Uptake of heavy metals by *Typha capensis* from wetland sites polluted by effluent from mineral processing plants: implications of metal–metal interactions

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Abstract The aim of the present work was to demonstrate the existence of metal–metal interactions in plants and their implications for the absorption of toxic elements like Cr. *Typha capensis*, a good accumulator of heavy metals, was chosen for the study. Levels of Fe, Cr, Ni, Cd, Pb, Cu and Zn were determined in the soil and roots, rhizomes, stems and leaves of *T. capensis* from three Sites A, B and C polluted by effluent from a chrome ore processing plant, a gold ore processing plant, and a nickel ore processing plant, respectively. The levels of Cr were extremely high at Site A at 5415 and 786–16,047 $\mu\text{g g}^{-1}$ dry weight in the soil and the plant, respectively, while the levels of Ni were high at Site C at 176 and 24–891 $\mu\text{g g}^{-1}$ in the soil and the plant, respectively. The levels of Fe were high at all three sites at 2502–7500 and 906–13,833 $\mu\text{g g}^{-1}$ in the soil and plant, respectively. For the rest of the metals, levels were modest at 8.5–148 and 2–264 $\mu\text{g g}^{-1}$ in the soil and plant, respectively. Pearson's correlation analysis confirmed mutual synergistic metal–metal interactions in the uptake of Zn, Cu, Co, Ni, Fe, and Cr, which are attributed to the similarity in the radii and coordination geometry of the cations of these elements. The implications of such metal–metal interactions (or effects of one metal on the behaviour of another) on the uptake of Cr, a toxic element, and possible Cr detoxification mechanism within the plant, are discussed.

Keywords *Typha capensis* · Bio-concentration factor · Chromium detoxification · Heavy metal uptake · Metal–metal interactions

Introduction

The possibility of plants to be used in phytoremediation of sites polluted by heavy metals has led to increased scientific interest in the mechanisms involved in the absorption of metals from the soil by plants, and their translocation within the plant. The metals iron (Fe), zinc (Zn), copper (Cu), nickel (Ni), cobalt (Co), manganese (Mn) and molybdenum (Mo) are essential for plant growth, while cadmium (Cd), chromium (Cr), lead (Pb) and mercury (Hg) are not essential and if present in high concentration in the plant, may have toxic effects on the plant's physiological processes (Rausser 1999; Williams et al. 2000; Nagajyoti et al. 2010). For normal growth and development, plants have, therefore, developed mechanisms designed to maintain internal concentrations of essential elements between their deficiency and toxicity levels, and those of non-essential elements below their toxicity levels (Rausser 1999). This is achieved by production of a range of enzymes that control not only the absorption of heavy metals from the soil, but also their translocation to the shoots. If we assume that metals compete for binding sites on such enzymes, then synergistic and antagonistic metal–metal interactions may be expected during the absorption and translocation of certain metals depending on the concentration, size and coordination geometry of their ions. The aim of the present work was to study the uptake of Zn, Cu, Cd, Cr, Ni, Fe and Pb by *Typha capensis* from wetland sites receiving effluent from chrome ore, nickel ore and gold ore processing plants in Zimbabwe, with a view to

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demonstrate the existence of metal–metal interactions in the plant, and to assess the potential of the plant for phytoremediation and phytoextraction of heavy metals from soils.

Typha capensis belongs to the *Typhaceae* family which comprises a single genus *Typha* (Morton 1975). Principal species of *Typha* (*T*) are *T. latifolia* L., *T. Angustifolia* L., *T. domingensis* Pers., *T. capensis* Rohrb., *T. orientalis* Presl., and *T. elephantina* Roxb. All inhabit slow moving shallow water and banks of freshwater ponds, lakes, rivers, dams, canals, ditches and swamps. *T. Capensis* is found throughout Southern Africa, Madagascar, the Philippines and New Guinea. The uptake of heavy metals by *T. capensis* was previously studied by Van der Merwe et al. (1990), Ma (2005) and Moodley et al. (2007), who reported that *T. capensis* exhibited high capacity to accumulate Cd, Cr, Ni, Zn, Mn, Fe, and Pb. The potential of *Typha* for use in phytoremediation of wastewaters has been reviewed by Singh et al. (2012), who concluded that *Typha* has immense potential for use in the phytoremediation of heavy metal contamination, and that more studies were required to gain better understanding of the mechanisms involved in the uptake and accumulation of heavy metals by the plant. In this paper we demonstrate the existence of synergistic metal–metal interactions in the adsorption of Cu, Co, Ni, Cr and Fe by the plant, and that *T. capensis* has potential for the phytoextraction of Zn, Cu, Cd and Fe from polluted soils.

Materials and methods

Sample collection and pre-treatment

Soil and *T. capensis* plant samples were collected from three wetland sites polluted by effluent from ore processing plants in Zimbabwe as follows: Sampling Site A is a chrome ore processing plant tailings dump site in the mining town of Kwekwe (18.9201°S, 29.8237°E). The tailings dump site is surrounded by a trench to catch any seepage from the tailings dumpsite. High levels of chromium are expected at Sampling Site A. Sampling Site B is a wetland along a stream receiving effluent from a gold ore processing plant at the north-western outskirts of the town of Bindura (17.300066°S, 31.301893°E). The plant employs the cyanidation process to recover gold from the ore. This necessitates use of large amounts of ferrous sulphate to neutralise the cyanide before the tailings from the cyanidation process can be deposited onto the slime dams. Thus, high levels of Fe pollution are expected at Site B. Sampling Site C is a wetland along a stream receiving effluent from a nickel ore processing plant at the southern

outskirts of the town of Bindura (17.29706°S, 31.33465°E). High levels of nickel pollution are expected at Site C.

Soil and plant samples were collected at 9 points about 50 m apart in the trench that surrounds the tailings dumpsite at Site A, and in the wetland along the stream at Sites B and C. Soil samples were collected using a manual auger 2.5 cm in diameter, to a depth of 15 cm. 9 whole *T. capensis* plants of nearly the same height were harvested at each Site A, B and C, by uprooting using a garden fork. Soil and plant samples were stored in polyethylene bags previously washed with tap water, followed by 10% nitric acid (HNO₃), and finally washed with distilled water.

At the laboratory, plant samples were washed with tap water followed by distilled water, and then separated into the different organs: roots, rhizomes, stems and leaves. The plant samples were left to air-dry at room temperature in a clean room, then oven-dried at 70 °C for 24 h. Soil samples were oven-dried at 105 °C overnight. Both dry soil and plant samples were ground into a fine powder using a silicon mortar and pestle. Soil samples from each sampling site were then combined into one composite sample and mixed thoroughly by quartering. Plant samples from each sampling site were similarly combined into one composite sample. The composite soil samples were then sieved through a 2-mm sieve (United States Standard Sieve no. 10, Endecotts Ltd, London, UK), while composite plant samples were sieved through a 0.5 mm sieve (Endecotts Ltd, London, UK). Both sieved soil and plant samples were stored in Teflon bags until required for analysis. Physico-chemical properties of the soil samples, see Table 1, were determined using standard methods (Page 1982).

Equipment

Heavy metals were determined using a Varian AA-1275 Atomic Absorption Spectrophotometer (AAS) (Varian, Australia), in conjunction with Phototron Hollow Cathode lamps (Phototron Pty, Ltd., Narre Warren, Victoria, Australia). Other equipment used includes an Analytical balance (Sartorius, Germany), hot plate (Stuart Scientific, Redhill, UK), Whatman no. 42 filter paper purchased from Vidovic Enterprises, Harare, Zimbabwe.

Materials and reagents

Hydrogen peroxide (Assay, 30%; Cl, 0.01%; residue on ignition, 0.03%) was purchased from Associated Chemical Enterprises, Johannesburg, SA, USA. The following were purchased from Skylab, Johannesburg, SA: nitric acid (AR grade), hydrochloric acid (AR grade), ZnSO₄·7H₂O (MW 287.54), CuSO₄·5H₂O (MW 249.68), CdCl₂·H₂O (MW, 201.32), K₂Cr₂O₇ (MW, 294.18), NiCl₂·6H₂O (MW,

Table 1 Physicochemical properties of soils sampled from wetlands receiving effluent from a chrome ore processing plant (Site A), a gold ore processing plant (Site B), and a nickel ore processing plant (Site C), in Zimbabwe

Property	Value $\pm$ standard deviation ($h = 9$, $n = 3$)		
	Site A	Site B	Site C
pH	6.5 $\pm$ 0.1	6.3 $\pm$ 0.1	6.9 $\pm$ 0.1
Clay (%)	15 $\pm$ 1	15 $\pm$ 2	20 $\pm$ 2
Silt (%)	16 $\pm$ 1	20 $\pm$ 2	12 $\pm$ 0
Sand (%)	69 $\pm$ 1	65 $\pm$ 1	68 $\pm$ 2
Organic carbon (OC) (%)	1.98 $\pm$ 0.004	3.89 $\pm$ 0.08	1.10 $\pm$ 0.07
Soil organic matter (SOM) (%)	3.96 $\pm$ 0.08	7.77 $\pm$ 0.15	2.2 $\pm$ 0.1
Cation exchange capacity (CEC) (me %)	20.6 $\pm$ 0.4	24.2 $\pm$ 0.2	29.3 $\pm$ 0.4

h number of samples, n number of replicate analyses, me milli-equivalent

237.71), $(\text{NH}_4)_2\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ (MW, 392.13) and $\text{Pb}(\text{NO}_3)_2$ (MW, 331.20).

Digestion of soil samples for total element determination

Total element in the soil samples was determined using the conventional hot plate aqua regia digestion procedure (Chen and Ma 2001). When digestion was complete, the solution was evaporated to near dryness at low heat (80 °C), then allowed to cool. The residue was then transferred to a 100 mL volumetric flask with 20 mL of 2% (v/v with H_2O) nitric acid through a Whatman No. 42 filter paper, then made up to volume with distilled deionised water. The same procedure was used to prepare reagent blanks.

Extraction of metal exchangeable fraction in soil samples

The metal exchangeable fraction was extracted using 1 M ammonium acetate solution (Gibson and Farmer 1986). Two grammes of the ground soil sample was divided into equal 1.0 g portions, and each portion was placed in an Erlenmeyer flask and 20 mL of 1 M ammonium acetate solution added. One of the flasks was labelled “extraction efficiency”, and spiked with 2.5 mL of each heavy metal working standard solution containing 5.0 mg L^{-1} of the metal. The flasks were tightly capped and shaken on a flask shaker for 2 h at room temperature, after which the samples were centrifuges at 4000 rpm for 10 min. The supernatant solution was transferred to a plastic vial for storage. To the residue in the centrifuge tube, 10 mL deionised water was added, the mixture centrifuged again, and the supernatant combined with the first extract. The soil sample, the spiked sample and blank sample were analysed using flame atomic absorption spectrometry (FAAS).

Digestion of plant samples

The digestion of plant samples was carried out using the wet ashing method as described previously by Campbell and Plank (1998) using concentrated nitric acid followed by 30% hydrogen peroxide. After completion of digestion, the residue was evaporated to near dryness at low heat (80 °C), allowed to cool, then transferred to a 100 mL volumetric flask containing dilute aqua regia (25 mL conc. HNO_3 and 75 mL conc. HCl , diluted to 1000 mL with distilled deionised water). All digestions were carried out in triplicate.

Heavy metal analysis

Soil and plant sample solutions prepared as described above were analysed for Zn, Cu, Cd, Cr, Ni, Fe and Pb using flame atomic absorption spectrometry (FAAS) (Campbell and Plank 1998). Quantification was done using the calibration curve technique. In all cases linear calibration curves were obtained. Validation was done by analysis of a plant-based (beech leaves) certified reference material (CRM), supplied by the Bureau of Community Reference (BCR), CRM 100: Minor and Trace Elements in Beech Leaves, subjected to the same treatment as the plant samples, and then analysed for Cr. All determinations were corrected for reagent blank readings, $(0.0010\text{--}0.0020) \pm 0.0006$ absorbance units, depending on the element. The results obtained are shown in Tables 2, 3 and 4.

Bio-concentration factors for *T. capensis* roots, rhizomes, stems and leaves were calculated and are shown in Table 5. Correlations between heavy metal concentrations in *T. capensis* organs (roots, rhizomes, stems and leaves) and total heavy metal in the soil were evaluated using Pearson's product moment correlation analysis using Minitab statistical package (Release 12, 2001). The results obtained are shown in Tables 6, 7 and 8.

Table 2 Mean exchangeable metal and total element concentration in soil ($\mu\text{g g}^{-1}$ soil) and total element concentration in *Typha capensis* organs ($\mu\text{g g}^{-1}$ plant) for samples from Site A ($h = 9$, $n = 3$)

Metal	Exchangeable ($\mu\text{g g}^{-1}$ soil)	Total ($\mu\text{g g}^{-1}$ soil)	Root ($\mu\text{g g}^{-1}$ plant)	Rhizome ($\mu\text{g g}^{-1}$ plant)	Stems ($\mu\text{g g}^{-1}$ plant)	Leaves ($\mu\text{g g}^{-1}$ plant)
Zn	0.6 ± 0.2	43.5 ± 1	162 ± 5	26 ± 3	29 ± 1	13 ± 1
Cu	nd	8.5 ± 1	35 ± 1	10 ± 1	16 ± 1	10 ± 1
Co	10.5 ± 0.6	32.5 ± 1	58 ± 0	5 ± 3	22 ± 2	36 ± 2
Cd	nd	7.0 ± 0.5	16 ± 2	18 ± 2	25 ± 0	28 ± 1
Cr	14.4 ± 0.8	5415 ± 10	$16,047 \pm 31$	786 ± 2	3353 ± 12	3768 ± 48
Ni	1.8 ± 0.3	58 ± 2	196 ± 2	17 ± 2	37 ± 2	28 ± 1
Fe	4.5 ± 0.3	7500 ± 1	9413 ± 12	8970 ± 231	$11,680 \pm 147$	906 ± 122
Pb	0.6 ± 0.2	43.5 ± 0.5	28 ± 1	6.00 ± 0.01	46 ± 2	35 ± 1

h number of samples, n number of replicate analyses, me milli-equivalent, nd not detected

Table 3 Mean exchangeable metal and total element concentration in soil ($\mu\text{g g}^{-1}$ soil) and total element concentration in *Typha capensis* organs ($\mu\text{g g}^{-1}$ plant) for samples from Site B ($h = 9$, $n = 3$)

Metal	Exchangeable ($\mu\text{g g}^{-1}$ soil)	Total ($\mu\text{g g}^{-1}$ soil)	Root ($\mu\text{g g}^{-1}$ plant)	Rhizome ($\mu\text{g g}^{-1}$ plant)	Stems ($\mu\text{g g}^{-1}$ plant)	Leaves ($\mu\text{g g}^{-1}$ plant)
Zn	5.4 ± 0.6	60.0 ± 0.5	87 ± 6	30 ± 2	82 ± 2	36 ± 2
Cu	nd	45.5 ± 0.5	211 ± 1	56 ± 1	45 ± 2	17 ± 3
Co	8.1 ± 0.3	31 ± 1	124 ± 3	10 ± 1	2 ± 1	24 ± 1
Cd	nd	8.5 ± 0.5	20 ± 1	21 ± 0	23 ± 1	27 ± 1
Cr	8.7 ± 0.3	148 ± 1	222 ± 2	73 ± 3	65 ± 1	74 ± 3
Ni	2.7 ± 0.3	67 ± 1	593 ± 1	73 ± 3	35 ± 1	51 ± 3
Fe	6.9 ± 0.3	5190 ± 10	$12,967 \pm 21$	4993 ± 72	8833 ± 45	5343 ± 21
Pb	0.9 ± 0.5	61.5 ± 0.5	27 ± 1	16 ± 2	13 ± 1	63 ± 2

h number of samples, n number of replicate analyses, me milli-equivalent, nd not detected

Table 4 Mean exchangeable metal and total element concentration in soil ($\mu\text{g g}^{-1}$ soil) and total element concentration in *Typha capensis* organs ($\mu\text{g g}^{-1}$ plant) for samples from Site C ($h = 9$, $n = 3$)

Metal	Exchangeable ($\mu\text{g g}^{-1}$ soil)	Total ($\mu\text{g g}^{-1}$ soil)	Root ($\mu\text{g g}^{-1}$ plant)	Rhizome ($\mu\text{g g}^{-1}$ plant)	Stems ($\mu\text{g g}^{-1}$ plant)	Leaves ($\mu\text{g g}^{-1}$ plant)
Zn	0.3 ± 0.2	44 ± 2	56 ± 2	24 ± 1	53 ± 1	25 ± 2
Cu	nd	60 ± 0	224 ± 2	41 ± 0	31 ± 1	17 ± 1
Co	9.3 ± 0.6	33.5 ± 0.0	88 ± 1	7 ± 1	8 ± 1	20 ± 11
Cd	nd	10 ± 0	22 ± 1	21 ± 1	23 ± 1	24 ± 1
Cr	5.4 ± 0.3	101.5 ± 0.5	264 ± 3	70 ± 1	73 ± 4	73 ± 1
Ni	4.8 ± 0.3	176 ± 1	891 ± 8	88 ± 3	34 ± 1	24 ± 1
Fe	5.4 ± 0.5	2502 ± 11	$13,833 \pm 92$	2303 ± 15	5447 ± 51	5267 ± 12
Pb	2.4 ± 0.3	51 ± 1	63 ± 4	8 ± 2	10 ± 1	5 ± 1

h number of samples, n number of replicate analyses, me milli-equivalent, nd not detected

Table 5 Bio-concentration factors (BCF) for Zn, Cu, Cd, Cr, Ni, Fe and Pb for *Typha capensis* from wetlands polluted by effluent from a chrome ore processing plant (Site A), a gold ore processing plant (Site B), and a nickel ore processing plant (Site C) in Zimbabwe

Metal	Site	BCF (roots)	BCF (rhizomes)	BCF (stems)	BCF (leaves)
Zn	A	3.74	0.60	0.67	9.30
	B	1.45	0.50	1.37	0.60
	C	1.27	0.55	1.20	0.57
	Mean $\pm$ SD	2.15 $\pm$ 1.38	0.55 $\pm$ 0.05	1.08 $\pm$ 0.37	3.49 $\pm$ 5.03
Cu	A	4.12	1.18	1.88	1.18
	B	4.64	1.23	0.99	0.37
	C	3.73	0.68	0.52	0.28
	Mean $\pm$ SD	4.16 $\pm$ 0.46	1.03 $\pm$ 0.30	1.13 $\pm$ 0.69	0.61 $\pm$ 0.50
Co	A	1.79	0.15	0.68	1.11
	B	4.00	0.32	0.06	0.77
	C	2.63	0.21	0.24	
	Mean $\pm$ SD	2.81 $\pm$ 1.12	0.23 $\pm$ 0.09	0.33 $\pm$ 0.32	
Cd	A	2.29	2.57	3.57	4.00
	B	2.35	2.47	2.71	3.18
	C	2.20	2.10	2.30	2.40
	Mean $\pm$ SD	2.28 $\pm$ 0.08	2.38 $\pm$ 0.25	2.86 $\pm$ 0.65	3.19 $\pm$ 0.80
Cr	A	2.96	0.15	0.62	0.70
	B	1.5	0.49	0.44	0.50
	C	2.60	0.69	0.72	0.72
	Mean $\pm$ SD	2.35 $\pm$ 0.76	0.44 $\pm$ 0.27	0.59 $\pm$ 0.14	0.64 $\pm$ 0.12
Ni	A	3.38	0.29	0.64	0.48
	B	8.85	1.09	0.52	1.10
	C	5.06	0.50	0.19	0.14
	Mean $\pm$ SD	5.76 $\pm$ 2.80	0.63 $\pm$ 0.41	0.45 $\pm$ 0.23	0.57 $\pm$ 0.49
Fe	A	1.26	1.20	1.56	0.12
	B	2.50	0.96	1.70	1.03
	C	5.53	0.92	2.18	2.11
	Mean $\pm$ SD	3.10 $\pm$ 2.20	1.03 $\pm$ 0.15	1.81 $\pm$ 0.33	1.09 $\pm$ 1.00
Pb	A	0.64	0.14	1.06	0.81
	B	0.44	0.26	0.21	1.02
	C	1.24	0.16	0.20	0.10
	Mean $\pm$ SD	0.77 $\pm$ 0.42	0.19 $\pm$ 0.06	0.49 $\pm$ 0.49	0.64 $\pm$ 0.48

SD standard deviation

Results and discussion

The results obtained for the physicochemical properties of the soil samples are shown in Table 1. The results obtained for the heavy metal exchangeable fraction and total element in soil samples, and total element in plant samples are shown in Tables 2, 3 and 4. Exchangeable metal extraction recovery ranged from 93.0% for Zn to 106.0% for Co, with a mean recovery of $97 \pm 8\%$. The concentration of Cr obtained for the plant-based (beech leaves) certified reference material (CRM), supplied by the Bureau of Community Reference (BCR), CRM 100, was $7.3 \pm 0.6 \mu\text{g g}^{-1}$ versus a certified value of

$8.0 \pm 0.6 \mu\text{g g}^{-1}$. The CRM did not have certified values for the other metals under consideration in this study.

Heavy metal levels and distribution in the plant

From Table 2, it is apparent that Site A is heavily contaminated with Cr at $5415 \mu\text{g g}^{-1}$, compared to $148 \mu\text{g g}^{-1}$ at Site B, and $101 \mu\text{g g}^{-1}$ at Site C. It is therefore not surprising that *T. capensis* plants growing in the site are also heavily contaminated with Cr. Chromium levels are highest in the roots at $16,047 \mu\text{g g}^{-1}$, followed by the leaves and stems at 3768 and $3353 \mu\text{g g}^{-1}$, respectively. The levels of Cr are lowest in the rhizomes at

Table 6 Metal/metal correlations at Site A

	Zn	Cu	Co	Cd	Cr	Ni	Fe
(a) Within the soil/plant system							
Cu	0.944						
	0.016						
Co	0.805	0.730					
	0.053	0.162					
Cd	−0.343	−0.041	−0.129				
	0.573	0.947	0.837				
Cr	0.971	0.915	0.924	−0.277			
	0.001	0.029	0.008	0.652			
Ni	0.992	0.938	0.870	−0.314	0.992		
	0.000	0.018	0.024	0.607	0.000		
Fe	0.478	0.372	0.157	−0.307	0.378	0.424	
	0.338	0.537	0.767	0.615	0.460	0.403	
Pb	0.196	−0.034	0.541	0.020	0.353	0.270	0.417
	0.710	0.956	0.268	0.974	0.492	0.604	0.410
(b) Within the plant only							
Cu	0.982						
	0.018						
Co	0.780	0.809					
	0.220	0.191					
Cd	−0.715	−0.616	−0.146				
	0.285	0.384	0.854				
Cr	0.968	0.971	0.912	−0.522			
	0.032	0.029	0.088	0.478			
Ni	0.992	0.986	0.854	−0.620	0.992		
	0.008	0.014	0.146	0.380	0.008		
Fe	0.331	0.391	−0.178	−0.550	0.158	0.253	
	0.669	0.609	0.822	0.450	0.842	0.747	
Pb	−0.037	0.141	0.394	0.642	0.149	0.065	−0.017
	0.963	0.859	0.606	0.358	0.851	0.935	0.983

The correlation coefficient in bold are statistically significant at $P \leq 0.05$

Cell contents: Pearson correlation

P value

786 $\mu\text{g g}^{-1}$. Soils levels of Fe are high at all three Sites A, B and C at 7500, 5190 and 2502 $\mu\text{g g}^{-1}$, respectively. The Cr ore processing plant at Site A uses iron in the production of ferrochrome, while the gold ore processing plant at Site C uses ferrous sulphate to neutralise cyanide ion following the cyanidation process to extract gold from the ore. Levels of Fe in *T. capensis* are highest in stems at 11,680 $\mu\text{g g}^{-1}$ and lowest in the leaves at 906 $\mu\text{g g}^{-1}$ at Site A. At Sites B and C, levels of Fe in *T. capensis* are highest in the roots at 12,967 and 13,833 $\mu\text{g g}^{-1}$, respectively, and lowest in the rhizomes at 4993 and 2303 $\mu\text{g g}^{-1}$, respectively. From Table 2, it is apparent that the concentrations of Zn, Cu, Co, Ni, Cd and Pb at Site A are much lower than those of Cr and Fe, both in the soil and *T. capensis* organs, while it is apparent from Tables 3 and 4 that the concentrations of

Zn, Cu, Co, Ni, Cd, Pb, as well as Cr are much lower than those of Fe at Sites B and C, both in the soil and *T. capensis* organs.

Bio-concentration factors

Table 5 shows the bio-concentration factors (BCF) obtained for *T. capensis* at the three sites. Of the eight elements studied (Zn, Cu, Co, Cd, Cr, Ni, Fe and Pb), bio-concentration factors for *T. capensis* roots are greater than unity for Zn, Cu, Co, Cd, Cr, Ni and Fe, showing that *T. capensis* roots are good accumulators (BCF >1) of these elements (Yoon et al. 2006). Mean BCFs for *T. capensis* rhizomes are greater than unity for Cu, Cd and Fe, while mean BCFs for stems are greater than unity for Zn, Cu, Cd

Table 7 Metal/metal correlations at Site B

	Zn	Cu	Co	Cd	Cr	Ni	Fe
(a) Within the soil/plant system							
Cu	0.575						
	0.311						
Co	0.517	0.938					
	0.294	0.018					
Cd	−0.156	−0.082	−0.113				
	0.802	0.896	0.857				
Cr	0.703	0.867	0.887	−0.456			
	0.119	0.057	0.019	0.440			
Ni	0.564	0.985	0.979	−0.023	0.855		
	0.244	0.002	0.001	0.971	0.030		
Fe	0.909	0.881	0.730	0.135	0.801	0.793	
	0.012	0.049	0.099	0.828	0.055	0.060	
Pb	0.160	−0.326	0.158	−0.279	0.399	0.006	0.128
	0.762	0.592	0.765	0.650	0.434	0.992	0.809
(b) Within the plant only							
Cu	0.588						
	0.412						
Co	0.475	0.945					
	0.525	0.055					
Cd	−0.403	−0.733	−0.486				
	0.597	0.267	0.514				
Cr	0.532	0.977	0.993	−0.579			
	0.468	0.023	0.007	0.421			
Ni	0.534	0.984	0.988	−0.610	0.999		
	0.466	0.016	0.012	0.390	0.001		
Fe	0.890	0.883	0.819	−0.564	0.859	0.860	
	0.110	0.117	0.181	0.436	0.141	0.140	
Pb	−0.423	−0.256	0.073	0.791	−0.046	−0.082	−0.291
	0.577	0.744	0.927	0.209	0.954	0.918	0.709

The correlation coefficient in bold are statistically significant at $P \leq 0.05$

Cell contents: Pearson correlation

P value

and Fe, and mean BCFs for leaves are greater than unity for Zn, Cd and Fe. High BCF values are desirable for phytoremediation of polluted soils so that phytoremediation can be achieved by periodic harvesting the shoots, thereby lowering the metal content of the soil (Rauser 1999). The fact that mean BCFs for stems and leaves are greater than unity shows that *T. capensis* is a particularly good candidate for the phytoextraction of Zn, Cu, Cd and Fe from polluted soils.

Metal–metal interactions

The metals Fe, Zn, Cu, Ni, and Co are essential for plant growth; hence the high BCFs displayed by the plant for these elements are understandable. On the other hand Cd,

Cr and Pb are not essential and can be toxic at high concentrations (Rauser 1999; Williams et al. 2000; Nagajyoti et al. 2010). For this reason, we expect the plant to develop some mechanism for exclusion of these metals, resulting in their reduced absorption by the roots. Such a mechanism has been proposed in Al-tolerant plants, whereby Al stimulates secretion of ligands (phosphate, organic acids and mucilage) that complex Al, resulting in reduced absorption by root-tip cells (Rauser 1999). The levels of Cr found in *T. capensis* from Site A ($16,047 \pm 31$, 786 ± 2 , 3353 ± 12 and $3768 \pm 48 \mu\text{g g}^{-1}$ dry weight for roots, rhizomes, stems and leaves, respectively) are at least 10^2 to 10^4 above the range of Cr levels normally found in plants, $0.2\text{--}1 \mu\text{g g}^{-1}$ plant dry weight (Nagajyoti et al. 2010). In addition Table 5 shows that *T. capensis* roots exhibit high

Table 8 Metal/metal correlations at Site C

	Zn	Cu	Co	Cd	Cr	Ni	Fe
(a) Within the soil/plant system							
Cu	0.575						
	0.311						
Co	0.517	0.938					
	0.294	0.018					
Cd	−0.156	−0.082	−0.113				
	0.802	0.896	0.857				
Cr	0.703	0.867	0.887	−0.456			
	0.119	0.057	0.019	0.440			
Ni	0.564	0.985	0.979	−0.023	0.855		
	0.244	0.002	0.001	0.971	0.030		
Fe	0.909	0.881	0.730	0.135	0.801	0.793	
	0.012	0.049	0.099	0.828	0.055		
Pb	0.160	−0.326	0.158	−0.279	0.399	0.006	0.128
	0.762	0.592	0.765	0.650	0.434	0.992	0.809
(b) Within the plant only							
Cu	0.635						
	0.365						
Co	0.577	0.968					
	0.423	0.032					
Cd	0.000	−0.350	−0.136				
	1.000	0.650	0.864				
Cr	0.639	0.994	0.989	−0.245			
	0.361	0.006	0.011	0.755			
Ni	0.611	0.999	0.979	−0.321	0.997		
	0.389	0.001	0.021	0.679	0.003		
Fe	0.734	0.929	0.968	0.013	0.961	0.936	
	0.266	0.071	0.032	0.987	0.039	0.064	
Pb	0.677	0.997	0.975	−0.289	0.997	0.996	0.953
	0.323	0.003	0.025	0.711	0.003	0.004	0.047

The correlation coefficient in bold are statistically significant at $P \leq 0.05$

Cell contents: Pearson correlation

P value

BCFs (2.20–2.35) for Cr. Cadmium has the lowest soil concentration at 7–10 $\mu\text{g g}^{-1}$ (Tables 2, 3, 4), yet it shows high BCFs for all organs of *T. capensis* (2.10–4.00) at all Sites A, B and C (Table 5). Lead has a fairly high soil concentration at 43.5–61.5 $\mu\text{g g}^{-1}$ (Tables 2, 3, 4), and the lowest mean root BCF at 0.77 (Table 5). Table 5 however shows that the bio-concentration factors for Pb, though low in the rhizomes at 0.14–0.26, are fairly high in *T. capensis* roots at 0.64–1.24. As noted above, these observations are contrary to what is expected for the absorption of non-essential toxic elements by plants. The possible explanations for these observations are discussed below.

Tables 6, 7 and 8 show Pearson's correlations between the metals (a) in the soil–plant system (i.e., including

concentration levels in the soil), and (b) within the plant (i.e., excluding concentration levels in the soil), at Sites A, B and C, respectively. Significant metal–metal correlations observed (in bold type) can be categorised into (a) those observed at all three sites, (b) those observed at Site A only, (c) those observed at Sites B and C only, and (d) those observed at Site C only.

Significant correlations observed at Sites A, B and C

Significant correlations involving Ni/Cu, Ni/Co, Ni/Cr, Cr/Cu and Cr/Co are observed at all three sites A, B and C. If we assume that the absorption of essential elements involves enzymes specific for each element (Rauser 1999),

and that metal ions with the same, or nearly the same, ionic radius and co-ordination geometry can be co-absorbed, then mutual co-absorption can be expected for essential elements with the same, or nearly the same, ionic radius and co-ordination geometry. The metals Cu, Co and Ni are essential to plant growth, hence significant positive correlations are understandable, and may be explained on the basis of similarity of ionic radii and coordination geometry, assuming that these elements are absorbed as Cu(II) (CN = 6, $r^+ = 0.73 \text{ \AA}$), Co(II) (CN = 6, $r^+ = 0.65 \text{ \AA}$), and Ni(II) (CN = 6, $r^+ = 0.69 \text{ \AA}$) (CN = coordination number, r^+ = ionic radius, see Table 9). Chromium is not essential and is toxic to most plants at $100 \mu\text{g kg}^{-1}$ dry weight (Davies et al. 2002; Nagajyoti et al. 2010). The significant metal–metal correlations observed between Cr and Cu, Co and Ni, can only be attributed to co-absorption of Cr(II) (CN = 6, $r^+ = 0.73 \text{ \AA}$) or Cr(III) (CN = 6, $r^+ = 0.62 \text{ \AA}$), with Cu(II) ($r^+ = 0.73 \text{ \AA}$), Co(II) ($r^+ = 0.65 \text{ \AA}$) and Ni(II) ($r^+ = 0.69 \text{ \AA}$).

Table 9 Coordination numbers and ionic radii of the metals studied (Evans Jr 1992)

Metal ion	Coordination number (CN)	Ionic radius (r^+) ($\text{\AA}$)
Zn(II)	4	0.60
	6	0.74
Zn(IV)	8	0.84
Cu(I)	2	0.46
	4	0.60
Cu(II)	4sq	0.57
	6	0.73
Cd(II)	4	0.78
	6	0.95
	8	1.10
Co(II)	6	0.65
Co(III)	6	0.55
Cr(II)	6	0.73
Cr(III)	6	0.62
Cr(VI)	4	0.26
Ni(II)	4sq	0.44
	6	0.69
Fe(II)	6	0.65
Fe(III)	4	0.49
	6	0.55
Pb(II)	6	1.19
	10	1.40
Pb(IV)	4	0.65
	6	0.78

Significant correlations observed at Site A only

Significant correlations involving Cu/Zn, Cr/Zn and Ni/Zn are observed at Site A only, and may be explained on the basis of the similarity of the ionic radius and coordination geometry of Zn(II) ions ($r^+ = 0.74 \text{ \AA}$, CN = 6) to those of Cu(II), Ni(II) and Cr(III). The absence of significant correlations involving Cu/Zn, Cr/Zn and Ni/Zn at Sites B and C may be related to the low concentrations of Cr at these sites, suggesting that the co-absorption of Zn at Site A is driven by the high concentration of Cr at Site A. It should be noted however that fairly strong but non-significant correlations (Pearson's correlation coefficient, $r = 0.575\text{--}0.703$) involving Cu/Zn, Cr/Zn and Ni/Zn are observed at Sites B and C.

Significant correlations observed at Sites B and C only

Significant correlations involving Co/Cu, Fe/Zn and Fe/Cu are observed at Sites B and C only. Fe can be absorbed as Fe(II) (CN = 6, $r^+ = 0.65$) or Fe(III) (CN = 6, $r^+ = 0.55$); hence since Co, Cu and Fe are all essential in plants, the observed significant correlation between these elements may be attributed to mutual co-absorption as a result of the similarity in their ionic radii and coordination geometry. Significant correlations between these elements are not observed at Site A, although weak but positive Fe/Zn and Fe/Cu correlations ($r = 0.331\text{--}0.478$) are observed. This may be attributed to competition from Cr(III) ions which are in very high concentration at Site A, and whose ionic radius at $r^+ = 0.62 \text{ \AA}$ is closer to those of Zn and Cu at $r^+ = 0.74 \text{ \AA}$ and $r^+ = 0.73 \text{ \AA}$ respectively, than that of Fe(II) or Fe(III). This would tend to reduce any co-absorption of Fe with Zn or Cu.

Significant correlations observed at Site C only

The following significant correlations are observed within the plant system only at Site C: Fe/Co, Fe/Cr, Pb/Cu, Pb/Co, Pb/Cr, Pb/Ni, and Pb/Fe. The fact that these metal–metal interaction are observed in the plant system only, suggests that these interactions occur during heavy metal translocation from the roots to the rhizomes, rhizomes to stems and stems to the leaves. If we assume that Cr is co-absorbed with the essential elements Cu, Ni, and Co as discussed above, then the significant correlations Fe/Co, Fe/Cr involving the essential elements Co and Fe and Cr may be attributed to direct mutual co-absorption or indirect co-absorption via co-absorption of Cr with Cu, Ni and Co, and Fe with Cu, Ni and Co.

Pb is not essential to plants and if present in high concentration, may be toxic to the plant (Rauser 1999;

Williams et al. 2000; Nagajyoti et al. 2010). We would have, therefore, expected the plant to develop some mechanism for its exclusion. Table 5 however shows that the bio-concentration factors for Pb, though low in the rhizomes at 0.14–0.26, are fairly high in *T. capensis* roots at 0.64–1.24. Pb can exist as Pb(II) (CN = 6, $r^+ = 1.19 \text{ \AA}$), Pb(II) (CN = 10, $r^+ = 1.40 \text{ \AA}$), Pb(IV) (CN = 4, $r^+ = 0.65 \text{ \AA}$) and Pb(IV) (CN = 6, $r^+ = 0.78 \text{ \AA}$). The combination of charge, coordination geometry and ionic radius for each of these Pb cations is not favourable for co-absorption of Pb with the essential elements Zn, Cu, Co, Ni and Fe. The fact that the significant correlations Pb/Cu, Pb/Co, Pb/Cr, Pb/Ni, and Pb/Fe are observed within the plant only, suggests that the absorption Pb by *T. capensis* roots may be attributed solely to diffusion across the root cell membrane driven by the high concentration of the its exchangeable fraction in the soil ($2.4 \mu\text{g g}^{-1}$ soil at Site C versus 0.6 and $0.9 \mu\text{g g}^{-1}$ soil at Sites A and B, respectively), and that once inside the plant, it induces the production of ligands involved in the translocation of metals to the rhizome, stem and leaves. A variety of such ligands have been proposed and include asparagines (Asn), histidine (His), citrate, malate, and oxalate (Rauser 1999). Rauser (1999) reports that in the xylem sap, citrate and histidine are the principal ligands for Cu, Zn and Ni. The resulting increase in the concentration of such ligands leads to an increase in the translocation of Cu, Co, Cr and Fe, thus explaining the observed significant correlation of Pb with these elements. Pb has also been reported to induce formation of phytochelatin for detoxification (Grill et al. 1987). An increase in phytochelatin would also lead to increased complexation of other metals for storage in the various plant organs, a further possible explanation for the significant correlation between Pb and Cu, Co Cr and Fe.

Cadmium

Cadmium does not show any significant correlations with any of the metals studied. It has the lowest soil concentration at $7\text{--}10 \mu\text{g g}^{-1}$ (Tables 2, 3, 4), yet it shows high BCFs for all organs of *T. capensis* (2.10–4.00) at all Sites A, B and C, see Table 5. In fact Cd shows weak negative correlations, or antagonistic metal–metal interactions with Cr, Ni and Fe. Cadmium can exist as Cd(II) (CN = 4, $r^+ = 0.78 \text{ \AA}$), Cd(II) (CN = 6, $r^+ = 0.95 \text{ \AA}$) and Cd(II) (CN = 8, $r^+ = 1.10 \text{ \AA}$). Since the ionic radius of the octahedral state is very high at $0.95 \text{ \AA}$, the absorption of Cd cannot be attributed to co-absorption with Cu, Zn, Ni, Fe and/or Cr. We, therefore, postulate that Cd may be essential in *T. capensis*, so that the plant produces an enzyme selective towards Cd. In this regard, we note that in plant cells and tissues, Cd has been reported to induce formation

of specific sulfhydryl-rich peptides, and was found to be necessary for the regulation of phytochelatin synthase (Chen et al. 1997; Rauser 1999). This suggests that Cd may become essential when high concentrations of toxic elements such as Cr are present.

Implications for the absorption of Cr by plants

As discussed above, the high BCFs (2.20–2.35) for Cr exhibited by *T. capensis* roots, and significant metal–metal correlations consistently observed between Cr and Cu, Zn, Co and Ni, can be attributed to co-absorption of Cr(II) (CN = 6, $r^+ = 0.73 \text{ \AA}$) or Cr(III) (CN = 6, $r^+ = 0.62 \text{ \AA}$), with Cu(II) ($r^+ = 0.73 \text{ \AA}$), Zn(II) ($r^+ = 0.74 \text{ \AA}$), Co(II) ($r^+ = 0.65 \text{ \AA}$) and Ni(II) ($r^+ = 0.69 \text{ \AA}$). If indeed Cr is co-absorbed with Cu, Zn and Ni, then plants cannot avoid the absorption of Cr by secreting ligands that complex Cr to reduce its absorption by the root tip, as any ligands that complex Cr are likely to complex Cu, Zn and Ni as well, thus reducing the absorption of these essential elements. The extremely high levels of Cr found in the roots, stem and leaves of *T. capensis* from Site A suggest that these organs are used as storage organs by the plant for the storage of excess Cr after conversion to harmless compounds by complexation, in a fashion similar to what has been proposed for Cd. Here it has been proposed that phytochelatin are synthesised in the cytosol in response to Cd stimulation, to give Cd-binding complexes which are then transferred to the vacuole where they are converted to sulphide-rich Cd-binding complexes (Ortiz et al. 1995; Rauser 1999). Phytochelatin have also been implicated in the detoxification of As and Tl in *Sinapis alba* L. (Kowalska et al. 2015). A consequence of the apparently unavoidable co-absorption of the Cr(III) ion with the essential elements cations Zn(II), Cu(II), Co(II), Ni(II), and Fe(II) is that plants that cannot detoxify the Cr by converting it to harmless complexes for storage cannot grow in high Cr concentration soils. Further work is underway in our laboratory to confirm this prediction.

Conclusions

From the foregoing discussion, the following conclusions can be made regarding the uptake of heavy metals by *T. capensis*. (a) Whereas *T. capensis* roots are hyper-accumulators (BCF > 1) for Zn, Cu, Co, Cd, Ni and Fe, *T. capensis* shoots are hyper-accumulators of Zn, Cu, Cd and Fe. (b) Synergistic metal–metal interactions exist in the absorption of the essential elements Zn, Cu, Co, Ni and Fe as a result of the similarity in their ionic radii and octahedral coordination geometry. (c) Cr is co-absorbed with the essential elements Zn, Cu, Co, and Ni, because of the

similarity in coordination geometry and closeness of its ionic radius to the ionic radii of the essential elements. (d) This unavoidable co-absorption of Cr with the essential elements Zn, Cu, Co and Ni, suggests that plants that cannot detoxify Cr by converting it to harmless complexes for storage, cannot grow in high Cr concentration soils.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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