Zinc and Cadmium Uptake by Hyperaccumulator *Thlaspi caerulescens* Grown in Nutrient Solution

S. L. Brown,* R. L. Chaney, J. S. Angle, and A. J. M. Baker

ABSTRACT

Phytoremediation of heavy-metal-contaminated soils can be an inexpensive means to remove hazardous metals from soil. Two metallophytes, *Thlaspi caerulescens* (J. & C. Presl, a Zn and Cd hyperaccumulator) from Prayon, Belgium, and a Zn-tolerant ecotype of bladder campion [*Silene vulgaris* (Moench.) Garcke L.] from Palmerton, PA, were compared with tomato [*Lycopersicon lycopersicum* (L.) Karsten, metal intolerant] in nutrient solution to characterize Zn and Cd uptake and tolerance. Zinc and Cd were added to solutions at a 50:1 molar ratio to simulate concentrations often found on contaminated sites. Seven treatment concentrations were used, ranging (in half-log increments) from 3.16 μ*M* Zn + 0.063 μ*M* Cd to 10000 μ*M* Zn + 200 μ*M* Cd. *Thlaspi caerulescens* showed much greater tolerance to Zn/Cd treatments than the other species, with toxicity stress only apparent at the 10000 μ*M* Zn/200 μ*M* Cd treatment. In this treatment, shoot concentrations of Zn and Cd were 33600 and 1140 mg kg^{-1}, respectively. *Thlaspi caerulescens* was also more effective at translocating both Zn and Cd from solution to shoots. Zinc concentration in shoots of *T. caerulescens* was higher than the other species at all Zn/Cd treatments. Cadmium concentration in shoots of *T. caerulescens* were significantly higher than in bladder campion only at the 316 μ*M* Zn/6.32 μ*M* Cd treatment. This genotype of *T. caerulescens* may not hyperaccumulate Cd. However, extreme Zn and Cd uptake and tolerance is evident in *T. caerulescens*, with >25000 mg Zn kg^{-1} and 1000 mg Cd kg^{-1} before yield is reduced. Results suggest that *T. caerulescens* may be a candidate for the phytoremediation of Zn-contaminated soils.

THE AMOUNT OF LAND contaminated with heavy metals has increased during the last century due to mining, smelting, and other industrial activities. At present, remediation technologies consist primarily of removal and replacement of contaminated soils (Geiger et al., 1993). An alternative soil remediation technology has been proposed that would use rare, heavy-metal-tolerant plant species that are able to hyperaccumulate metals in plant shoots (Chaney, 1983; Baker and Brooks, 1989; Baker et al., 1991). Possibly, these tolerance mechanisms could be exploited to remove heavy metal pollutants from soil (Chaney, 1983; Baker et al., 1991). This proposed technology, called *phytoremediation*, involves successive croppings of hyperaccumulator plants to translocate polluting metals from soil to plant shoots. Shoots of some species accumulate as much as 4 g Ni or Zn kg^{-1} in dry matter, and the plant ash is similar to low-grade metal ores (Baker and Walker, 1990). Harvested plant shoot biomass could then be smelted to recycle the accumulated metals. Before phytoremediation can be developed for commercial uses, the behavior of hyperaccumulator species must be more clearly understood. An understanding of the patterns of heavy metal uptake and the specificity of tolerance will enable growers to maximize metal pollutant concentrations in plant shoots.

Plants able to tolerate high soil concentrations of particular metals were first described several centuries ago (Cannon, 1960; Baker et al., 1988). Metal-tolerant plants are currently used to revegetate sites denuded due to excessive soil metal concentrations (e.g., Oyler, 1988). Recent studies investigated the evolution of tolerance (Antonovics et al., 1971; Baker et al., 1990), specific mechanisms of tolerance (Ernst, 1978; Baker, 1987; Baker and Walker, 1990; Cumming and Tomsett, 1992), and the specificity of metal tolerance (Baker, 1987; Cumming and Tomsett, 1992).

Hyperaccumulator species are defined as those whose leaves contain >100 mg Cd kg^{-1}, 1000 mg Ni and Cu kg^{-1}, or >10000 mg Zn and Mn kg^{-1} (dry weight) when grown in metal-rich soils (Baker and Brooks, 1989; Baker et al., 1994). Species also accumulate metals when grown in conventional potting media (Reeves and Brooks, 1983). Possibly, hyperaccumulator plants have a higher requirement for metals such as Zn, which are essential micronutrients, and show a positive response to increased soil or solution concentrations of these elements (Hajar, 1987).

Thlaspi caerulescens and bladder campion are both metallophytes. *Thlaspi caerulescens* can be considered an endemic Zn hyperaccumulator (Baker, 1987). *Endemic metallophytes* are defined as ancient colonizing species that are only competitive on contaminated sites. *Thlaspi caerulescens* grows as a small basal rosette up to 15 cm high. Its habitat is generally confined to metal-contaminated sites. *Thlaspi caerulescens* is related to the perennial weed pennycress (*Thlaspi arvense* L.). Bladder campion has ecotypes tolerant of a variety of metals (Baker, 1978; Verkleij et al., 1990; Schat and Kalff, 1992). It can grow on unpolluted as well as contaminated sites. Both species may prove useful in the remediation of contaminated sites, *T. caerulescens* through phytoremediation and bladder campion through revegetation.

Previous work with *T. caerulescens* has concentrated on defining metal concentrations and growth patterns of plants found on contaminated sites (Rascio, 1977; Reeves

S.L. Brown and J.S. Angle, Dep. of Agronomy, Univ. of Maryland, College Park, MD 20742; R.L. Chaney, USDA-ARS, Environmental Chemistry Lab., Beltsville, MD 20705; and A.J.M. Baker, Dep. of Animal and Plant Sciences, Sheffield Univ., Sheffield, UK S10 2UQ. Received 25 Oct. 1993. *Corresponding author.

Published in Soil Sci. Soc. Am. J. 59:125–133 (1995).

and Brooks, 1983; Reeves et al., 1983). Limits of tolerance and cotolerance have also been addressed by some researchers (Reeves and Baker, 1984; Ingrouille and Smirnoff, 1986; Hajar, 1987; Baker et al., 1994). Rascio (1977) measured the Zn content of *Thlaspi rotundifolium* ssp. *cepaeifolium* (Wulfen) Rouy & Foucaud roots, shoots, and leaves from a mine site at different intervals during a 1-yr period. Leaf concentrations were consistently higher than root or stem concentrations.

For phytoremediation to prove effective, it is necessary to delineate patterns of uptake and limits of tolerance of potential phytoremediation plant species. It is also important to define accumulation patterns at lower concentrations of soil metals to determine if phytoremediation efficiency will decline as soils approach natural metal concentrations. This study was conducted using nutrient solution to observe Zn and Cd uptake across a wide range of solution metal concentrations. Zinc and Cd uptake and distribution within the three plant species were compared to develop more specific insight into the functioning of a hyperaccumulator species (*T. caerulescens*) in comparison with a tolerant species (bladder campion) and a sensitive species (tomato). Mineral nutrient concentrations in *T. caerulescens* were also measured to determine the effect of high Zn concentrations on the distribution and uptake of necessary plant nutrients by a hyperaccumulator.

MATERIALS AND METHODS

A nutrient solution study was conducted to define patterns of Zn and Cd uptake by two Zn- and Cd-tolerant races of bladder campion and *T. caerulescens* compared with a nontolerant species ('Rutgers' tomato) at varying solution concentrations of Zn and Cd. Seed for bladder campion was collected from plants growing on the mountainside <1.6 km directly downwind from a Zn smelter in Palmerton, PA. Seed for *T. caerulescens* was obtained from plants growing near a Zn/Cd smelter in Prayon, Belgium (Vazquez et al., 1992). The study was conducted in an environmental growth chamber in which temperature was maintained at 25°C day and 19°C night, and relative humidity was set at 70%. The day period was 16 h, with >400 μmol photon m^{-2} s^{-1} photosynthetically active radiation from a combination of cool-white fluorescent and incandescent lamps.

Thlaspi caerulescens was germinated by placing seeds in an aerated dilute 0.5 concentrated Hoagland solution (2.5 m*M* $CaNO_3$ and KNO_3; 10 μ*M* Fe as FeEDDHA; 0.1 m*M* K_2HPO_4; 75 μ*M* KCl; 10 μ*M* H_3BO_3; 1 m*M* Mg as $MgSO_4$; 4 μ*M* Mn as $MnCl_2 \cdot 4H_2O$; 0.5 μ*M* Cu as $CuSO_4 \cdot 5H_2O$; 1 μ*M* Zn as $ZnSO_4 \cdot 7H_2O$; and 0.2 μ*M* Mo as $Na_2MoO_4 \cdot 2H_2O$). After 2 d, radicals and two primary leaves emerged and seedlings were transplanted to standard seed germination papers. The papers were kept moist in a germination solution containing 1 m*M* $MgSO_4$, 0.1 m*M* K_2HPO_4, 2.5 m*M* $Ca(NO_3)_2$, and 2.5 m*M* KNO_3. After 14 d, *T. caerulescens* was transferred from the seed germination papers into 8-L buckets containing the same nutrient solution described above. Seedlings were maintained in these buckets for an additional 14 d before treatments were initiated.

Bladder campion seed was placed in standard seed germination papers kept moist with the same germination solution described above. Seeds germinated within 6 d. Bladder campion was transferred to 8-L buckets containing the nutrient solution described above 2 wk after seeds germinated and was maintained in the growth medium for 7 d until metal treatments were initiated. Tomato seeds were also germinated in seed germination papers for 10 d. Seedlings were transplanted into 8-L buckets containing the above nutrient solution 4 d before metal treatments were begun.

Two *T. caerulescens*, three bladder campion, and two tomato plants were placed into each of the 2-L polyethylene beakers used for the experiment. The continuosly aerated beakers were arranged in a randomized complete block design with three replications. Beakers were filled to 2 L with the previously described nutrient solution minus Zn. Seven treatments with Zn and Cd added at a 50:1 molar ratio were then applied. Zinc was supplied as $ZnCl_2$ and Cd was added as $3CdSO_4 \cdot 8H_2O$. Treatments were 3.16/0.063, 31.6/0.632, 100/2, 316/6.32, 1000/20, 3160/63.2, and 10000/200 μ*M* Zn/Cd. Normal Zn concentrations for nutrient solutions are 1 μ*M* Zn. In most uncontaminated soils, water-soluble Zn and Cd concentrations are <1 μ*M*. In the soil directly behind the smelter in Palmerton, total metals were 0.738 mol Zn kg^{-1} and 9.1 mmol Cd kg^{-1}. Water-extractable (5 g soil per 25 mL H_2O) concentrations (which may have some relationship to soil solution concentrations) were 2.74 m*M* Zn and 32.9 μ*M* Cd (Brown et al., 1994).

The activities of free Zn and Cd as well as other nutrients in the nutrient solutions were calculated using GEOCHEM-PC (Parker et al., 1995). Levels of Zn and Cd treatments were too high to consider using chelator-buffer techniques to buffer metal ion activities in the nutrient solution. Differences in concentration and calculated activity are reported in Table 1. Solution pH was maintained near 6.0 by the addition of 2 m*M* MES (2-morpholinoethanesulfonic acid) buffer and by adjusting pH as necessary by the addition of KOH. This was done to control the precipitation of Zn phosphate across all units. All plants were equally replicated in all treatments, except for the 10000 μ*M* treatment in which only *T. caerulescens* was included because earlier studies showed that the other species would not survive this treatment. Within each beaker, plant roots were separated daily to prevent interspecies contamination. Each day, 10 μ*M* K_2HPO_4, 0.333 μ*M* $CaNO_3$, 0.024 m*M* $MgSO_4$, 106 n*M* Mn, and 269 n*M* B were added to each bucket to maintain nutrient supply. Deionized water was added to buckets to maintain solution volume. Sixteen days after initiation of treatment, all solutions were completely replaced.

Table 1. Total Zn and Cd concentrations and calculated activity (using GEOCHEM-PC) of free Zn^{2+} and Cd^{2+} for seven Zn/Cd treatment additions (Cd/Zn molar ratio 1:50) to 0.5 strength Hoagland solution.

Treatment	Concentration	Calculated activity
μ*M*	— log mol/L —	
Zinc		
3.16	5.50	5.76
31.6	4.50	4.76
100	4.00	4.26
316	3.50	3.84
1000	3.00	3.33
3160	2.50	2.82
10 000	2.00	2.37
Cadmium		
0.063	7.20	7.47
0.632	6.20	6.48
2.00	5.70	5.98
6.32	5.20	5.49
20.0	4.70	5.02
63.2	4.20	4.62
200	3.70	4.34

Harvest

Eight days after the application of treatments, tomato plants in the two highest Zn treatments were harvested. All tomato plants in these treatments had died. Death, or senescence, was defined as the loss of viability of the terminal bud. Bladder campion in the 3160 μM Zn/63.2 μM Cd treatment was harvested 13 d into the experiment. Fifteen days after treatment was initiated, the remaining tomato plants were harvested. Tomato plants in the 3.16 μM Zn/0.063 μM Cd treatment were starting to shade the other species. All remaining bladder campion were harvested at 22 d. At this time, the plants in the 1000 μM Zn/20 μM Cd treatment had also senesced. Bladder campion in all remaining treatments had entered the reproductive growth stage. *Thlaspi caerulescens* was harvested 28 d after beginning the treatments.

At harvest, roots and shoots of all species were separated. Roots were rinsed in deionized water. *Thlaspi caerulescens* shoots were further separated into leaf and stem tissue. All samples were dried at 70°C.

Sample Analysis

Dry plant samples were weighed and placed in a 480°C oven for 16 h. Ash was digested with concentrated HNO_3 and taken to dryness, and the residue was dissolved with 3 M HCl. Samples were brought to 25 mL in 1 M HCl. Necessary dilutions were made in 1 M HCl to maintain constant viscosity. The accuracy of dilutions was checked by diluting known standards. Zinc and Cd concentrations of plants were determined using a flame atomic absorption spectrometer. *Thlaspi caerulescens* samples were also analyzed for Ca, Mg, Mn, Cu, P, Mo, Fe, and K using an inductively coupled plasma spectrometer. Standards were prepared with Zn and Cd concentrations approximately equal to plant sample concentrations to assure that these high concentrations would not interfere with readings of other elements. Cobalt was added to all standards and samples (40.0 mg Co L^{-1} reference standard) as an internal standard for inductively coupled plasma analysis.

Statistical Analysis

Data was analyzed using SAS-PC (SAS Institute, 1989). Data required logarithmic transformation to attain homogeneity. Log-transformed treatment means were separated using the Waller–Duncan K-ratio t-test after it was determined that there was a significant ($P < 0.05$) treatment effect using the GLM procedure. Data presented are arithmetic means of untransformed data. All figures show standard errors calculated from this data. Root mass from bladder campion in the 3160 μM Zn/63.2 μM Cd and tomato in the 1000 μM Zn/20 μM Cd treatments were combined across all replicates due to low yield. Because of this, standard errors were not calculable.

RESULTS AND DISCUSSION

Visual Symptoms

Two days after the initiation of treatments, both bladder campion and tomato leaves receiving the 3160 μM Zn/63.2 μM Cd treatment became purple. This may be from a Zn-induced P deficiency. Tomato leaves in this treatment also had necrotic spots. *Thlaspi caerulescens* was chlorotic in all treatments at the start of treatments. This chlorosis may be related to an abnormally high Zn requirement that was not met by the concentration of Zn in the dilute 0.5 concentrated Hoagland solution in which the plants were maintained prior to the beginning of the experiment (Hajar, 1987). On the fourth day of the trial, tomato plants in the 3160 μM Zn/63.2 μM Cd treatment were dead and those in the 1000 μM Zn/20 μM Cd treatments were also showing signs of toxicity. Bladder campion plants in the 1000 μM/20 μM Cd treatment were showing some purpling in older leaves. *Thlaspi caerulescens* remained chlorotic in all treatments.

Seven days after treatment initiation, tomato remained vigorous only in the 3.16 μM Zn/0.063 μM Cd treatment. Chlorosis and lateral root development were apparent on all higher treatments, with growth suppression significant ($P = 0.01$) in the 31.6 μM Zn/0.632 μM Cd treatment (Fig. 1). All bladder campion seedlings receiving the 3160 μM Zn/63.2 μM Cd treatment died. Bladder campion supplied with 1000 μM Zn/20 μM Cd exhibited severe toxicity. At 316 μM Zn/6.32 μM Cd, bladder campion plants showed some purpling of older leaves but otherwise appeared vigorous. *Thlaspi caerulescens* remained chlorotic, but showed no growth effects or loss of vigor in all treatments.

At 15 d, tomato was harvested for all remaining treatments. At 21 d, *T. caerulescens* at 3160, 1000, 100, and 31.6 μM Zn/63.2, 20, 2, and 0.632 μM Cd treatments were no longer chlorotic. *Thlaspi caerulescens* in the 10000 μM Zn/200 μM Cd treatment was severely chlorotic, with root and shoot growth inhibited. *Thlaspi caerulescens* in both the 316 μM Zn/6.32 μM Cd and 3.16 μM Zn/0.0632 μM Cd treatments remained chlorotic and

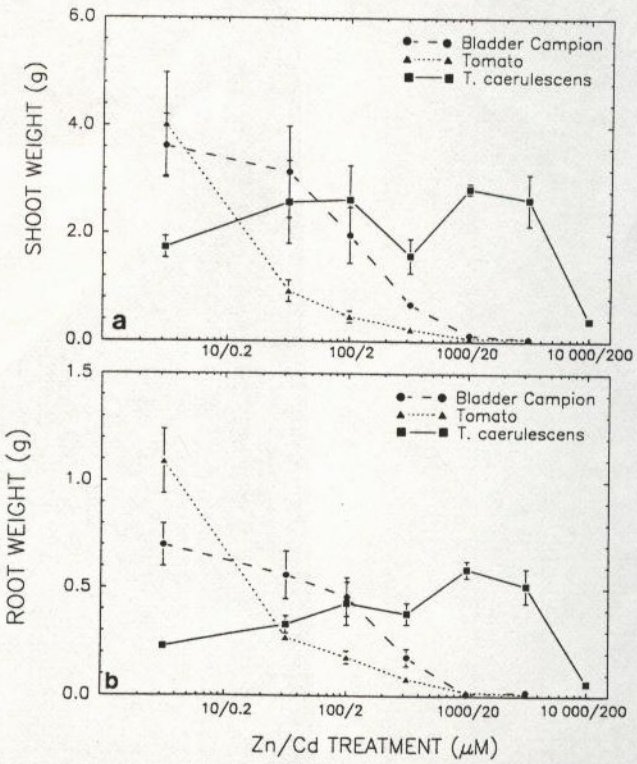

Fig. 1. Dry yield of (a) shoots and (b) roots of *Thlaspi caerulescens* (hyperaccumulator), bladder campion (indicator), and tomato (susceptible species) grown in nutrient solution with Zn added at a 50:1 ratio to Cd across seven Zn/Cd treatments. Bars represent standard errors of values averaged across replications.

plants were smaller than the other treatments. While this chlorosis may be related to a Zn deficiency in the 3.16 μ*M* Zn/0.063 μ*M* Cd and 31.6 μ*M* Zn/0.632 μ*M* Cd treatments, there is no apparent explanation for the visual symptoms in the 316 μ*M* Zn/6.32 μ*M* Cd treatment. Bladder campion receiving the 1000 μ*M* Zn/20 μ*M* Cd treatment died but at all lower Zn/Cd treatments had entered the reproductive growth phase. The effect of plant growth cycle on metal uptake is not known. Bladder campion was harvested at 22 d. At 28 d, all *T. caerulescens* were harvested; plants in the highest treatment were showing signs of toxicity (chlorosis and significant [$P < 0.01$] yield reduction) although they maintained their turgor. Plant roots and shoots in the 3.16 μ*M* Zn/0.063 μ*M* Cd and 316 μ*M* Zn/6.32 μ*M* Cd treatments were smaller across all replicates compared with other treatments ($P < 0.05$).

Concentration of Zinc and Cadmium in Plant Tissues

Bladder campion had significantly higher root concentrations of Cd than *T. caerulescens* and tomato in the lower Zn/Cd treatments ($P < 0.05$) (Fig. 2). Root Cd concentrations in tomato were also significantly higher than *T. caerulescens* ($P < 0.05$). At treatments higher than 316 μ*M* Zn/20 μ*M* Cd, this relationship for bladder campion and *T. caerulescens* changed, with root Cd concentrations no longer significantly different between the two species. Also for these treatments, bladder campion showed severe phytotoxic leaf symptoms. Because *T. caerulescens* was more tolerant of Zn and Cd, much higher root Cd concentrations were reached in *T. caerulescens* than in tomato or bladder campion before yield was reduced significantly.

Shoot Cd concentrations were similar for all species at the two lowest treatments. At the 2 μ*M* Cd and higher, concentrations of Cd in *T. caerulescens* shoots was significantly higher than in the other species. The difference increased as Cd concentration in solution increased. Concentrations of Cd in shoot tissue of *T. caerulescens* reached 1290 mg kg^{-1} in the 3160 μ*M* Zn treatment with no visible symptoms of Cd or Zn toxicity.

Zinc concentration in plants followed a different pattern than Cd concentration (Fig. 3). *Thlaspi caerulescens* had significantly lower concentrations of Zn in roots than the other species to 316 μ*M* Zn/6.32 μ*M* Cd. Above that level, root concentrations of Zn were not significantly different for bladder campion, *T. caerulescens*, and tomato. Shoot concentrations of Zn were significantly higher for *T. caerulescens* in all treatments ($P < 0.001$). The shoot Zn concentrations of *T. caerulescens* reached 26000 mg kg^{-1} in the 3160 μ*M* Zn treatment without any visible signs of Zn toxicity.

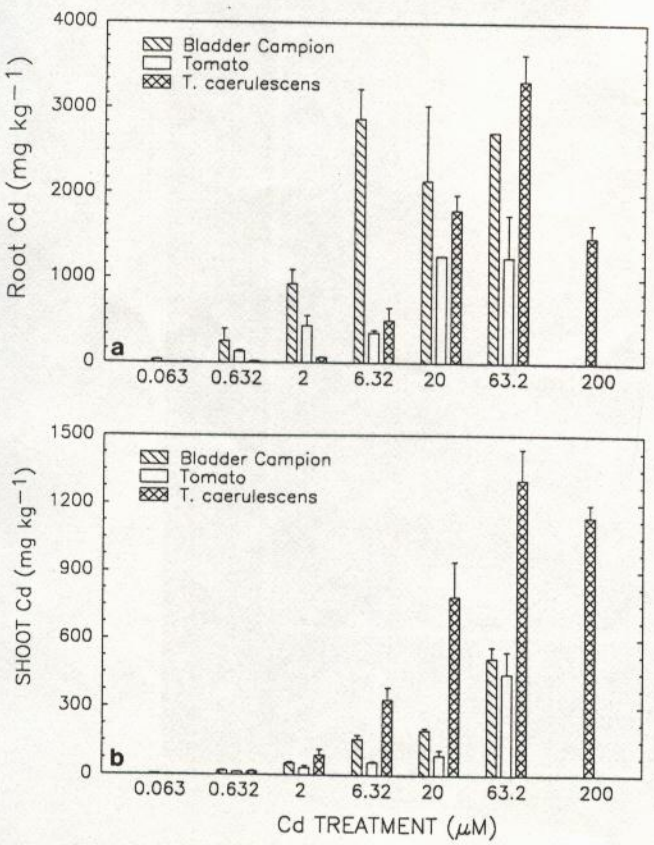

Fig. 2. Cadmium concentration in (a) roots and (b) shoots of *Thlaspi caerulescens* (hyperaccumulator), bladder campion (indicator), and tomato (susceptible species) grown in nutrient solution with Zn added at a 50:1 ratio to Cd across seven Zn/Cd treatments. Bars represent standard errors of values averaged across replications.

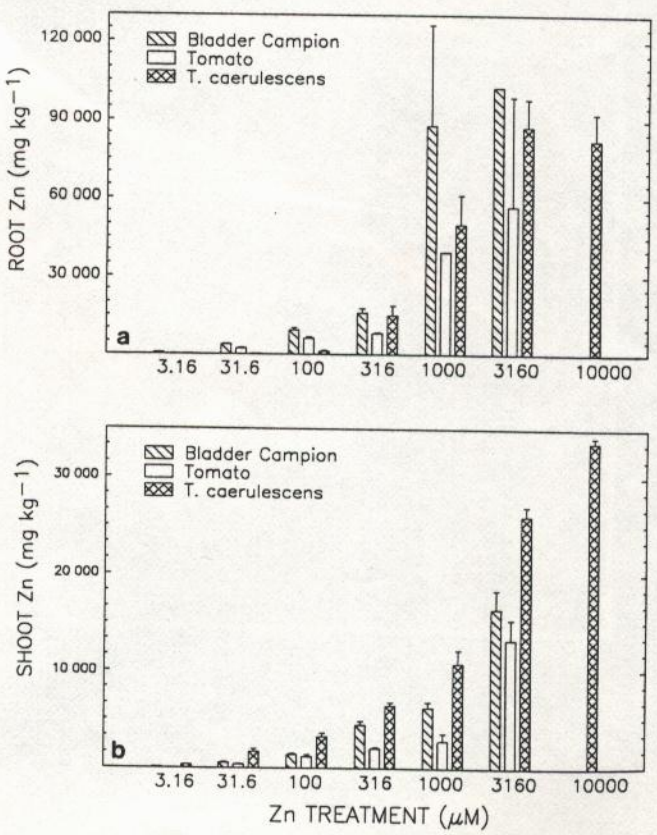

Fig. 3. Zinc concentration in (a) roots and (b) shoots of *Thlaspi caerulescens* (hyperaccumulator), bladder campion (indicator), and tomato (susceptible species) grown in nutrient solution with Zn added at a 50:1 ratio to Cd across seven Zn/Cd treatments. Bars represent standard errors of values averaged across replications.

Zinc and Cadmium Translocated

Total Zn and Cd translocated to shoot tissue (concentration of metal in dry matter × dry weight) was calculated. *Thlaspi caerulescens* translocated significantly more Zn and Cd to shoots than either bladder campion or tomato, with maximum metals translocated at the 3160 μ*M* Zn/63 μ*M* Cd treatment ($P < 0.001$) (Fig. 4). Bladder campion translocated more Cd from solution to shoots than tomato, although translocation of Zn was not significantly different.

For *T. caerulescens*, higher tolerance to both metals was responsible for higher yields in all but the 3.16 μ*M* Zn treatment for tomato and in all treatments >31.6 μ*M* Zn for bladder campion. Despite generally lower biomass, hyperaccumulation of Zn at low solution concentrations made *T. caerulescens* more efficient than bladder campion and tomato at translocating Zn to shoot tissue. For phytoremediation purposes, *T. caerulescens* is much more effective than the other plant species used in this study.

Shoot/Root Partitioning of Zinc and Cadmium

The lower Zn or Cd tolerance of bladder campion and tomato compared with *T. caerulescens* suggest that interspecies comparisons of internal distribution of Zn and Cd may provide an understanding of different internal tolerance mechanisms (Baker and Walker, 1990.) However, lower tolerance reduced the yield of bladder campion and tomato at higher treatment concentrations, making comparisons difficult across the range of treatments used in this study. Shoot/root ratios of metals are <1 when nontolerant or "indicator" species are grown on contaminated soils (Antonovics et al., 1971; Baker and Walker, 1990; Peterson, 1983).

With regard to shoot/root ratios of Zn and Cd, bladder campion behaved like tomato, with a more severe restriction of Zn and Cd translocation to shoots (Fig. 5). Tolerance thresholds (as defined by shoot/root partitioning) for both plants were reached at the 316 μ*M* Zn treatment. *Thlaspi caerulescens* showed ratio values >1 for shoot/root Zn partitioning up to the 316 μ*M* Zn/6.32 μ*M* Cd treatment. Above 316 μ*M* Zn, the shoot/root ratio decreased to 0.22 at 1000 μM Zn and gradually increased in the higher treatments.

Shoot/root distribution of Cd and Zn followed similar patterns in *T. caerulescens* (Fig. 5). Distribution of both Zn and Cd within *T. caerulescens* was different than distribution of these metals in bladder campion and tomato. *Thlaspi caerulescens* translocated a greater percentage of Zn and Cd to shoot tissue. A different partitioning pattern for both Zn and Cd in *T. caerulescens* compared with bladder campion and tomato may suggest that the internal mechanisms that control the translocation

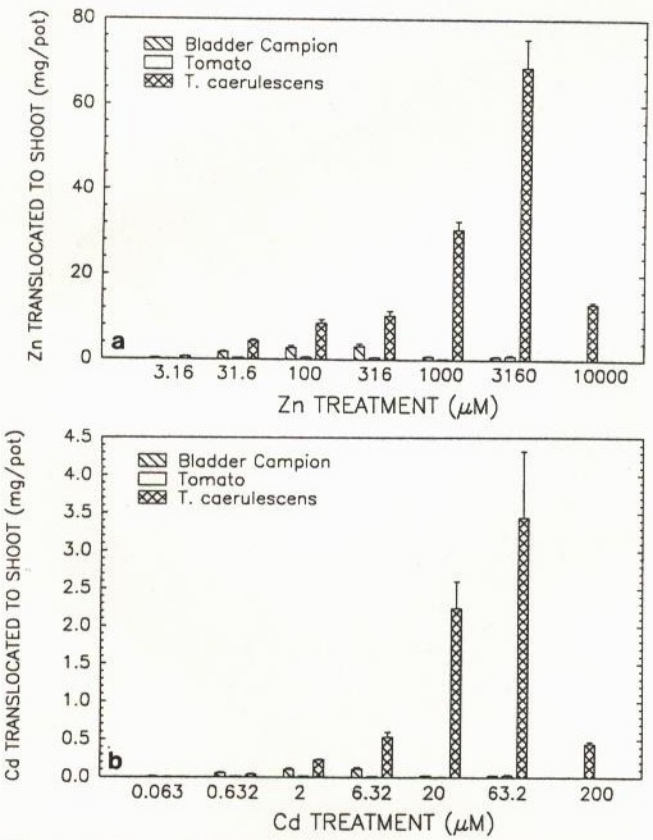

Fig. 4. (a) Zinc and (b) Cd translocated to harvestable biomass by *Thlaspi caerulescens* (hyperaccumulator), bladder campion (indicator), and tomato (susceptible species) grown in nutrient solution with Zn added at a 50:1 ratio to Cd across seven Zn/Cd treatments. Bars represent standard errors of values averaged across replications.

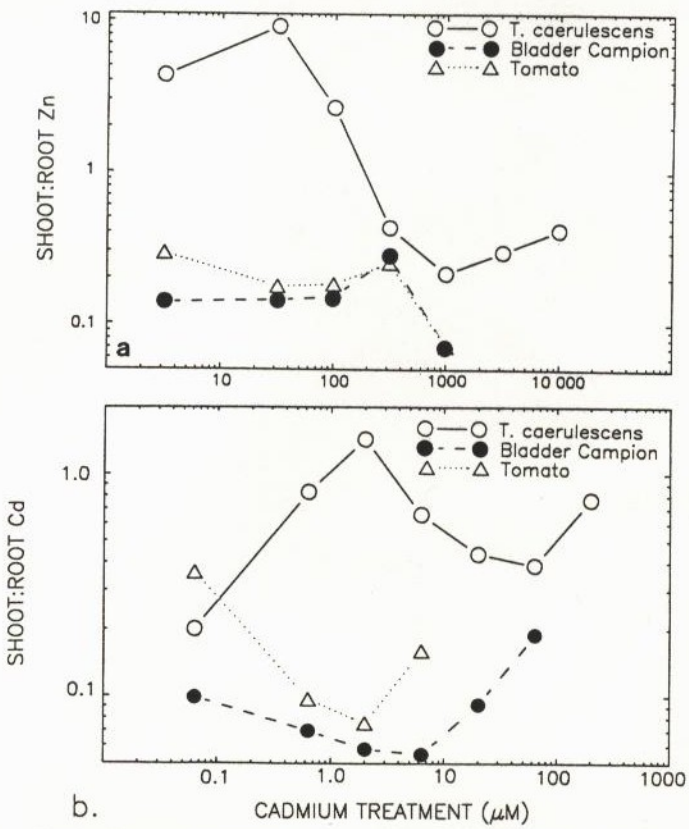

Fig. 5. Shoot/root ratios of (a) Zn and (b) Cd concentrations in *Thlaspi caerulescens* (hyperaccumulator), bladder campion (indicator), and tomato (susceptible species) grown in nutrient solution with Zn added at a 50:1 ratio to Cd across seven Zn/Cd treatments.

of Cd and Zn in *T. caerulescens* may be similar (Baker and Walker, 1990). Hajar (1987) also found shoot/root ratios >1 for both elements through a range of metal additions in a pot study. Although his highest Zn rate was greater than the highest Zn treatment used in this study, the available fraction of total metal added was not reported.

Average Zn concentration in plant material (sum of root and shoot) was higher for *T. caerulescens* than the other plants in the 3.16 and 31.6 μM Zn treatments (data not shown). This implies that, even at lower solution concentrations of Zn, Zn uptake by *T. caerulescens* follows a different pattern than bladder campion and tomato. This different uptake pattern was not followed by *T. caerulescens* for Cd uptake. For Cd, average total concentration in plants (sum of root and shoot) was higher for bladder campion in the same treatments. These results may suggest that the specialized mechanism for Zn uptake in *T. caerulescens* does not control Cd uptake. Although this study did not examine uptake of Zn or Cd by *T. caerulescens* when supplied singly, different accumulation patterns for Zn and Cd from solution to root cell plasma may suggest that different mechanisms are involved for each metal. A field study (Brown et al., 1994) showed *T. caerulescens* shoots with >1000 mg Zn kg^{-1} when grown on uncontaminated soils. Zinc concentrations in *T. caerulescens* were significantly different ($P = 0.01$) from other plants used in the study. In the same study, Cd uptake by *T. caerulescens* was not significantly different from bladder campion or lettuce (*Latuca sativa* L.). These results may indicate that competition for uptake between Zn and Cd may not be a factor.

Separation of *Thlaspi caerulescens* Tissues

Separation of the plant tissue of *T. caerulescens* into leaves, stems, and roots and subsequent analysis showed that distribution of Zn and Cd in plant parts was not consistent across treatments (Fig. 6). *Thlaspi caerulescens* had significantly higher concentrations of Zn in leaf tissue than stem or root in all of the low-Zn treatments. This relationship changed at the 316 μM Zn/6.32 μM Cd treatment, with root concentration exceeding leaf concentration for all higher treatments. The observed chlorosis at the 316 μM Zn treatment may have some relationship to the change in this partitioning. The concentration of Zn in shoots in the 316 μM Zn treatment was significantly lower than at the three higher treatments (Fig. 3).

Cadmium concentrations in leaf and stem material of *T. caerulescens* were significantly lower than for roots in the lowest Zn/Cd treatment ($P < 0.05$). At the 31.6 μM Zn/0.63 μM Cd treatment, leaf Cd was not significantly different from root Cd concentrations, although stem Cd concentrations remained significantly lower. The pattern of Cd distribution between root, stem, and leaf remained consistent at all higher treatments. Stem Cd concentrations increased in proportion to leaf and root Cd concentrations, but never reached the concentrations found in leaves or roots.

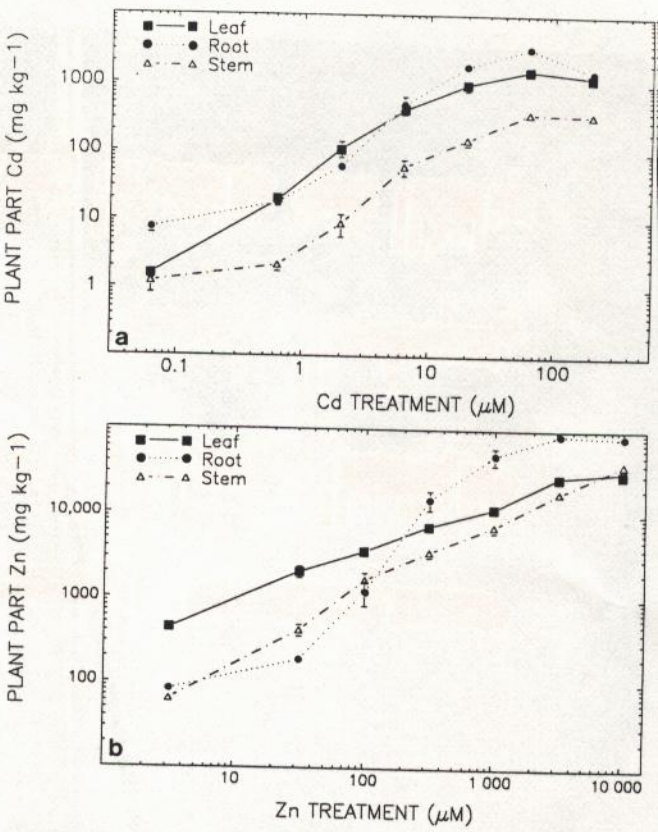

Fig. 6. Distribution of (a) Cd and (b) Zn in root, stem, and leaf tissue of *Thlaspi caerulescens* grown in nutrient solution with Zn added at a 50:1 ratio to Cd across seven Zn/Cd treatments.

Nutrient Partitioning in *Thlaspi caerulescens*

Phosphorus concentrations in leaves remained similar in lower Zn/Cd treatments, increased at the 1000 μM Zn/20 μM Cd concentration and then decreased sharply to possibly deficient levels for the 10000 μM Zn/200 μM Cd treatment (Table 2). Stem concentrations of P were significantly higher in the 316 μM Zn/6.32 μM Cd than in the two lowest treatments as well as in the 10000 μM Zn/200 μM Cd treatment. Concentrations were not significantly different from 100 μM Zn/2 μM Cd to 3160 μM Zn/63.2 μM Cd. Root concentrations of P increased starting at 316 μM Zn, the same treatment at which the shoot/root Zn ratio became <1. This decrease continued until the highest Zn/Cd treatment, where P concentrations significantly decreased. GEOCHEM calculations indicated that a significant amount of P would precipitate as $Zn_3(PO_4)_2$ beginning at the 316 μM Zn treatment. Precipitation could have occurred on plant roots or in the solution. At 1000 μM Zn and higher, >97% of nutrient solution PO_4 was calculated to be precipitated. However, leaf P did not fall greatly until the 10000 μM Zn treatment. These results may indicate that internal precipitation of $Zn_3(PO_4)_2$ inhibited transfer of absorbed P to shoots.

Iron concentrations in leaf tissue were at or below deficiency levels for conventional plant species ($\leq$50 mg kg^{-1} dry weight) in all Zn treatments (Marschner, 1986). There have been no studies to determine Fe

Table 2. Mineral nutrient distribution within leaf, stem, and root tissues of *Thlaspi caerulescens* grown in nutrient solution with Zn and Cd added (50:1) at seven concentrations.

Zn treatment	P	Cu	Mn	Fe	Mg	Ca	K	Mo
μM	g kg^{-1}	mg kg^{-1}			g kg^{-1}			mg kg^{-1}
3.16								
Leaf	2.4 a	4.7 a	29.7 a	16.8 ab	1.46 a	19.1 b	42.1 a	1.3 a
Stem	2.6 a	2.5 a	10.4 a	25.1 a	1.63 a	12.7 b	38.2 ab	0.4 b
Root	3.7 a	19.8 a	391 c	432 a	2.46 c	8.2 cd	16.9 a	8.3 a
31.6								
Leaf	2.50 a	5.7 ab	52.7 b	22.6 ab	1.88 a	22.5 cd	45.7 ab	2.3 a
Stem	2.8 ab	4.5 a	11.1 ab	27.2 a	2.00 a	14.7 b	41.8 ab	1.5 bc
Root	4.0 ab	22.1 a	249 b	510 a	2.38 bc	10.4 d	18.2 a	11.6 a
100								
Leaf	2.8 a	5.4 a	72.8 c	22.8 ab	2.46 cd	23.0 d	52.1 abc	2.8 ab
Stem	3.1 abc	5.1 a	13.6 b	20.7 a	2.20 ab	13.6 b	50.0 b	2.0 bc
Root	4.5 ab	21.7 a	141 a	557 a	2.00 b	7.5 cd	25.3 ab	14.4 a
316								
Leaf	3.9 b	7.4 ab	60.5 b	16.8 ab	2.85 de	23.4 d	61.0 c	2.9 ab
Stem	3.6 bc	4.1 a	16.2 b	25.3 a	2.21 ab	13.2 b	48.9 b	1.2 b
Root	9.6 ab	51.2 b	52 a	1020 b	1.94 b	7.8 cd	31.3 b	13.4 a
1000								
Leaf	4.7 c	6.4 ab	49.5 b	24.0 b	3.30 e	21.0 cd	58.5 cd	5.7 c
Stem	3.8 c	5.0 a	12.4 ab	20.3 a	3.00 c	12.0 b	46.8 b	3.9 c
Root	17.1 c	47.7 b	62 a	1100 b	2.00 b	5.9 bc	25.1 ab	17.8 a
3160								
Leaf	3.0 a	7.0 ab	38.9 a	24.6 b	3.22 e	16.5 b	56.2 abc	4.9 bc
Stem	3.4 abc	6.2 a	12.2 ab	21.8 a	2.82 bc	8.0 a	42.4 ab	2.8 bc
Root	23.0 c	46.2 b	38 a	1850 c	1.50 a	3.1 ab	22.7 a	13.8 a
10 000								
Leaf	1.2 d	9.0 b	28.3 a	15.1 a	2.25 bc	13.0 a	45.5 ab	1.3 a
Stem	3.0 ab	15.7 b	33.3 c	71.5 b	1.67 a	8.9 a	34.1 a	<1 a
Root	10.3 b	34.7 ab	56 a	454 a	1.41 a	2.3 a	24.2 ab	7.1 a

For given elements, means followed by the same letter across treatments and within a plant part are not significantly different using the Duncan Waller *K*-ratio *t*-test procedure ($P < 0.05$, df = 20).

uptake patterns and requirements of *T. caerulescens*. Low concentrations of Fe in *T. caerulescens* shoots (stem and leaf) may be indicative of a reduced Fe requirement. However, in another study, *T. caerulescens* grown in metal-contaminated soil contained concentrations of Fe in shoot tissue comparable with other plant species (Brown et al., 1994). There may be some interaction between Zn and Fe uptake by *T. caerulescens*.

Calcium and Mg exhibited significantly lower root concentrations at higher treatment Zn levels ($P < 0.05$). For Ca, root concentrations were significantly lower at 3160 μM Zn/63.2 μM Cd and 10000 μM Zn/200 μM Cd than at all treatment concentrations <316 μM Zn/6.32 μM Cd. Magnesium concentrations in roots in the 3160 μM Zn/63.2 μM Cd and 10000 μM Zn/200 μM Cd treatments were significantly lower than in all other treatments. Leaf concentrations of Ca were lowest ($P < 0.05$) in the 10000 μM Zn/200 μM Cd treatment. Stem concentrations of Ca were significantly lower in the 3160 μM Zn/63.2 μM Cd and 10000 μM Zn/200 μM treatments. Leaf concentrations of Mg were lowest in the two lowest Zn/Cd treatment concentrations. Stem concentrations of Mg were significantly different only in the 1000 μM Zn/20 μM Cd treatment with concentrations of 3.00 g kg^{-1}.

Increasing K concentrations within the plant leaf may be a response to balance the ionic charge in cells that are accumulating organic acids (Marschner, 1986). In this case although concentrations of K increased, this increase was not significant ($P = 0.05$). *Thlaspi caerulescens* may produce increased concentrations of organic acids to complex foliar Zn and Cd (Ernst, 1978; Baker et al., 1990). In order to maintain a charge balance between acid production and metal complexation, *T. caerulescens* may take up excess K in relation to the production of organic acids.

Manganese concentrations in roots were highest in the lowest Zn treatment, and were significantly lower in all other treatments. Concentrations in all treatments at and above 100 μM Zn/2 μM Cd were significantly lower than in other treatments. Stem concentrations of Mn were highest in the 10000 μM Zn/200 μM Cd treatment. Leaf concentrations of Mn were highest in the 100 μM Zn/2 μM Cd treatment. Leaf Mn concentrations were above deficiency concentrations for conventional plant species in all treatments.

Although concentrations of other elements measured in *T. caerulescens* tissue were generally above deficiency concentrations for conventional plants, changes in concentrations and distribution within the plant varied with Zn treatment. Potentially, increasing P supply is related to increasing Zn concentrations in roots. Van Steveninick et al. (1987) observed precipitation of Zn with P in roots of tufted hair grass [*Deschampsia caespitosa* (L.) P. Beauv.]. Although P is not a component of glutathione or any of the organic acids implicated in metal tolerance mechanisms, it may be involved in some type of secondary tolerance system. Multiple tolerance mechanisms within a particular species have been suggested as a response to elevated concentrations of one or more metals (Cumming and Tomsett, 1992). At the 10000 μM Zn/200 μM Cd treatment, concentrations of P in root and shoot tissue significantly decreased. While decreasing P in shoots may be related to P precipitation in roots, it is also possible that decreasing P concentrations in shoot tissue at the 10000 μM Zn treatment may be related to

decreasing concentrations of Mg, Mo, and Ca and the observed chlorosis and growth suppression. These findings indicate that *T. caerulescens*'s tolerance limits were exceeded in our study. Possibly, solution concentrations of Zn were high enough in this treatment to cause some precipitation of $Zn_3(PO_4)_2$, and limit plant availability of P. The exact cause of toxicity is not known nor could we clearly define the elemental interactions involved since the precipitation of P could have occurred either within the symplasm or within the root apoplasm.

CONCLUSIONS

Thlaspi caerulescens shows much greater accumulation of, and tolerance to, both Zn and Cd than the other plant species studied. Toxicity was evident for *T. caerulescens* receiving the 10000 μ*M* Zn/200 μ*M* Cd treatment, with shoot Zn and Cd concentrations of 32500 and 1270 mg kg^{-1}, respectively. In this study, the 50:1 Zn/Cd ratio was representative of the ratio of these elements found on sites contaminated by mining and smelting activities. Further studies with different Zn/Cd ratios are necessary to describe potential uptake patterns of *T. caerulescens* grown on sites contaminated by other activities.

Thlaspi caerulescens was much more efficient than bladder campion or tomato at translocating both elements from solution to shoots. Except for low biomass, the metal-accumulating properties of *T. caerulescens* are those necessary for plants that could be used for phytoremediation of metal-contaminated soils. Concentrations of Zn in harvestable shoots are high enough that plant tissue could be treated as a low-grade ore (Chaney, 1983). Zinc and Cd could be recycled by smelting dried *T. caerulescens* shoots. Low yield and slow growth rate are the two major factors that limit the potential for the phytoremediation of Zn/Cd-contaminated soils by successive croppings of *T. caerulescens*. In addition, *T. caerulescens* is a low-growing plant, which makes any mechanical harvesting problematic. Based on the more extensive literature on Ni hyperaccumulators (>200 species are known), there is no inherent requirement for slow growth in metal hyperaccumulator species (Baker and Brooks, 1989). Our results suggest *T. caerulescens* plants or genes from this species could be used to develop phytoremediation technology for Zn- and Cd-contaminated soils. A higher yielding crop with similar Zn- and Cd-accumulating properties could be used to remediate Zn/Cd-contaminated soils in situ within a shorter time frame.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of C.E. Green and M.L. Leech in collecting and increasing the bladder campion seed from Palmerton, of C.E. Green for her advice on nutrient solution methodology, and of Ed Henke.

REFERENCES

Antonovics, J., A.D. Bradshaw, and R.G. Turner. 1971. Heavy metal tolerance in plants. Adv. Ecol. Res. 7:1–85.

Baker, A.J.M. 1978. Ecophysiological aspects of zinc tolerance in *Silene maritima*. New Phytol. 80:635–642.

Baker, A.J.M. 1987. Metal tolerance. New Phytol. 106(Suppl.):93–111.

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

Baker, A., R. Brooks, and R. Reeves. 1988. Growing for gold ... and copper ... and zinc. New Scientist 117(1603):44–48.

Baker, A.J.M., K. Ewart, G.A.F. Hendry, P.C. Thorpe, and P.L. Walker. 1990. The evolutionary basis of cadmium tolerance in higher plants. p. 23–29. *In* J. Barcelo (ed.) Proc. Int. Conf. Environ. Contam. 4th, Barcelona. CEP Consultants, Edinburgh.

Baker, A.J.M., R.D. Reeves, and S.P. McGrath. 1991. In situ decontamination of heavy metal polluted soils using crops of metal-accumulating plants—A feasibility study. p. 600–605. *In* R.L. Hinchee and R.F. Olfenbuttel (ed.) In situ bioreclamation. Butterworth-Heinemann, Boston, MA.

Baker, A.J.M., R.D. Reeves, and A.S.M. Hajar. 1994. Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). New Phytol. (in press).

Baker, A.J.M., and P.L. Walker. 1990. Ecophysiology of metal uptake by tolerant plants. p. 155–177. *In* A.J. Shaw (ed.) Heavy metal tolerance in plants: Evolutionary aspects. CRC Press, Boca Raton, FL.

Brown, S.L., R.L. Chaney, J.S. Angle, and A.J.M. Baker. 1994. Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc- and cadmium-contaminated soils. J. Environ. Qual. 23:1151–1157.

Cannon, H.L. 1960. Botanical prospecting for ore deposits. Science (Washington, DC) 132:591–598.

Chaney, R.L. 1983. Plant uptake of inorganic waste constituents. p. 50–76. *In* J.F. Parr et al. (ed.) Land treatment of hazardous wastes. Noyes Data Corp., Park Ridge, NJ.

Cumming, J.R., and A.B. Tomsett. 1992. Metal tolerance in plants: Signal transduction and acclimation mechanisms. p. 329–364. *In* D. Adriano (ed.) Biogeochemistry of trace metals. Lewis Publ., Chelsea, MI.

Ernst, W.H.O. 1978. Physiology of heavy metal resistance in plants. p. 121–136. *In* T.C. Hutchinson et al. (ed.) Proc. Symp. Int. Conf. heavy metals in the environment, Toronto. 27–31 Oct. 1975. Vol. 2. Part 1. CEP Consultants, Edinburgh.

Geiger, G., P. Federer, and H. Sticker. 1993. Reclamation of heavy metal contaminated soils: Field soils germination experiments. J. Environ. Qual. 22:201–207.

Hajar, A.S.M. 1987. Comparative ecology of *Minuartia verna* (L.) Hiern and *Thlaspi alpestre* L. in the southern Pennines, with special reference to heavy metal tolerance. Ph.D. diss. Univ. of Sheffield, Sheffield, UK.

Ingrouille, M.J., and N.W. Smirnoff. 1986. *Thlaspi caerulescens* J. and C. Presl. (*Thlaspi alpestre* L.) in Britain. New Phytol. 102: 219–233.

Marschner, Horst. 1986. Mineral nutrition of higher plants. Academic Press, London.

Oyler, J.A. 1988. Remediation of metals-contaminated site near a zinc smelter using sludge/fly ash amendments: Herbaceous species. p. 306–320. *In* D.D. Hemphill (ed.) Trace substances in environmental health. Proc. Univ. Missouri's Annu. Conf. 22nd, St. Louis. 23–26 May. Univ. of Missouri Press, Columbia.

Parker, D.R., R.L. Chaney, and W.A. Norvell. 1995. Equilibrium computer models: Applications to plant nutrition research. *In* R. Loeppert et al. (ed.) Chemical equilibrium and reaction models. SSSA Spec. Publ. ASA and SSSA, Madison, WI (in press).

Peterson, P.J. 1983. Adaptation to toxic metals. p. 51–69. *In* D.A. Robb and W.S. Pierpoint (ed.) Metals and micronutrients: Uptake and utilization by plants. Academic Press, London.

Rascio, W. 1977. Metal accumulation by some plants growing on zinc mine deposits. Oikos 29:250–253.

Reeves, R.D., and A.J.M. Baker. 1984. Studies on metal uptake by plants from serpentine and nonserpentine populations of *Thlaspi goesingense* Halacsy (Cruciferae). New Phytol. 98:191–204.

Reeves, R.D., and R.R. Brooks. 1983. Hyperaccumulation of lead and zinc by two metallophytes from mining areas of central Europe. Environ. Pollut. A31:277–285.

Reeves, R.D, R.M. MacFarlane, and R.R. Brooks. 1983. Accumulation of nickel and zinc by western North American genera containing serpentine-tolerant species. Am. J. Bot. 70:1297–1303.

SAS Institute. 1989. SAS procedures guide. Version 6, 3rd ed. SAS Inst., Cary, NC.

Schat, H., and M.M.A. Kalff. 1992. Are phytochelatins involved in differential metal tolerance or do they merely reflect metal-imposed strain? Plant Physiol. 99:1475–1480.

Van Steveninick, R.F.M., D.R. Fernando, D.L. Godbold, W.J. Horst, and H. Marschner. 1987. Identification of zinc-containing globules in roots of a zinc-tolerant ecotype of *Deschampsia caespitosa*. J. Plant Nutr. 10:1239–1246.

Vazquez, M.D., J. Barcelo, Ch. Poschenrieder, J. Madico, P. Hatton, A.J.M. Baker, and G. Coupe. 1992. Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can hyperaccumulate both metals. J. Plant Physiol. 140:350–355.

Verkleij, J.A.C., P. Koevoets, J. van't Riet, R. Bank, Y. Nijdam, and W.H.O. Ernst. 1990. Poly (gamma-glutamylcysteinyl) glycines or phytochelatins and their role in cadmium tolerance of *Silene vulgaris*. Plant Cell Environ. 13:913–921.