1} for Ni, 62.2–852.6 mg kg^{-1} for Cr and 30.5–107 mg kg^{-1} for Cd. Except *B. juncea* for Ni, no other plant species exceeded the hyperaccumulation threshold value for metal extraction; yet they accumulated conspicuously higher concentrations of heavy metals. *B. juncea* accumulated Ni up to 3916 mg kg^{-1}, exceeding the threshold limit (> 1000 mg kg^{-1}) [6] and thus could be regarded as a Ni hyperaccumulator.

Table 1. Average plant biomass (g) and total metal accumulation (mg kg^{-1}) in different plant species after 60 days of exposure ($n = 3$).

	Biomass yield		Zn		Ni		Cr		Cd	
Plant species	Per plant	Per pot	Roots	Shoot	Roots	Shoot	Roots	Shoot	Roots	Shoot
A. thaliana	16.6 ± 1.6	51.2 ± 3.3	2175 ± 17.2[a]	4732 ± 23.4[a]	179.3 ± 4.5[a]	714.6 ± 9.4[a]	33.4 ± 2.3[a]	53.6 ± 2.5[a]	25.4 ± 2.1[a]	81.5 ± 4.4[a]
B. juncea	22.5 ± 2.4	68.3 ± 5.8	1240 ± 13.6[b]	3863 ± 21.3[ab]	1132.6 ± 3.7[b]	2784.3 ± 6.2[b]	36.8 ± 3.5[a]	61.4 ± 3.2[a]	14.2 ± 3.3[ab]	77.4 ± 3.8[a]
C. juncea	31.3 ± 2.8	92.8 ± 7.2	1105 ± 12.3[b]	3145 ± 16.7[b]	194.7 ± 5.2[a]	632.8 ± 7.3[a]	24.0 ± 2.6[a]	38.2 ± 2.7[a]	17 ± 2.4[ab]	37.6 ± 3.5[b]
C. dactylon	4.6 ± 0.2	14.6 ± 1.4	580 ± 6.5[c]	454 ± 4.2[d]	187.6 ± 3.4[a]	383.2 ± 5.3[c]	106.0 ± 4.5[b]	746.6 ± 9.4[b]	11.3 ± 1.3[b]	19.2 ± 2.2[c]
P. integrifolium	23.8 ± 3.7	70.4 ± 5.7	486 ± 7.2[c]	712 ± 9.4[d]	238.2 ± 4.1[a]	464.2 ± 4.4[ac]	28.8 ± 3.2[a]	65.8 ± 4.6[a]	15 ± 1.6[ab]	23.8 ± 1.5[bc]
P. communis	18.3 ± 1.8	56.2 ± 4.9	562 ± 11.5[c]	365 ± 13.3[c]	270 ± 5.2[a]	510 ± 9.1[ac]	35.4 ± 2.3[a]	50.4 ± 2.8[a]	13.4 ± 1.5[ab]	29.3 ± 3.4[bc]

Note: ± Indicates standard deviation; different letters in the same column indicate significant differences ($p < 0.05$).

In most cases, the metal transport was higher in shoots indicating high mobility of metals from root to shoot. Maximum accumulation of Zn and Cd was recorded in the shoots of *A. thaliana* whereas the minimum was found in *C. dactylon*. The maximum accumulation of Ni and Cr was observed in the shoots of *B. juncea* and *C. dactylon* respectively, whilst the minimum value was recorded in *C. dactylon* and *C. juncea*. *C. dactylon* accumulated greater amounts of Zn in the roots, showing similarity with the report of [10]. Greater accumulation of metal in the roots could be attributed to the increased metal adsorption on the root surface, being facilitated by relatively less mobility of metals in the root zone [20–22]. The results revealed that the metal accumulation was plant specific, which may be due to several factors, including environmental conditions, metal species and their available forms [23,24].

3.2. *Phytoremediation capacity*

Plant remediation capacity, determined by calculating the remediation factor (Rf), revealed that the plant species with low metal concentrations in their shoots compensated the remediation effectiveness with more biomass production compared to those species with higher metal concentrations and less biomass production. The results presented in Figure 1 show that *B. juncea* and *C. juncea*, with lower Zn and Cd accumulation in shoots (Table 1), compensated the remediation effectiveness by producing more biomass, whilst *A. thaliana* with higher Zn and Cd accumulation compensated the remediation effectiveness by less biomass production. Although these plant species differed strategically they may have similar effects on the phytoextraction of Zn and Cd from contaminated sites. Ebbs et al. [8] reported that *B. juncea*, accumulating one-third of the concentration of Zn in its tissue, was a more promising phytoextractor than *Thlaspi caerulescens* (a known hyperaccumulator of Zn) because of producing ten-times more biomass than the latter. *B. juncea* showed an extremely high remediation capacity for Ni whereas no other species except *C. dactylon* performed well for Cr. The results depicted that the plants with good remediation capacity for a particular metal may be regarded as potential phytoextractors.

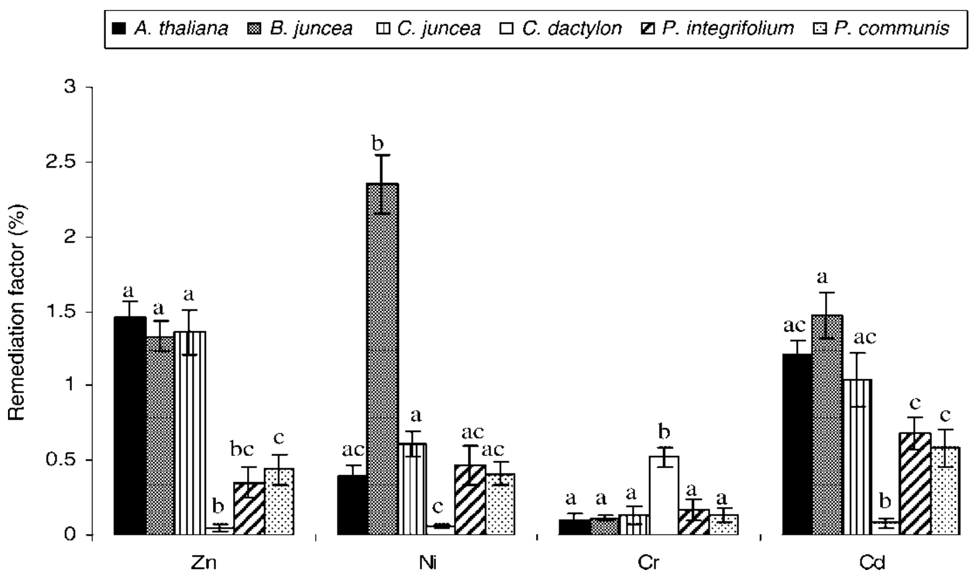

Figure 1. Average remediation factor (Rf in %) for metals in different plant species. Mean values (± SD) marked with different letters in a column differed significantly at $p < 0.05$.

3.3. *Bioconcentration factors (BCF) and translocation factors (TF) for heavy metals*

The results presented in Table 2 showed BCFs and TFs for different heavy metals, which are the key measurements to estimate a plant's potential for metal phytoextraction. Plants exhibiting TF and BCF values > 1 are suitable for phytoextraction [13]. Among tested plant species, *A. thaliana* had the highest BCF for Zn and Cd (i.e. 3.72 ± 0.14 and 2.59 ± 0.11 respectively), followed by *B. juncea* and *C. juncea*, though their total Zn and Cd accumulation was less than the hyperaccumulation threshold limit (Table 1). However, in the cases of Ni and Cr, all plant species had BCF values < 1 except *B. juncea* (4.46 ± 0.13) and *C. dactylon* (1.16 ± 0.07). The results matched well with those of Cardoso et al. [25] and Abou-Shanab et al. [10], who reported higher concentrations of Ni and Cr in roots of *C. juncea* and *C. dactylon* respectively. The experimental results showed that *A. thaliana, B. juncea* and *C. juncea* had good phytoextraction potential for Zn and Cd. However, *B. juncea* and *C. dactylon* could also be used for phytostabilisation of Ni and Cr respectively from contaminated sites, as they exhibited BCF values > 1. In addition to BCFs, greater TF values were also recorded for tested metals by all plant species (Table 2). *B. juncea* appeared most efficient in translocating Cd (4.5 ± 0.14) and Zn (3.12 ± 0.15) from root to shoot, whereas *A. thaliana* was better for Ni (3.98 ± 0.16) and *C. dactylon* for Cr (7.04 ± 0.28). However, other plant species also contributed in translocation of Zn and Cd from root to shoot as they exhibited TF > 1. This may be due to the fact that Cd and Zn are more mobile in nature and are retained less strongly by the soil [26]. The results are in accordance with Fischerova et al. [27], who reported higher TF values for Zn and Cd compared to other metals. In the present study, plant species, viz. *A. thaliana*, *B. juncea*, *C. juncea*, with high uptake and translocation of Zn, Ni and Cd from root to shoot emphasised their strong potential for phytoextraction.

3.4. *Available metal content in soil*

In general, metal content determined in $CaCl_2$ extraction exceeded that of soil solution (Figure 2). The available metal content in $CaCl_2$ extracts ranged from 196–993 $\mu g\, l^{-1}$ for Zn, 19.5–77.8 $\mu g\, l^{-1}$ for Cd, 123–820 $\mu g\, l^{-1}$ for Ni and 92–548 $\mu g\, l^{-1}$ for Cr. The values for Ni and Cd significantly differed ($p < 0.05$) in soil solution and $CaCl_2$ extract, thus verifying the applicability of $CaCl_2$ extraction procedures determining available Zn, Ni and Cd concentration in soil.

Heavy metal availability in soil solutions of different plant species were determined with respect to time (Figure 3). Zn content in *A. Thaliana* and *C. juncea* and Ni in *B. juncea* decreased significantly ($p < 0.05$) during successive suctions, as compared to the control. Decrease in Cd content followed suit with moderate magnitude, which may be accounted for by its high toxicity to plants. In contrast, Cr exhibited a slight increase in soil solution by all the plants except *C. dactylon*, which may be due to a restriction in the metal uptake by most of the plant species [27]. The relatively stable concentration of metals in the soil solution during vegetative growth conforms to the theory of continuous metal supply from the soil [28]. However, the metal uptake is largely influenced by its availability, which is in turn determined by both external (soil-associated) and internal (plant-associated) factors [10]. The higher concentration of available Zn and Cd was found in soil solutions of *A. thaliana*, whereas that of Ni and Cr was obtained in the soil solutions of *B. juncea* and *C. dactylon* respectively during all suctions. This might be due to the excretion of root exudates of organic and inorganic nature, leading to changes in the soil's biochemical and physical properties [29] and metal mobilisation. The results elucidated a significant role of plants enabling bioavailability of heavy metals in soil through excretion of root exudates, and thus facilitating the extraction of metals from the contaminated soils. However, the increase in Cd bioavailability in control pots may be associated with the acidic soil pH, suitable redox potential, cation exchange capacity, organic matter content and presence of other metals etc. [30,31].

Table 2. Bioconcentration and translocation factors (mean ± SD) for Zn, Ni, Cr and Cd in different plant species ($n = 3$).

Plant species	BCF (Bioconcentration factor)				TF (Transfer factor)			
	Zn	Ni	Cr	Cd	Zn	Ni	Cr	Cd
A. thaliana	3.72 ± 0.14^{a}	0.70 ± 0.06^{a}	0.37 ± 0.04^{a}	2.59 ± 0.11^{a}	2.18 ± 0.08^{a}	3.98 ± 0.16^{a}	1.60 ± 0.05^{a}	3.20 ± 0.09^{a}
B. juncea	2.12 ± 0.08^{b}	4.46 ± 0.13^{b}	0.40 ± 0.04^{a}	1.75 ± 0.06^{b}	3.12 ± 0.15^{b}	2.45 ± 0.10^{b}	1.67 ± 0.07^{a}	4.50 ± 0.14^{b}
C. juncea	1.89 ± 0.06^{b}	0.76 ± 0.08^{a}	0.26 ± 0.01^{a}	1.73 ± 0.08^{b}	2.85 ± 0.06^{ab}	3.26 ± 0.11^{a}	1.59 ± 0.05^{a}	2.21 ± 0.08^{c}
C. dactylon	0.99 ± 0.06^{c}	0.73 ± 0.02^{a}	1.16 ± 0.07^{b}	1.15 ± 0.05^{b}	0.78 ± 0.04^{c}	2.04 ± 0.07^{b}	7.04 ± 0.28^{b}	1.70 ± 0.06^{cd}
P. integrifolium	0.83 ± 0.03^{c}	0.93 ± 0.05^{a}	0.32 ± 0.03^{a}	1.53 ± 0.05^{b}	1.47 ± 0.08^{d}	1.95 ± 0.04^{b}	2.28 ± 0.13^{c}	1.59 ± 0.06^{d}
P. communis	0.96 ± 0.05^{c}	0.86 ± 0.06^{ac}	0.39 ± 0.02^{a}	1.36 ± 0.06^{b}	2.42 ± 0.13^{ab}	1.89 ± 0.08^{b}	1.42 ± 0.06^{a}	2.18 ± 0.11^{c}

Note: Different letters in the same column indicate significant differences ($p < 0.05$).

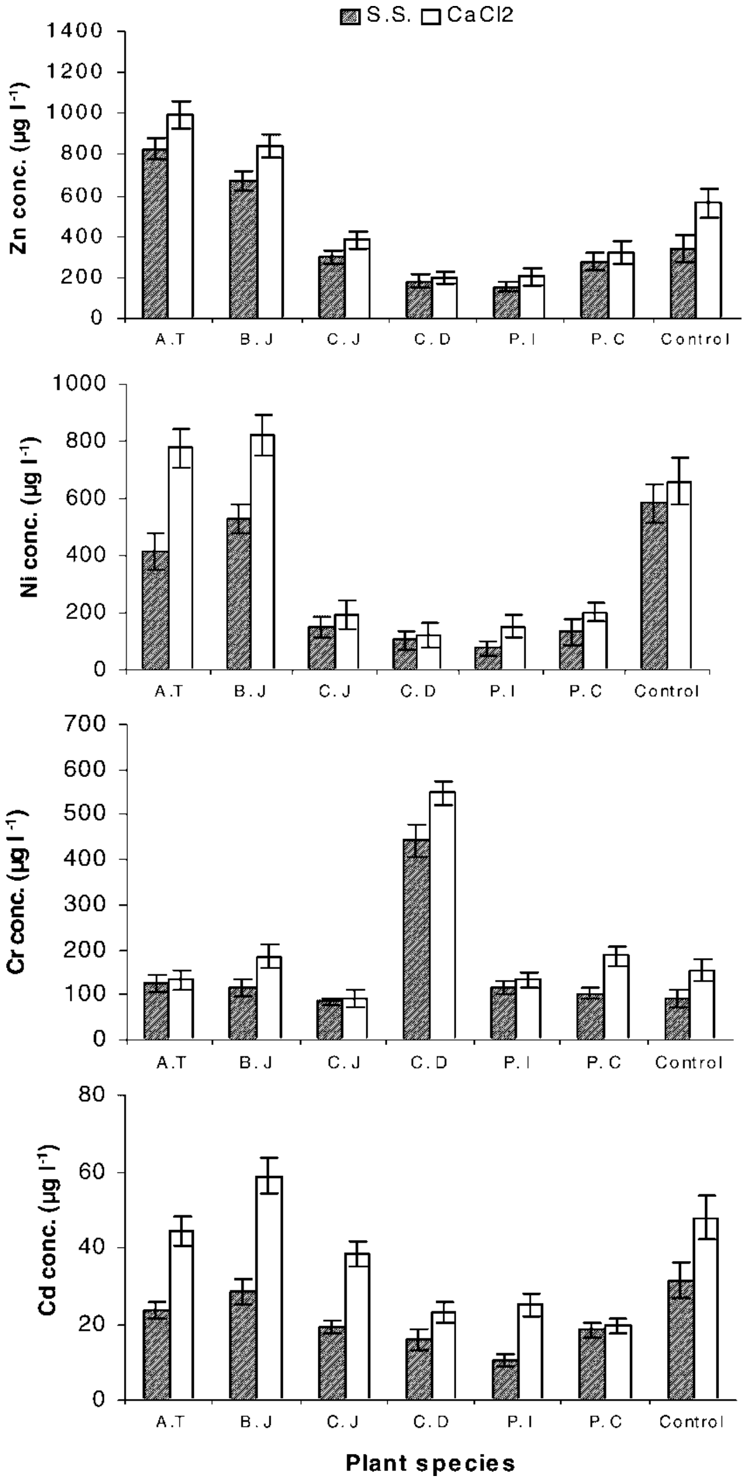

Figure 2. Available metal content ($\mu g\, l^{-1}$) in soil solution (S.S.) and in $CaCl_2$ extract ($n = 3$) in response to different plant species: A.T., *A. thaliana*; B.J., *B. juncea*; C.J., *C. juncea*; C.D., *C. dactylon*; P.I., *P. integrifolium*; P.C., *P. communis*.

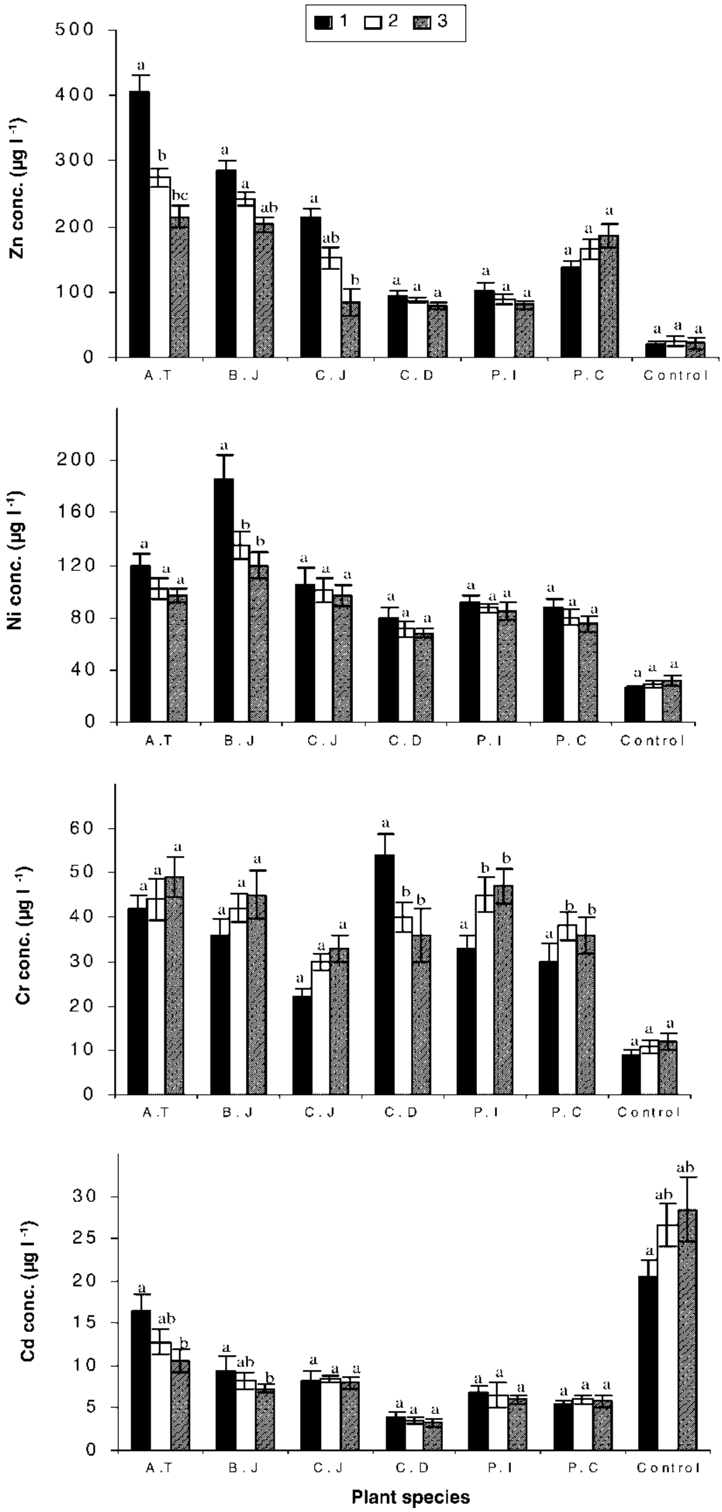

Figure 3. Available metal content in soil solution ($n = 3$) at different suction periods (i.e. 1, 20 days; 2, 40 days and 3, 60 days) in response to different plant species; A.T., *A. thaliana*; B.J., *B. juncea*; C.J., *C. juncea*; C.D., *C. dactylon*; P.I., *P. integrifolium*; P.C., *P. communis*.

4. Conclusions

Among the tested plants, *A. thaliana*, *B. juncea* and *C. juncea* confirmed their phytoextraction capability for Zn, Ni and Cd, *C. dactylon* for Cr and only *B. juncea* behaved as a Ni hyperaccumulator. The plant species adopted different strategies for maintaining phytoextraction potential. For example, *A. thaliana* extracting more metal produced less above ground biomass, whilst *B. juncea* and *C. juncea* accumulated a comparatively smaller amount of metals but compensated their remediation effectiveness by high biomass production. However, the rest of the three plant species behaved arbitrarily. Based on the experimental results, it is concluded that the application of these plant species could reduce metal pollution up to acceptable levels, and thus remediate the contaminated soil effectively.

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