Plant and Soil (2006) 281:325–337
DOI 10.1007/s11104-005-4642-9

Soil pH effects on uptake of Cd and Zn by *Thlaspi caerulescens*

Autumn S. Wang[1,5], J. Scott Angle[1], Rufus L. Chaney[2], Thierry A. Delorme[3] & Roger D. Reeves[4]

[1]*Department of Natural Resources and Landscape Architecture, University of Maryland at College Park, College Park, MD, 20742, USA.* [2]*USDA-ARS Animal Manure and By-products Laboratory, Beltsville, MD, 20705, USA.* [3]*Kent State University, Ashtabula, OH, 44004, USA.* [4]*Massey University, Palmerston North, New Zealand.* [5]*Corresponding author**

Received 1 August 2005. Accepted in revised form 31 October 2005

Key words: cadmium, phytoextraction, soil pH, *Thlaspi caerulescens*, zinc

Abstract

For phytoextraction to be successful and viable in environmental remediation, strategies that can optimize plant uptake must be identified. *Thlaspi caerulescens* is an important hyperaccumulator of Cd and Zn, whether adjusting soil pH is an efficient way to enhance metal uptake by *T. caerulescens* must by clarified. This study used two soils differing in levels of Cd and Zn, which were adjusted to six different pH levels. *Thlaspi caerulescens* tissue metal concentrations and 0.1 *M* $Sr(NO_3)_2$ extractable soil metal concentrations were measured. The soluble metal form of both Cd and Zn was greatly increased with decreasing pH. Lowering pH significantly influenced plant metal uptake. For the high metal soil, highest plant biomass was at the lowest soil pH (4.74). The highest shoot metal concentration was at the second lowest pH (5.27). For low metal soil, due to low pH induced Al and Mn toxicity, both plant growth and metal uptake was greatest at intermediate pH levels. The extraordinary Cd phytoextraction ability of *T. caerulescens* was further demonstrated in this experiment. In the optimum pH treatments, *Thlaspi caerulescens* extracted 40% and 36% of total Cd in the low and high metal soils, respectively, with just one planting. Overall, decreasing pH is an effective strategy to enhance phytoextraction. But different soils had various responses to acidification treatment and a different optimum pH may exist. This pH should be identified to avoid unnecessarily extreme acidification of soils.

Introduction

Phytoextraction uses hyperaccumulator plants to remove contaminants from soil (Chaney, 1983). As a promising alternative soil remediation technology, it has been the focus of extensive research over the past decade. However, progress in making phytoextraction a practical commercial technology is hindered by a lack of strategies to optimize plant uptake of metals. Although the mechanisms of hyperaccumulation remain unclear, it is generally agreed that hyperaccumulation involves three major processes: rapid uptake of heavy metals by roots, high rate of translocation from roots to shoots, and high storage capacity by vacuolar compartmentalization (Chaney et al., 1997; McGrath et al., 2002; Pollard et al., 2002). The first step is likely to be rate-limiting. Plant uptake of metals is limited by metal solubility/availability in soil.

Among known hyperaccumulators, *Thlaspi caerulescens* is one of the most studied species. It is an endemic metallophyte and primarily a Zn hyperaccumulator (Baker and Brooks, 1989; Brooks, 1998; Reeves and Brooks, 1983). *Thlaspi*

* FAX No: +1-301-314-9089.
E-mail: wspring@wam.umd.edu

caerulescens actually requires high amounts of Zn to grow normally (Shen et al., 1997). Concentrations can exceed 3% Zn in shoot dry matter. *Thlaspi caerulescens* is also able to hyperaccumulate Cd. Different populations of *T. caerulescens* possess different levels of Cd hyperaccumulation ability (Lombi et al., 2000; Roosen et al., 2003). The southern French populations have a superior ability to hyperaccumulate Cd (Lombi et al., 2000, 2001b; Zhao et al., 2003). The highest shoot Cd concentrations observed in the field reach 3600 mg kg^{-1} dry weight (DW) (Reeves et al., 2001; Robinson et al., 1998).

Although *T. caerulescens* has extraordinary ability to transfer high amounts of Zn and Cd from soil into the shoot, its use for commercial remediation of contaminated soils is limited by several factors. Numerous observations have suggested that metal supply rate in soil is inadequate when compared to uptake. Studies have suggested that specific distribution of roots in soil rich in Zn is an important factor in determining the efficient removal of metals by *T. caerulescens* (Schwartz et al., 1999; Whiting et al., 2000). Once roots have proliferated in metal rich soil, the concentration of soluble or plant available metal must be high enough to meet the extraordinary requirement of *T. caerulescens* (Whiting et al., 2003).

Acidification and release of root exudates are two common mechanisms by which plants modify the rhizosphere to acquire nutrients. However, Luo et al. (2000) found that *T. caerulescens* rhizosphere soil had higher pH than non-rhizosphere soils. This suggests that rhizosphere acidification is not an important mechanism for mobilizing metals in soil by *T. caerulescens*. Similar results were found in a pot study by McGrath et al. (1997). Further, root exudates do not appear to play a role in metal mobilization by *T. caerulescens* hyperaccumulation (Zhao et al., 2001).

It was repeatedly found that metal uptake of *T. caerulescens* exceeded the original soluble metal fractions in soil. In McGrath's (1997) study, decreases in the mobile fraction of Zn accounted for less than 10% of total uptake by *T. caerulescens*, that is, more than 90% of the Zn must have come from previously less-mobile fractions. These authors also found that rhizosphere soils tended to have higher concentrations of mobile Zn than non-rhizosphere soils. Similarly, in a study by Knight et al. (1997), the decrease of Zn in the soil solution after growth accounted for only 1% of the total Zn uptake by *T. caerulescens*. The authors suggested that either *T. caerulescens* was highly efficient at mobilizing Zn which was not initially soluble, or the soil could replenish solution Zn rapidly due to high buffering capacity.

Whiting et al. (2001a) used co-cultivated plants to assess whether mobilization of Zn by *T. caerulescens* increases Zn concentrations of co-cultivated indicator plants (*Thlaspi arvense* or *Festuca rubra*) provided that they shared the same rhizosphere. *Thlaspi caerulescens* did not increase Zn concentrations in either of the indicator plants, suggesting that *T. caerulescens* does not "strongly" mobilize Zn in its rhizosphere. In another experiment, Whiting et al. (2001b) used five Zn compounds of different solubility [ZnS, $Zn_3(PO_4)_2$, ZnO, $ZnCO_3$, and $ZnSO_4 \cdot 7H_2O$] to test how Zn hyperaccumulation was influenced by Zn phytoavailability. Zinc hyperaccumulation of the less soluble form – ZnS treatment was less than that from the other four treatments. Again, this indicated that the solubilization effect of Zn by *T. caerulescens* was not strong.

If *T. caerulescens* is not able to strongly mobilize non-labile metals, then uptake will depend on the soil's potential to replenish the metal supply and root growth to access more soil volume. Both Zn and Cd are present in many different forms with different levels of solubility in soil. These forms include: soluble-exchangeable, comprised of hydrated free metal ions and soluble complexes, specifically sorbed, metals primarily complexed or co-precipitated with Fe, Mn oxides or phosphates, and residual metals held in the primary mineral matrix (Ahnstrom and Parker, 1999; Aualiitia and Pickering, 1987; Hall et al., 1996; Hickey and Kittrick, 1984; Kim and Fergusson, 1991; Shuman, 1982, 1983). Usually, the hydrated free metal ions and soluble complexed metals are the forms that plants can take up. Distribution of metals among different forms is influenced by numerous physicochemical processes in soils. Among them, pH is the most important factor. For example, heavy metals can be retained by the permanent charge sites of layered silicate clays through non-specific electrostatic forces or specific chemisorption. The irreversibility and the specificity are increased at

higher pH (Farrah and Pickering, 1976, 1977; Tiller et al., 1979, 1984).

Studies conducted on crops have shown a negative correlation between soil pH and metal uptake (Castilho and Chardon, 1995; Narwal et al., 1983). Specific studies on the pH effect on metal uptake by *T. caerulescens* hyperaccumulation, however, are lacking. Previously, Brown et al. (1994, 1995) conducted both a greenhouse and field study investigating soil pH effect on *Thlaspi caerulescens* metal uptake. In the greenhouse study, they did not conduct salt leaching process after S addition. Due to salt toxicity, the yield reduction offset the increase in biomass metal concentration. Therefore they did not observe increased total metal translocation from soil into plant biomass at low pH treatments. The field study did not show a consistent pH effect, either. Yanai et al. (2005) studied effect of soil characteristics, including soil pH on Zn/Cd uptake by *Thlaspi caerulescens*, and concluded that variations in shoot Cd concentration were mostly due to total and extractable Cd in soils. Based on the previous discussion about mechanisms of *T. caerulescens* metal uptake, we believe soil acidification should be an effective way to enhance phytoextraction. However, until now, no existing experimental evidence supports this hypothesis. For phytoextraction to be successful, it is important to determine whether adjusting soil pH is an efficient way to enhance *T. caerulescens* hyperaccumulation. Therefore, the primary objective of this work was to examine the effect of pH on metal solubility and *T. caerulescens* growth and Cd and Zn hyperaccumulation.

Materials and methods

site description and soil sampling

Soil samples were collected from the A horizon of two cultivated fields near a former Zn smelter that had been in operation for nearly 100 years at Palmerton, Pennsylvania. Metals released to the environment were primarily Zn and Cd resulting in a metal concentration gradient according to the distance and direction from the smelter. Two soils were sampled, one was about 4.5 km up wind from the smelter and was characterized by relatively low metal concentrations; the other soil was collected about 1.4 km down wind from the smelter, and had a greater metal content. Both soils belong to Montevallo series (loamy-skeletal, mixed, subactive, thermic, shallow Typic Dystrudepts). Soils were first passed through a 1-cm sieve to remove pieces of stone and large plant residues, then passed through a 4-mm sieve. Soils were then homogenized and stored in closed containers to avoid dehydration.

Soil characterization

Total Zn and Cd concentrations were extracted by digesting with concentrated hot nitric acid and measured by flame atomic absorption spectrometry. Soil particle size distribution was determined by the hydrometer method (Gee and Bauder, 1986). Soil pH was measured in a soil water suspension (10 g soil to 20 ml deionized water) after shaking 1 h at 180 rev min^{-1} on a reciprocal shaker (Eckert and Sims, 1995). Organic matter content was determined by loss on ignition (Storer, 1984). Plant available Ca^{2+}, Mg^{2+}, K^{+}, and P were extracted with Mehlich (I) and determined on a Technicon Auto-Analyzer using a colorimeter for Mg and a flame photometer for K, Ca and P (Flannery and Markus, 1980). Total N was determined by the combustion method (Campbell, 1992).

Soil pH adjustment and salt leaching

Different amounts of elemental sulfur (S) were used to adjust soil pH to desired levels based on a preliminary acid incubation experiment (Table 1). Soil pH was monitored periodically by taking 10 g soil and measuring pH. Soil was thoroughly mixed every day to ensure equal distribution of S and to accelerate the S oxidation process. Incubation was terminated when pH did not change for 3 consecutive weeks. It took 17 weeks to reach the final pH. Next, 500 ml of deionized water was added to each pot (1 kg soil) to leach salts from soil. This procedure was repeated two additional times. The total amount of water used (1500 ml) equals to more than 3 times of soil total pore volume in each pot (Small, 1995). By doing this, the excess salts formed during S oxidation was removed.

Table 1. Amount of S needed to reach target soil pH and the equilibrium pH values

Soil	Target pH	Equilibrium pH	H^+(mmol) kg^{-1} soil	S g kg^{-1} soil
Low	7.3	7.27	0	0
metal soil	6.9	6.88	19.52	0.312
	6.5	6.37	70.12	1.122
	6.0	6.07	120.94	1.935
	5.5	5.28	146.34	2.341
	5.0	4.74	171.85	2.749
High	6.9	6.88	0	0
metal soil	6.5	6.37	11.64	0.186
	6.0	6.07	35.71	0.571
	5.5	5.28	88.24	1.412
	5.0	4.74	180.15	2.883

Plant growth

Thlaspi caerulescens seeds were collected from Viviez, France with high Cd hyperaccumulation potential. Seeds were germinated in growth media (Metro-mix) and seedlings were grown for 60 days and watered daily to maintain relatively constant moisture. Plants were grown in a controlled-environment growth chamber, which was set at 16 h/8 h day/night cycle at 25 °C/22 °C. Light intensity exceeded 400 μmol photon m^{-2} s^{-1} and relative humidity was 65%. Peters™ 20–20–20 general purpose fertilizer was used as liquid spray when needed. Seedlings were then transplanted into 15 cm (diameter) by 14 cm (height) plastic pots. Each pot contained 1 kg soil and received three plants. Pots were put into growth chambers with the same settings as for seedling growth. After transplanting, fertilizer was only supplied twice during the growth period at the rate of 0.04 g Peters™ 20–20–20 general purpose fertilizer pot^{-1}. After 6 months of growth, plants were harvested.

Rhizosphere soil sampling

Rhizosphere soil is defined as the portion of soil adjacent to and influenced by plant roots (Metting, 1993). *Thlaspi caerulescens* has a very prolific root system. After 6 months of growth, all soil in the pot was filled with fine roots and considered as rhizosphere soil. At harvest, the shoots were cut at the base using stainless steel scissors. The whole soil/root mass was removed from the pot. Root and soil were manually separated. Both shoots and roots were carefully washed with deionized water for numerous times to avoid soil contamination.

Experimental design

A completely randomized block design with factorial treatment combination was used with the following factors: (1) soil type (low metal soil and high metal soil), (2) presence of plant (with and without plant, i.e., rhizosphere soil and non-rhizosphere), and (3) soil pH (6.88, 6.37, 6.07, 5.28, 4.74). For the low metal soil, soil pH was adjusted to 6 levels; an additional pH treatment of 7.27 which was the initial soil pH was used. The initial soil pH for the high metal soil was 6.88. There were 4 replications for each of the treatments which were randomly placed into one of the four growth chambers.

Plant available soil metal extraction

Soil extractable forms of Cd and Zn are usually extracted with dilute Ca or Mg salt solutions (Shuman, 1991). Because we wanted to monitor Ca and Mg concentrations after acidification treatment, we used Sr salt instead. Chloride salts were avoided because Cl^- tends to form complexes with Cd which may cause an overestimate. So we chose 0.1 *M* $Sr(NO_3)_2$ as the extracting reagent (Ahnstrom and Parker, 1999).

Prior to extraction, 6–8 g of each soil was air-dried overnight, and ground to pass a 150-μm sieve. Duplicate 2 g samples were added to 50-ml polycarbonate centrifuge tubes and extracted with 15 ml 0.1 *M* $Sr(NO_3)_2$ in a reciprocal shaker (2400 oscillations h^{-1}) for 2 h at room temperature. A successive extraction was repeated (Ahnstrom and Parker, 1999). Concentrations of Cd and Zn were determined using an inductively coupled plasma spectrometer (ICP-OES). Detection limits (DL) were 0.400, 1.00, 0.060, 0.020, 0.010, and 0.020 mg L^{-1} for Mg, Ca, Al, Mn, Cd, and Zn, respectively. Laboratory standards were routinely included for analysis.

Plant biomass metal extraction

Plant shoot and root tissue were separately washed in deionized water, and dried at 70 °C.

Shoot tissue was ground when it weighed more than 4 g. Dry plant biomass was weighed and ashed in a muffle oven at 480 °C for about 16 h. After cooling, 2-ml concentrated HNO_3 was added to the beaker. Beakers were then placed on the surface of a hot plate and allowed to evaporate for 1 h to near dryness. Then 10 ml of 3 N HCl was added and the beaker was covered with a watch glass and refluxed on a hot plate for 2 h. The mixture in the beaker was then filtered into a 25-ml volumetric flask through a Whatman #40 filter paper; HCl (0.1 N) was added to volume. Yttrium (20 mg L^{-1}) was added as an internal standard. Element concentrations were determined using an ICP-OES. Detection limits were 0.051 and 0.209 mg L^{-1} for Cd, and Zn, respectively. National Institute of Standards and Technology (NIST) plant standards were included in all analyses.

Statistical analysis

Statistical analyses were conducted using SAS version 8.2 (SAS Institute Inc., 1999–2001). The assumption of normality was tested by examining the plot of residuals and calculating the Shapiro–Wilk statistic. The homogeneity of variance was assessed by examining a plot of predicted values versus residual values. The Spearman test was used to assess the significance of the correlation between the predicted value and absolute value of the residual. Logarithmic transformation of data was performed for shoot Cd and shoot Zn concentrations. After checking that data met assumptions, the PROC MIXED procedure was used for univariate ANOVA to determine the significance of the main factors and their interactions with block as a random factor. The pH treatment of 7.27 in the low metal soil was omitted when doing this analysis. When significant effects were detected, pair-wise treatment mean comparisons were made using a Least Significance Difference (LSD) *t*-test on pH treatment means. Linear or quadratic regression equations were calculated by the least-squares method. Differences between non-rhizosphere soil and rhizosphere soil treatment means were compared by a paired *t*-test. The association between two variables was estimated by the Pearson product-moment correlation coefficient. Unless otherwise indicated, all statistical significance levels were set as $P \leq 0.05$.

Results

Soil properties

The two soils had very different levels of heavy metals (Table 2). The low metal soil had a total Zn and Cd concentration of 450 and 5.0 mg kg^{-1}, respectively. For the high metal soil, it contained 1500 and 25.4 mg kg^{-1}, respectively. Soil pH, organic matter content, and particle size distribution were similar for the two soils. However, more elemental sulfur was generally needed to achieve lower pH in the low metal soil than was required for the high metal soil.

Plant yield

Soil type ($F = 32.3$, $P < 0.001$) and pH by soil interaction ($F = 11.5$, $P < 0.001$) had significant effects on yield of *T. caerulescens*. For the high metal soil, plant dry weight ranged from 5.1 to 6.8 g and highest shoot yield was at the lowest pH treatment (Figure 1). For the low metal soil, highest yield was observed at pH 6.07. The lowest pH treatment caused a dramatic yield reduction. Plant growth at the lowest pH treatment was also noticeably slower with a much smaller rosette, and fewer leaves. Root development in the lowest pH treatment of the low metal soil was also characterized by an unhealthy, stunted root system, lacking small side branches and fine roots. These are typical symptoms of Al toxicity.

Table 2. Soil properties

Soil	Total Zn mg kg^{-1}	Total Cd mg kg^{-1}	Texture	pH	O.M. %	Sand %	Silt %	Clay %	M (I) – P mg kg^{-1}	M (I) – K mg kg^{-1}	N%
Low metal	450	5.0	Loam	7.3	4.7	36.5	38.0	25.5	68.4	249	0.075
High metal	1500	25.4	Loam	6.9	5.2	39.5	34.5	26.0	265	295	0.096

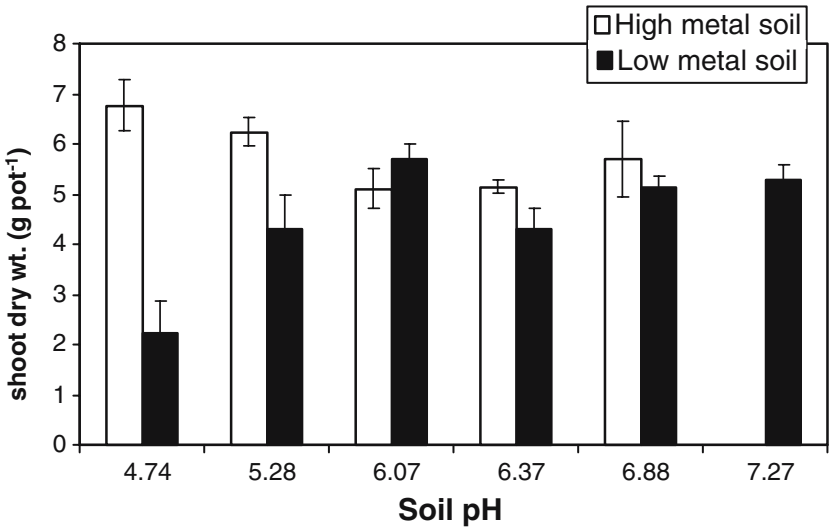

Figure 1. Means ($n = 4$) and standard errors of *T. caerulescens* shoot dry weight with different pH treatments.

Effect of decreasing pH on the concentrations of 0.1 M $Sr(NO_3)_2$ extractable Al, Ca, Cd, Mg, Mn, and Zn from soils.

Concentrations of 0.1 *M* $Sr(NO_3)_2$ extractable Al, Ca, Cd, Mg, Mn and Zn were strongly affected by soil pH. Decreasing pH drastically increased the concentration of extractable Al, Cd, Mn, and Zn while it decreased the extractable concentrations of Ca and Mg (Figures 2 and 3). The extractable Al concentration for the pH treatments was not equal for the high and low metal soils. For high metal soil, from the highest pH to the lowest, Al increased about 30%. However, for low metal soil, there was 8 to 11 fold increase in Al concentration. The final concentration reached 49 and 71.8 mg kg^{-1} for rhizosphere and non-rhizosphere soil, respectively. This difference may be due to the difference in the clay mineralogy. In the low pH treatments of the low metal soil, released extractable Al due to decomposition of clay minerals added extra buffering capacity to the soil. Therefore higher S was required to reduce soil pH in the low metal soil. There was little difference in the concentrations of Ca, Mn and Mg between these two soils. Rhizosphere soil generally had lower 0.1 *M* $Sr(NO_3)_2$ extractable metal concentrations, which indicating the uptake by the plant roots lowered the extractable metal concentrations.

Effect of pH on plant tissue Cd and Zn concentration

Plants grown in the high metal soil had much higher shoot Cd concentration than those in the lower metal soil. For the high metal soil, shoot Cd concentration ranged from 937–1456 mg kg^{-1} DW and linearly increased with decreasing of soil pH ($\text{LogCd} = 7.73 - 0.12 \times \text{pH}$, adjusted $R^2 = 0.24$, $P < 0.01$) (Figure 4). For the low metal soil, shoot Cd concentration ranged from 86 to 355 mg kg^{-1} DW. Cadmium concentration increased with decreasing pH from pH 7.27 to 6.07, then rapidly decreased in the lower pH treatment. It fit a quadratic pH regression model ($\text{LogCd} = -16.24 + 7.07 \times \text{pH} - 0.56 \times \text{pH}^2$, adjusted $R^2 = 0.79$, $P < 0.0001$) (Figure 4).

Unlike the shoot, root Cd concentrations did not respond to pH changes. For all pH treatments, the root Cd concentrations were not significantly different from each other. However, roots from high metal soil still had much higher Cd concentrations than the low metal soil. The possible reason was that the differences in available Cd concentrations caused by pH treatments within each soil usually were still smaller than the differences between high and low metal soils at the same pH treatment.

Soil pH had a significant effect on shoot Zn concentration. For high metal soil, shoot Zn concentrations present an irregular pattern

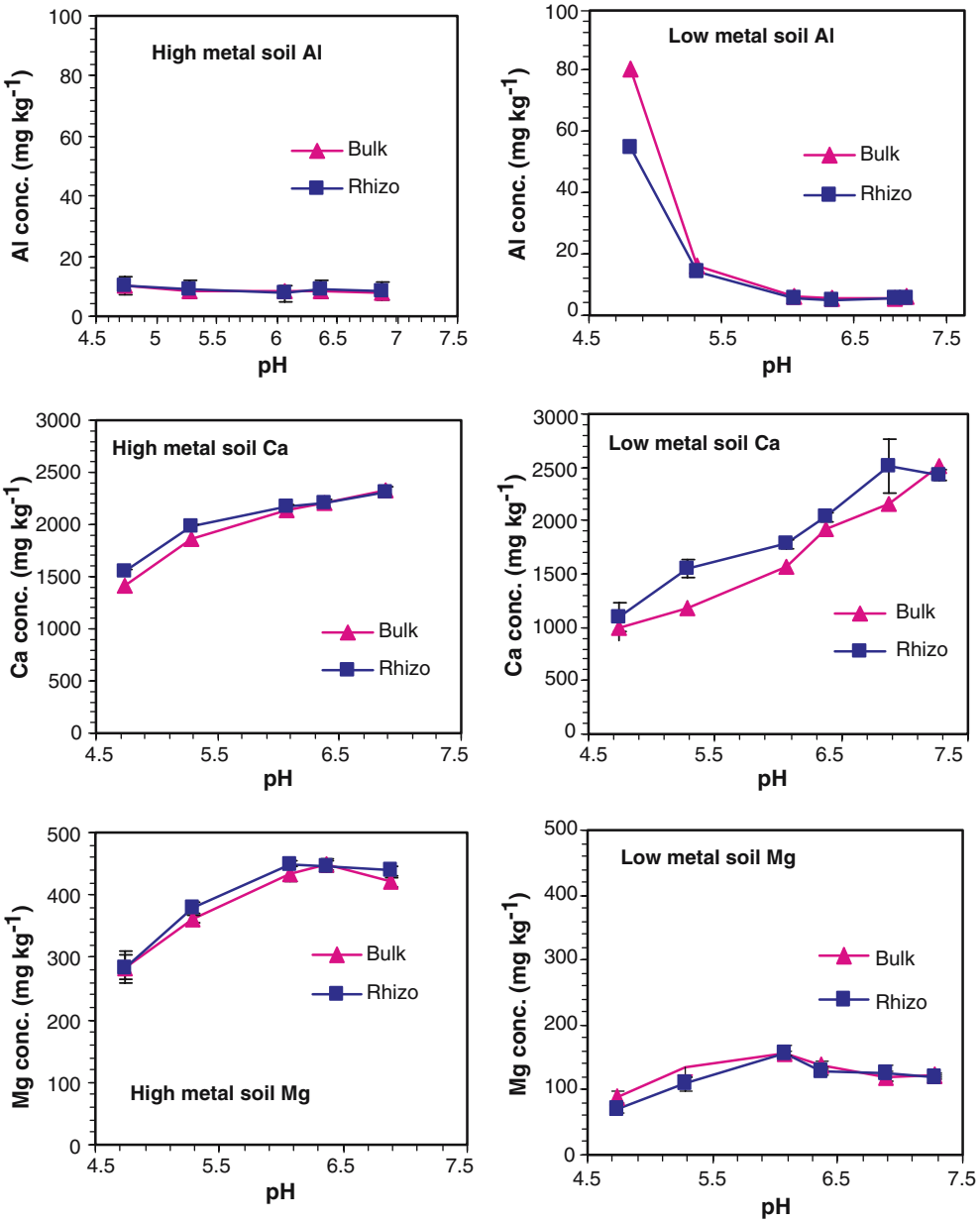

Figure 2. Means ($n = 8$) and standard errors of 0.1 *M* $Sr(NO_3)_2$ extractable Al, Ca, and Mg concentrations in high metal soil and low metal soil with different pH treatments.

(Figure 5). For low metal soil, the highest concentration was at pH 6.07 then decreased with increasing distance from this pH. It well fit a quadratic pH regression model (Log Zn = $-16.37 + 8.11 \times \text{pH} - 0.67 \times \text{pH}^2$, adjusted $R^2 = 0.85$, $P < 0.0001$).

Effect of pH on total amount of Cd and Zn accumulated in shoot

Lowering pH significantly affected total Cd accumulated in shoots ($F = 55.6$, $P < 0.001$). For the high metal soil, values ranged from 4.8 to

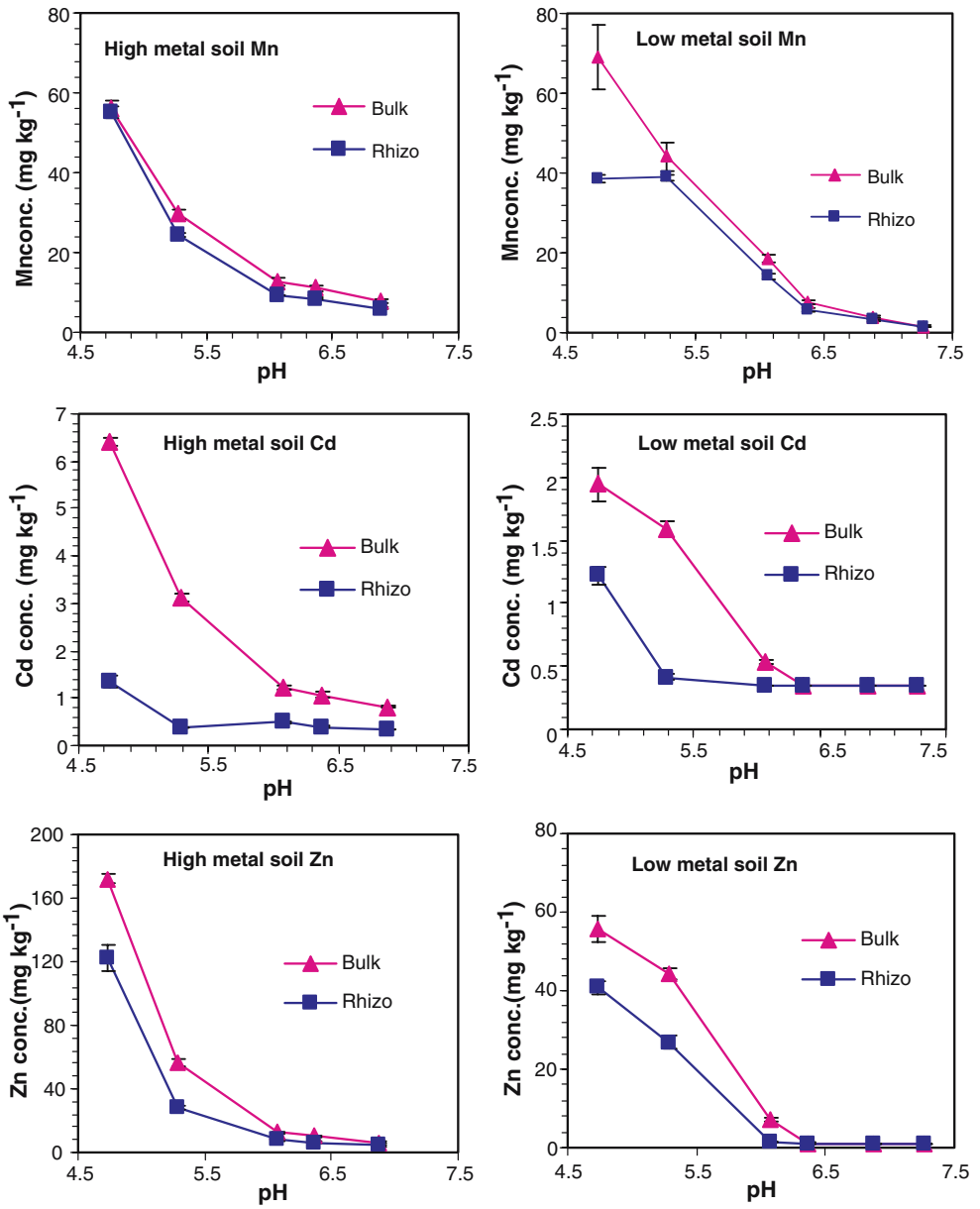

Figure 3. Means ($n = 8$) and standard errors of 0.1 *M* $Sr(NO_3)_2$ extractable Mn, Cd, and Zn concentrations in high metal soil and low metal soil with different pH treatments.

9.1 mg kg^{-1}. Total Cd extracted at the two lowest pH treatments were significantly higher than the other three higher pH treatments (Table 3). For the low metal soil, the values ranged from 0.2 to 2.0 mg kg^{-1}. The highest extraction rate was at pH 6.07. Total Cd extraction at pH 5.28, 6.37, 6.88, and 7.27 were not significantly different. However, when pH was reduced to 4.74, there was a drastic reduction in total Cd phytoextraction. This was due to the combination of significant yield reduction and lowered metal concentration in the shoot. There was also a significant difference between the two soils. Plants grown in the high metal soil extracted much higher Cd and Zn than those in the low metal soil at most pH treatments. *Thlaspi caerulescens*

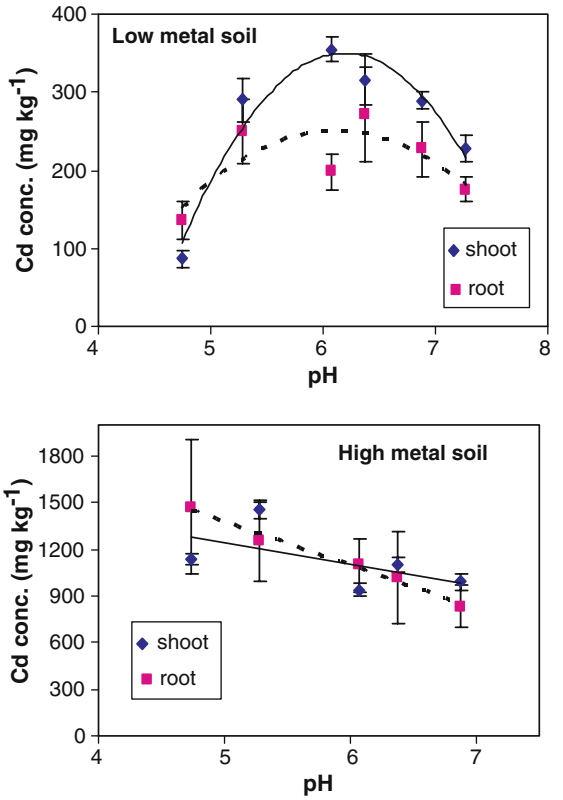

Figure 4. Changes of *T. caerulescens* tissue Cd concentration in high metal soil and low metal soil with pH treatments.

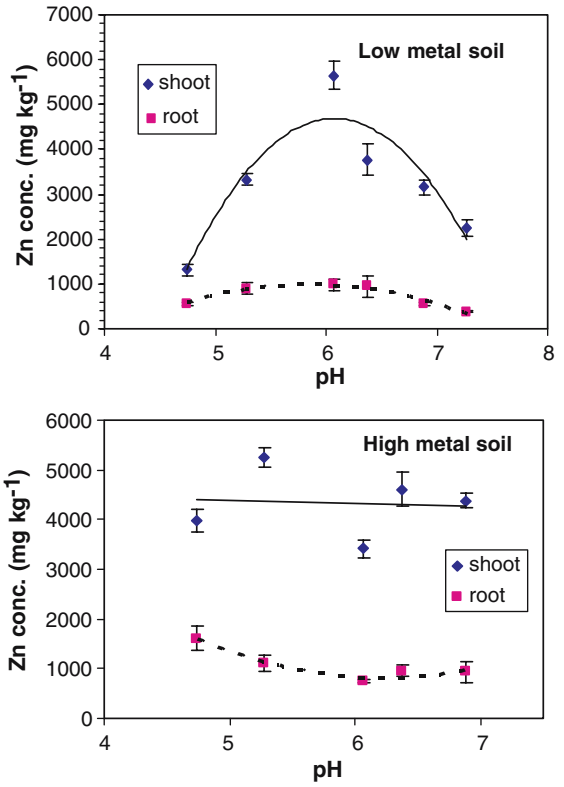

Figure 5. Changes of *T. caerulescens* tissue Zn concentration in high metal soil and low metal soil with pH treatments.

extracted as high as 9.1 mg Cd, about 36% of the total soil Cd in a pot, in the high metal soil pH treatment of 5.28.

Total Zn phytoextracted by shoots followed a similar pattern as Cd. For the high metal soil, values ranged from 17 to 33 mg kg^{-1}, which was about 1–2% of total soil Zn. For the low metal soil, values ranged from 3 to 32 mg kg^{-1}, or about 0.6–7% of the total soil Zn.

Discussion

A major limit to plant metal uptake is the solubility of metals in soil. Whiting et al. (2003) reported that in order to have efficient Zn uptake by *T. caerulescens*, soil solution Zn concentration should be no less than 27 μM. Lowering pH increased easily available Cd and Zn concentrations and enhanced metal uptake. Strontium nitrate (0.1 *M*) extractable Zn concentration increased more than 20-fold in both soils from the

Table 3. Total Cd and Zn translocated (shoot metal concentration × shoot dry weight) and percent of Cd and Zn translocated (total biomass metal/total metal in soil) by *Thlaspi caerulescens* in different pH treatments

soil	pH	Total Cd mg kg^{-1} soil	%Cd	Total Zn mg kg^{-1} soil	%Zn
High	4.74	7.70a*	30.3	26.99ab	1.8
metal soil	5.28	9.08a	35.8	32.82a	2.2
	6.07	4.78b	18.8	17.44c	1.2
	6.37	5.67b	22.3	23.67bc	1.6
	6.88	5.64b	22.2	24.96ab	1.7
Low	4.74	0.19c	3.8	2.91c	0.6
metal soil	5.28	1.25b	25.0	14.33b	3.2
	6.07	2.02a	40.4	32.08a	7.1
	6.37	1.36b	27.2	16.21b	3.6
	6.88	1.49b	29.8	16.26b	3.6
	7.27	1.21b	24.2	11.89b	2.6

*Means with different letters for the same soil within each column are significantly different (LSD, $P < 0.05$).

highest pH treatment to the lowest pH treatment. Extractable Cd also significantly increased with decreasing of soil pH.

Few studies have investigated the relationship between pH and *T. caerulescens* metal uptake. In a greenhouse study, Brown et al. (1994) used three soils adjusted to three different pH levels. Although metal concentrations in the plant biomass tended to increase in the lower pH treatments, the dramatic yield reduction in lower pH treatments made the final total extraction of these treatments even less than the higher pH treatments. This difference in result may be caused by a major difference in pH adjustment method. Although they, too, used S addition to lower soil pH, apparently, this process was not followed by salt leaching. Kayser et al. (2000) mentioned the high sensitivity of *T. caerulescens* to S addition. Presumably this sensitivity was due to salt toxicity rather than low pH effect. As we can see, there was no yield reduction even at the lowest pH treatment in the high metal soil in our study. Therefore, salt leaching is important to observe the true pH effect. In a field study of Brown et al. (1995), soil pH had no effect on Zn uptake. Lowering pH increased Cd uptake at the two highest metal treatments. In the control and low metal treatments, there was no significant difference in uptake. Since there were only two pH levels for each soil, it was not possible to conclude the pH and uptake relationships. Kayser et al. (2000) used sulfur to reduce soil pH and observed a more consistent effect of enhancing Zn and Cd uptake by other plant species including Indian mustard (*Brassica juncea)*, tobacco *(Nicotiana tabacum)*, willows *(Salix viminalis)*, sunflower *(Helianthus annuus)*, and maize *(Zea mays)*. Sulfur caused a small decrease in soil pH but a significant increase in Zn and Cd mobility. These authors therefore attributed the effect to soluble salts rather than a direct pH influence.

This research provided strong evidence of lowering pH in enhancing *T. caerulescens* phytoextraction. It also revealed that *T. caerulescens* can grow well under acidified soil pH as long as the acidification management is handled properly. The difference in the two tested soils also suggests that it is important to identify soil characteristics before considering decreasing soil pH as a strategy to enhance phytoextraction. Results may vary according to different soil types. For some soils, lowering soil pH could be a double-edged sword: on the one hand, decreasing soil pH can increase plant metal uptake because more metals become easily available for plants; on the other hand, in addition to increased available Cd and Zn, some toxic elements, e.g. Al and Mn, will also be increased and may reach toxicity levels at low pH. This will damage *T. caerulescens* root development and undermine metal uptake ability. This is especially critical since a dense and prolific root system seems to be strongly associated with hyperaccumulation. Higher plants vary widely in their sensitivity to Al toxicity. Susceptible plants can tolerate no more than 1–2 mg L^{-1} in the nutrient solution while other plants can tolerate more than 100 mg L^{-1} (Ligon and Pierre, 1932; McLean and Gilbert, 1927; Peiffer, 1976). Generally, a soil with potential Al toxicity has soil pH <5.0 and >1–2 mg Al L^{-1} in soil solution. While there is no documented threshold soil solution concentration of Al toxicity for *T. caerulescens*, in the pH treatment of 4.74 and an Al concentration of 71.8 mg L^{-1} there is no doubt that the great yield loss in the low metal soil was caused by Al or a combination of Al and Mn toxicity.

Researchers have unsuccessfully tried to correlate soil metal concentrations with plant concentrations using different extraction methods (Sims and Kline, 1991; Tsadilas et al., 1995). In fact, correlation studies are usually of limited use in interpreting bioavailability. This was supported by our Cd data. Non-rhizosphere soil extractable Cd was significantly correlated with shoot Cd concentration ($r = 0.53$, $P < 0.001$). Comparing total Cd in the plant shoot and extractable Cd pool (except pH 4.74), total Cd accumulated in plant biomass was larger than the extractable soil Cd pool. *Thlaspi caerulescens* must have used Cd from other pools. In these treatments, uptake by *T. caerulescens* is limited by the amount of "direct available" metal ions and must rely on soils replenishing ability for a continuous metal supply. Therefore the original extractable Cd pool is the available metal content at soil equilibrium. The larger the initial available Cd pool, the higher the soils ability to replenish Cd.

If we remove the treatments when the labile pool exceeded metal uptake, the extractable

metal amount should be a better indicator of metal "bioavailability". After we removed the pH 4.74 treatment from the high metal soil, and the 4.74 and 5.28 treatments from the low metal soil, the correlation coefficient of shoot Cd concentration and extractable Cd in soil indeed increased to 0.82 ($P < 0.0001$). Similar results were found for Zn, too. This showed that care must be exercised when interpreting correlations of plant metal concentrations with soil metal concentrations. A valid connection only occurs when two conditions concurrently exist: metal phytoavailability is limited for plant uptake and the rate of metal re-supply through replenishing mechanisms cannot meet the need for rapid uptake; therefore, the plant is constantly under the pressure of metal limitation.

The results also demonstrated that the Southern French population of *T. caerulescens* was much more efficient in reducing soil Cd concentrations than Zn. Total soil Cd was reduced by 19 to 36% in the high metal soil and the 0.1 *M* $Sr(NO_3)_2$ extractable pool was nearly depleted. While soil total Zn concentration was only reduced by 1 to 2% in the high metal soil. This was also because polluted soils usually contain many times more Zn than Cd; the difference of Zn concentration caused by plant uptake is much less dramatic than that of Cd. It is likely that under field conditions, total metal reduction due to *T. caerulescens* uptake would be less considering that in pot studies uptake has been enhanced by growing plants in the confined conditions of pots (Hammer and Keller, 2003).

A study where phytoextraction of three continuous crops of *T. caerulescens* were investigated (Keller and Hammer, 2004) indicated that relatively constant uptake of Zn can be achieved in subsequent croppings. However, Cd concentrations were significantly lower after the third cropping due to decreased Cd concentrations in the soil solution. They also pointed out that solution Cd and Zn tended to increase after phytoextraction to reach a new equilibrium due to a large buffering capacity. Therefore, in our experiment, by acidifying soil to increase the ability of soil to replenish solution Cd and Zn, one can expect more efficient uptake of Cd in subsequent croppings. If the constant uptake rate can be achieved, lowering pH would shorten the required remediation time from 5 year (the original soil pH) to 3 year (the optimum soil pH 5.28 in the high metal soil and 6.07 in the low metal soil) to remove all the Cd from both soils. For Zn, lowering pH would shorten the remediation time from 60 to 46 year for the high metal soil and 38 to 14 year for the low metal soil. However, in our experiment, removal of 30–40% of soil Cd in a single cropping is remarkable and whether this high rate can be kept in the following croppings needs further experimental evidence.

In the literature, there is a wide range of planting density used in different experiments. A density of 4 plants kg^{-1} soil was used in a pot study of Lombi et al. (2001a) while Hammer and Keller (2003) used a density of 100 plants m^{-2} in their field trials. Field experiment at Woburn, UK demonstrated that the biomass of *T. caerulescens* varied between 2 and 5 t ha^{-1} (McGrath et al., 2000). It has been suggested that an average biomass yield of 5 t ha^{-1} should be achievable with good agronomic management (Zhao et al., 2003). We used 3 plants kg^{-1} soil, which roughly equals to 300 plants m^{-2} in the field, and the average yield was 5.14 g kg^{-1} soil. This would result a yield of 5.14 t ha^{-1} assuming the soil surface area in a pot is 0.01 m^{-2}.

Complete sequential extraction experiments are needed to assess the possible changes in metal distribution. Our data also show that due to the ability of soils to replenish specific pools, *T. caerulescens* uptake was not exclusively confined by original available forms of metals. Similarly, other studies have found that for *T. caerulescens*, metals released from formerly non-available forms could reach more than 50% of the metals accumulated in plants (Knight et al., 1997; Whiting et al., 2001a, 2001b). In other words, depletion of soluble metal pool is not "definitive". Soils can rapidly replenish and reach a new equilibrium. Therefore, significant reduction in total metal concentration is more relevant since this will force soil to have lower bioavailable metals even under new equilibrium at the same environmental conditions.

Some may concern about the risk of off site movement of heavy metals after solubilization. We used the total metal content in soils before phytoextraction to subtract the amount of total metal extracted by plant and the metals in the soil after phytoextraction (data not shown).

The results indicated that the loss of metal was minimal, especially if pH only went down to the optimum pH levels instead of the lowest ones we used here. This may due to the rapid uptake of *Thlaspi caerulescens* and as a result the depletion of soluble metal pools.

In conclusion, lowering pH is an effective method to enhance metal bioavailability and *T. caerulescens* uptake for both Cd and Zn. It also can greatly shorten the time span for phytoremediation to be complete and has the potential to overcome the problems associated with long-term requirement of phytoremediation. Using S to reduce soil pH followed by salt leaching is advantageous over chelating-induced methods because it is cost-effective and with few toxic effects on plant growth. Salt leaching is an important step in soil pH adjustment. The possible reason that previous similar work failed to observe a consistent and more apparent pH effect is the great yield reduction caused by salt toxicity. However, care must be taken to avoid unnecessary extreme acidification treatments as in some cases this may induce Al and Mn toxicity. The efficiency of acidification in enhancing phytoextraction as well as the proper and effective pH range for maximum metal uptake may differ for individual soils and therefore must be identified to ensure more efficient and successful phytoremediation. Assuming a "one-size-fits-all" protocol of soil acidification is impractical.

Acknowledgments

We gratefully acknowledge Carrie E. Green for her advice and assistance.

References

Ahnstrom Z A S and Parker D R 1999 Development and assessment of a sequential extraction procedure for the fractionation of soil cadmium. Soil Sci. Soc. Am. J. 63, 1650–1658.

Aualiitia T U and Pickering W F 1987 The specific sorption of trace metal amounts of Cu, Pb, and Cd by inorganic particulates. Water Air Soil Poll. 35, 171–185.

Baker A J M and Brooks R R 1989 Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. Biorecovery 1, 81–126.

Brooks R R 1998 Plants that Hyperaccumulate Heavy Metals. CAB International, Wallingford, UK.

Brown S L, Chaney R L, Angle J S and Baker A J M 1994 Phytoextraction potential of *Thlaspi caerulescens* and Bladder Campion for zinc and cadmium-contaminated soil. J. Environ. Qual. 23, 1151–1157.

Brown S L, Chaney R L, Angle J S and Baker A J M 1995 Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris* grown on sludge-amended soils. Environ. Sci. Technol. 29, 1581–1585.

Campbell C R 1992 Determination of total nitrogen in plant tissue by combustion. *In* Plant Analysis Reference Procedures for the Southern Region of the United States. Ed. C O Plank. pp. 21–23. Southern Cooperative Series Bulletin 368.

Castilho P D and Chardon W J 1995 Uptake of soil cadmium by three field crops and its prediction by a pH-dependent Freundlich sorption model. Plant Soil 171, 263–266.

Chaney R L 1983 Plant uptake of inorganic waste constituents. *In* Land Treatment of Hazardous Wastes. pp. 50–77. Noyes Data Corporation, Park Ridge, NJ.

Chaney R L, Malik M, Li Y M, Brown S L, Brewer E P, Angle J S and Baker A J M 1997 Phytoremediation of soil metals. Curr. Opin. Biotech. 8, 279–284.

Eckert D and Sims J T 1995 Recommended soil pH and lime requirement test. *In* Recommended Soil Testing Procedures for the Northeastern United States. Eds. J T Smims and A Wolf. pp. 16–21. Northeastern Regional Publication No. 493.

Farrah H and Pickering W F 1976 The sorption of copper species by clays. II. Illite and montmorillonite. Aust. J. Chem. 29, 1177–1184.

Farrah H and Pickering W F 1977 The sorption of lead and cadmium species by clay and minerals. Aust. J. Chem. 30, 1417–1422.

Flannery R L and Markus D K 1980 Automated analysis of soil extracts for P, K, Ca, and Mg. J. Assoc. Off. Anal. Chem. 63, 779–787.

Gee G W and Bauder J W 1986 Particle size analysis. *In* Methods of Soil Analysis, Part 1. Agronomy Monograph 9. pp. 383–411. American Society of Agronomy, Madison, WI.

Hall G E M, Vaive J E, Beer R and Hoashi M 1996 Selective leaches revisited with emphasis on the amorphous Fe oxyhydroxide phase extraction. J. Geochem. Explor. 56, 59–78.

Hammer D and Keller C 2003 Phytoextraction of Cd and Zn with *Thlaspi caerulescens* in field trials. Soil Use Manage. 19, 144–149.

Hickey M G and Kittrick J A 1984 Chemical portioning of cadmium, copper, nickel, and zinc in soils and sediments containing high levels of heavy metals. J. Environ. Qual. 13, 372–376.

Kayser A, Wenger K, Keller A, Attinger W, Felix H R, Gupta S K and Schulin R 2000 Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: the use of NTA and sulfur amendments. Environ. Sci. Technol. 34, 1778–1783.

Keller C and Hammer D 2004 Metal availability and soil toxicity after repeated croppings of *Thlaspi caerulescens* in metal contaminated soils. Environ. Pollut. 131, 243–254.

Kim N D and Fergusson J E 1991 Effectiveness of a commonly used sequential extraction technique in determining the speciation of cadmium in soils. Sci. Tot. Environ 105, 191–209.

Knight B P, Zhao F J, McGrath S P and Shen Z G 1997 Zinc and cadmium uptake by the hyperaccumulator *Thlaspi caerulescens* in contaminated soils and its effects on the

concentration and chemical speciation of metals in soil solution. Plant Soil 197, 71–78.
Ligon W S and Pierre W H 1932 Soluble aluminum studies. II. Minimum concentrations of aluminum found to be toxic to corn, sorghum and barley in culture solutions. Soil Sci. 34, 307–321.
Lombi E, Zhao F J, Dunham S J and McGrath S P 2000 Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesingense*. New Phytol. 145, 11–20.
Lombi E, Zhao F J, Dunham S J and McGrath S P 2001a Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextracion. J. Environ. Qual. 30, 1919–1926.
Lombi E, Zhao F J, McGrath S P, Young S D and Sacchi G A 2001b Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. New Phytol. 149, 53–60.
Luo Y M, Christie P and Baker A J M 2000 Soil solution Zn and pH dynamics in non-rhizosphere soil and in the rhizosphere of *Thlaspi caerulescens* grown in a Zn/Cd contaminated soil. Chemosphere 41, 161–164.
McGrath S P, Dunham S J and Correll R L 2000 Potential for phytoextraction of zinc and cadmium from soils using hyperaccumulator plants. *In* Phytoremediation of Contaminated Soil and Water. Eds. N Terry and G Bañuelos. pp. 109–128. Lewis Publishers, Boca Raton, FL.
McGrath S P, Shen Z G and Zhao F J 1997 Heavy metal uptake and chemical changes in the rhizosphere of *Thlaspi caerulescens* and *Thlaspi ochroleucum* grown in contaminated soils. Plant Soil 188, 153–159.
McGrath S P, Zhao F J and Lombi E 2002 Phytoremediation of metals, metalloids, and radionuclides. Adv. Agron. 75, 1–56.
McLean F T and Gilbert B E 1927 The relative aluminum tolerance of crop plant. Soil Sci. 24, 163–176.
Metting B F 1993 Soil Microbial Ecology. Marcel Dekker, New York.
Narwal R P, Singh B R and Panhwar A R 1983 Plant availability of heavy metals in a sludge-treated soil: I. Effect of sewage sludge and soil pH on the yield and chemical composition of rape. J. Environ. Qual. 12, 358–365.
Peiffer R A 1976 Inter- and intra-specific plant difference in tolerance to aluminum. Ph.D. Thesis. The Pennsylvania State University, University Park, PA.
Pollard A J, Harper K D and Smith J A C 2002 The genetic bases of metal hyperaccumulation in plants. Crit. Rev. Plant Sci. 21, 539–566.
Reeves R D and Brooks R R 1983 European species of *Thlaspi* L. (Cruciferae) as indicators of nickel and zinc. J. Geochem. Explor. 18, 275–283.
Reeves R D, Schwartz C, Morel J L and Edmondson J 2001 Distribution and metal-accumulation behavior of *Thlaspi caerulescens* and associated metallophytes in France. Int. J. Phytoremediation 3, 145–172.
Robinson B H, Leblanc M, Petit D, Brooks R R, Kirkman J H and Gregg P E H 1998 The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant Soil 203, 47–56.
Roosens N, Verbruggen N, Meerts P, Ximénez-Embún P and Smith J A C 2003 Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. Plant Cell Environ. 26, 1657–1672.
SAS Release 8.2 1999–2001 SAS Institute Inc., Cary, NC.
Schwartz C, Morel J L, Saumier S, Whiting S N and Baker A J M 1999 Root development of the zinc-hyperaccumulator plant *Thlaspi caerulescens* as affected by metal origin, content and localization in soil. Plant Soil 208, 103–115.
Shen Z G, Zhao F J and McGrath S P 1997 Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum*. Plant Cell Environ. 20, 898–906.
Shuman L M 1982 Separating soil iron- and manganese-oxide fractions for microelement analysis. Soil Sci. Soc. Am. J. 46, 1099–1102.
Shuman L M 1983 Sodium hypochlorite methods for extracting microelements associated with organic matter. Soil Sci. Soc. Am. J. 47, 656–660.
Shuman L M 1991 Chemical forms of micronutrients in soil. *In* Micronutrients in Agriculture. Ed. J J Mortvedt. pp. 113–144. 2nd edition, SSSA Book Ser. 4. SSSA, Madison, WI.
Sims J T and Kline J S 1991 Chemical fractionation and plant uptake of heavy metals in soils amended with co-composted sewage sludge. J. Environ. Qual. 20, 387–395.
Small E P 1995 Field capacity is not saturation. Soil Profiles 5, No 2.
Storer D A 1984 A simple high sample volume ashing procedure for determining soil organic matter. Commun. Soil Sci. Plan. 15, 759–772.
Tiller K G, Gerth J and Brümmer G 1984 The relative affinities of Cd, Ni and Zn for different soil clay fractions and goethite. Geoderma 34, 17–35.
Tiller K G, Nayyar V L K and Clayton P M 1979 Specific and nonspecific sorption of cadmium by soil clays as influenced by zinc and calcium. Aust. J. Soil. Res. 17, 17–28.
Tsadilas C D, Matsi T, Barbayiannis N and Dimoyiannis D 1995 Influence of sewage sludge application on soil properties and on distribution and availability of heavy metal fraction. Commun. Soil Sci. Plan. 26, 2603–2619.
Whiting S N, Broadley M R and White P J 2003 Applying a solute transfer model to phytoextraction: Zn acquisition by *Thlaspi caerulescens*. Plant Soil 249, 45–56.
Whiting S N, Leake J R, McGrath S P and Baker A J M 2000 Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. New Phytol. 145, 199–210.
Whiting S N, Leake J R, McGrath S P and Baker A J M 2001a Assessment of Zn mobilization in the rhizosphere of *Thlaspi caerulescens* by bioassay with non-accumulator plants and soil extraction. Plant Soil 237, 147–156.
Whiting S N, Leake J R, McGrath S P and Baker A J M 2001b Zinc accumulation by *Thlaspi caerulescens* from soils with different Zn availability: a pot study. Plant Soil 236, 11–18.
Yanai J, Zhao F J, McGrath S P and Kosaki T, 2005 Effect of soil characteristics on Cd uptake by the hyperaccumulator *Thlaspi caerulescens*. Environ. Pollut. 139, 167–175.
Zhao F J, Hamon R E and McLaughlin M J 2001 Root exudates of the hyperaccumulator *Thlaspi caerulescens* do not enhance metal mobilization. New Phytol. 151, 613–620.
Zhao F J, Lombi E and McGrath S P 2003 Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. Plant Soil 249, 37–43.

Section editor: F. J. Zhao