Plant Physiol. (1995) 109: 1427–1433

Mechanisms of Cadmium Mobility and Accumulation in Indian Mustard[1]

David E. Salt*, Roger C. Prince, Ingrid J. Pickering, and Ilya Raskin

Center for Agricultural and Molecular Biology, Rutgers University, Cook College, New Brunswick, New Jersey 08903 (D.E.S., I.R.); Exxon Research and Engineering, Annandale, New Jersey 08801 (R.C.P.); and Stanford Synchrotron Radiation Laboratory, Stanford University, Stanford Linear Accelerator Center, Stanford, California 94309 (I.J.P.)

Indian mustard (*Brassica juncea* L.), a high biomass crop plant, accumulated substantial amounts of cadmium, with bioaccumulation coefficients (concentration of Cd in dry plant tissue/concentration in solution) of up to 1100 in shoots and 6700 in roots at nonphytotoxic concentrations of Cd (0.1 μg/mL) in solution. This was associated with a rapid accumulation of phytochelatins in the root, where the majority of the Cd was coordinated with sulfur ligands, probably as a Cd-S_4 complex, as demonstrated by x-ray absorption spectroscopy. In contrast, Cd moving in the xylem sap was coordinated predominantly with oxygen or nitrogen ligands. Cd concentrations in the xylem sap and the rate of Cd accumulation in the leaves displayed similar saturation kinetics, suggesting that the process of Cd transport from solution through the root and into the xylem is mediated by a saturable transport system(s). However, Cd translocation to the shoot appeared to be driven by transpiration, since ABA dramatically reduced Cd accumulation in leaves. Within leaves, Cd was preferentially accumulated in trichomes on the leaf surface, and this may be a possible detoxification mechanism.

Cd is a potentially toxic metal that can accumulate in the human body with a half-life exceeding 10 years. There is evidence that low-level exposure to Cd, derived from the diet, is associated with renal dysfunction (Buchet et al., 1990). Cd exposure has also been linked with pulmonary emphysema (Ryan et al., 1982) and possibly bone demineralization (Bhattacharyya et al., 1988). Pollution of the biosphere with this toxic metal has accelerated dramatically since the beginning of the industrial revolution (Nriago, 1979), and Cd accumulation in soil and water now poses a major environmental and human health problem, which is in need of an effective and affordable solution. The use of metal-accumulating plants to remove toxic metals, including Cd, from soil and aqueous streams has been proposed as a possible solution to this problem (reviewed by Salt et al., 1995). This process of using plants for environmental restoration is termed "phytoremediation." Cd is a particularly favorable target metal for this new technology because it is readily transported and accumulated in the shoots of several plant species (Wagner, 1994).

[1] This research was supported by the U.S. Department of Environment, grant No. R818619; Phytotech Inc.; New Jersey Commission for Science and Technology, grant No. 93-240380-1; New Jersey Agricultural Experimental Station; and the Stanford Synchrotron Radiation Laboratory, proposal No. 2301.

* Corresponding author; e-mail salt@mbcl.rutgers.edu; fax 1-908-932-6535.

The primary point of entry for Cd into plants is through the roots; however, for its efficient removal from the soil it must first be translocated to the harvestable parts of the shoot. To understand this translocation process, we undertook a study of Cd transport and accumulation in *B. juncea*, a high biomass crop plant within the Brassicaceae family, which has been identified as a potentially useful plant for phytoremediation (Banuelos and Meek, 1990). For comparison we have investigated a low biomass wild species within the same family, *Thlaspi caerulescens*, known to accumulate high levels of Cd in its shoots under certain conditions (Baker et al., 1994; Brown et al., 1994, 1995).

MATERIALS AND METHODS

Plant Material

Seeds of Indian mustard (*Brassica juncea* L., cv 426308), identified as a metal accumulator (Kumar et al., 1995), and *Thlaspi caerulescens* J. & C. Presl. were germinated on vermiculite and grown for 3 and 6 weeks, respectively, in a hydroponic system containing 500 mL of modified Hoagland solution containing the following nutrients: 28.7 mg/L $NH_4H_2PO_4$, 0.71 mg/L H_3BO_3, 164.1 mg/L $Ca(NO_3)_2$, 0.02 mg/L $CuSO_4$, 2.66 mg/L ferric tartrate, 60.19 mg/L $MgSO_4$, 0.45 mg/L $MnCl_2$, 0.004 mg/L MoO_3, 151.65 mg/L KNO_3, and 0.055 mg/L $ZnSO_4$. Seedlings were grown in hydroponic solution in a greenhouse equipped with supplementary lighting (16-h photoperiod; 24–28°C). The solutions were continuously aerated with an aquarium air pump and changed every 3 d. On the day of treatment, Cd was added to the hydroponic medium as $CdSO_4$ and the solutions were changed daily thereafter.

Cd Accumulation in Plants

Total shoot and root accumulation of Cd, Mn, and Cu in *B. juncea* and *T. caerulescens* were determined after 7 d of exposure to Cd, using a direct current plasma spectrometer (model SS-7, Fisons, Beverly, MA). Roots and shoots were harvested, washed in deionized water for 2 min, air dried at 60°C for 2 d, and then ground into a fine powder using a pestle and mortar. A known amount of this powder was then ashed in a muffle furnace at 500°C for 6 h. The ash was dissolved in 2 parts of 1 M HNO_3 and 1 part of 2 M HCl, and

Abbreviations: EXAFS, extended x-ray absorption fine structure; PC, phytochelatin; PC_3, (γ-glutamylcysteinyl)$_3$glycine; XAS, x-ray absorption spectroscopy.

the solution was analyzed by direct current plasma spectrometer. Accumulation of Cd was also monitored using ^{109}Cd as a radioactive tracer (1 μCi/μmol) added as $CdCl_2$ (5.35 mCi/mg). Tissue from plants exposed to ^{109}Cd was washed in deionized water and analyzed directly using a table-top γ-counter (model C5002, Packard Instrument Co., Meriden, CT). ^{109}Cd accumulation in the intact leaves of *B. juncea* was monitored by placing an NaI scintillation detector (2 × 2 inch crystal) against the underside of the leaf of interest (usually leaf No. 3, counting the first clearly identifiable leaf as 1). The NaI crystal was connected to a photon multiplier tube base (ScintiPack, 296 PMT base, EG & G, Oak Ridge, TN), and the signal was processed on a 286 personal computer containing a multichannel analyzer (Micro-Ace, EG & G), running with MAESTRO II emulation software (EG & G). This system will be referred to as the Cd probe. The software was programmed to collect data every 30 min, with a collection time of 10 min. The γ-radiation from the ^{109}Cd was shielded with 2-mm-thick lead so that Cd accumulation in a known area of leaf, usually 9 cm^2, could be measured. By cutting out that portion of the leaf that had been monitored and measuring the ^{109}Cd it contained using a γ-counter (see above), we calculated a counting efficiency for the Cd probe to be between 15 and 20%. Using this counting efficiency, we were then able to quantify the amount of Cd accumulated within the leaf. A similar methodology for measuring Cd and Ca transport in vivo using a semiconductor radiation detector was previously reported (Petit and Van de Geijn, 1978, and refs. therein).

Chl Content of Leaves

Chl content of partially expanded (leaf 3) and fully expanded (leaf 5) leaves of *B. juncea* was measured by extraction in acetone following the procedure of Strain et al. (1971).

PC Analysis

B. juncea roots exposed to 0.2 μg/mL Cd for 3 d were collected, washed in deionized water for 2 min, blotted dry, and ground to a fine powder in liquid nitrogen using a pestle and mortar. Chilled 500 mM HCl was added to the frozen powder (tissue:buffer ratio of 1), and the tissue was ground until thawed. The crude root extract was then centrifuged at 13,000 rpm for 10 min at 4°C in a microcentrifuge. Ascorbic acid was added to the supernatant to give a final concentration of 100 mM, and incubated for 20 min at room temperature. The sample was then centrifuged at 13,000 rpm for 10 min at 4°C in a microcentrifuge, and aliquots were applied to a Spherosorb C_{18} reverse-phase HPLC column equilibrated with 0.1% TFA. PCs were eluted over 30 min with a linear gradient of 0 to 15% acetonitrile in 0.1% TFA. PCs were detected using Ellman's reagent (Grill et al., 1985) following published procedures (Rauser, 1991). PCs were quantified as thiols, based on a glutathione standard. Using standard PC_3 isolated from the fission yeast *Schizosaccharomyces pombe* and sequenced using tandem MS spectroscopy (Salt and Rauser, 1995), we were able to identify one of these PCs in *B. juncea* roots as PC_3. The tentative identification of the other PCs was based on their relative elution position. Glutathione was identified using a standard.

XAS Data Collection and Analysis

B. juncea roots exposed to 0.6 μg/mL Cd for 7 d were rapidly frozen and ground to a fine powder in liquid nitrogen using a pestle and mortar. The powder was then packed into 2-mm-path-length aluminum frames with Mylar tape windows for XAS. Samples of xylem sap were collected from plants exposed to 0.6 μg/mL Cd for 10 h or 7 d, lyophilized to about 60% of their original volume, pipetted into Lucite sample holders with a 10-mm path length, and also subjected to XAS. Cd K-absorption edge x-ray absorption spectra of these samples were collected on beamline 4–3 of the Stanford Synchrotron Radiation Laboratory[2] using an Si (220) double-crystal monochromator with no focusing optics and an upstream vertical aperture of 1 mm. The incident x-ray intensity was monitored using an argon-filled ion chamber, and the Cd K_α fluorescence (23,110 electron volt) was recorded using a Canberra 13-element array germanium detector. The samples were maintained at 10 K using an Oxford Instruments (Concord, MA) flowing liquid helium cryostat. Spectra were calibrated using the spectrum of Cd metal foil, collected simultaneously with each data set; the first energy inflection point of Cd foil was assumed to be 26,714 electron volt. Data reduction and analysis were carried out according to standard methods (Koningsberger and Prins, 1988) using the EXAFSPAK suite of programs.[3] The EXAFS oscillations X(k) were quantitatively analyzed by curve fitting. The EXAFS total amplitude and phase-shift functions were calculated by using the program FEFF (version 6.01) (Mustre de Leon et al., 1991; Rehr et al., 1991).

Cd Content of Xylem Sap

Xylem sap from *B. juncea* seedlings was collected by decapitating the plant just above the root and collecting the xylem sap that was exuded under root pressure. Sap was usually collected for up to 1 h after decapitation. The Cd concentration in the xylem sap was monitored using ^{109}Cd. To confirm the results obtained using ^{109}Cd, some samples of xylem sap were also analyzed for Cd directly using graphite furnace atomic absorption spectroscopy (Perkin-Elmer model Zeeman 4100L). Sap was collected 10 h after exposure to Cd, because this time corresponded to the linear phase of Cd accumulation into the leaf (see "Results").

---

[2] Stanford Synchrotron Radiation Laboratory is funded by the Department of Energy, Office of Basic Energy Sciences. The Biotechnology Program is supported by the National Institutes of Health, Biomedical Research Technology Program, Division of Research Resources. Further support is provided by the Department of Energy, Office of Health and Environmental Research.

[3] EXAFSPAK, a suite of computer programs for analysis of x-ray absorption spectra, was written by Dr. Graham N. George of the Stanford Synchrotron Radiation Laboratory and is available from him on request.

The Effects of ABA on Cd Accumulation

The stomatal apertures of *B. juncea* seedlings were manipulated by the addition of ABA dissolved in methanol to the hydroponic nutrient solution to obtain a final concentration of 100 μM. After 24 h of incubation with ABA, the nutrient solution was replaced with fresh solution without ABA. Cd accumulation into leaves of ABA-treated plants was then monitored using the Cd probe as described above. The stomatal resistance during these experiments was measured using a steady-state porometer (model LI-1600, Li-Cor, Lincoln, NE).

Cd Localization in Leaves

Excised leaves of *B. juncea* plants exposed for 24 h to 0.1 μg/mL Cd, containing 10 μCi/μmol ^{109}Cd, were placed abaxial side against x-ray-sensitive film for 24 h at room temperature. Localization of Cd within the leaf was determined by examining the exposed film. Trichomes from these leaves were collected by carefully brushing the leaves after they had dried. The Cd content of the trichomes was then determined directly by measuring ^{109}Cd.

RESULTS

Cd Accumulation in *B. juncea* and *T. caerulescens*

B. juncea and *T. caerulescens* exposed to Cd in the nutrient solution accumulated substantial amounts of Cd in their roots and shoots (Fig. 1). Bioaccumulation coefficients (concentration of Cd in dry plant tissue/concentration of Cd in nutrient solution) were up to 1178 for shoots and 6705 for roots of *B. juncea* and 376 for shoots and 875 for roots of *T. caerulescens*. Cd appeared to accumulate preferentially in the youngest leaves of both *B. juncea* and *T. caerulescens* (Table I). Over the range of Cd concentrations used, neither *B. juncea* nor *T. caerulescens* showed any significant reduction in fresh weight of roots or shoots (data not shown). However, a significant reduction in the Chl content of the young leaves of *B. juncea* was observed above 0.1 μg/mL Cd in solution (Fig. 2). This concentration of Cd had no effect on Chl content in the leaves of *T. caerulescens* (data not shown). Cd exposure in *B. juncea* also produced a significant decrease in the Mn content of both the roots and shoots, with the highest Cd concentration (0.6 μg/mL) causing a 38% decrease of Mn in the shoots and an 80% decrease in the roots. Cu in the shoots was depleted 50% under these conditions, although no decrease in roots was observed. These trends were not observed in *T. caerulescens*. Roots of *B. juncea* exposed to 0.2 μg/mL Cd for 3 d accumulated 238, 424, and 504 nmol thiol/g fresh weight of $(\gamma\text{-glutamylcysteinyl})_2$glycine, PC_3, and $(\gamma\text{-glutamylcysteinyl})_4$glycine, respectively, confirming the presence of PCs in this tissue (Speiser et al., 1992).

Table I. *Distribution of Cd in B. juncea and T. caerulescens exposed to 0.1 μg/mL Cd for 24 h*

B. juncea		*T. caerulescens*	
Leaf No.[a]	Cd content	Leaf No.	Cd content
	μg/g dry wt		μg/g dry wt
1	34.8	1	24.4
2	42.0	2	20.3
3	40.3	3	10
4	35.8	4	2
5	16.9	5	5.6
6	16.2	7	1.3
7	17	9	1.4
8	8.1	11	0.5
9	1.2	13	0.4

[a] Leaf number was from the top of the plant, counting the first clearly identifiable leaf as No. 1.

Cd K-Edge XAS of *B. juncea*

The Cd K-absorption near-edge spectra of *B. juncea* root tissue and xylem sap after 10 h and 7 d of exposure are shown in Figure 3A. The xylem sap spectra were similar for

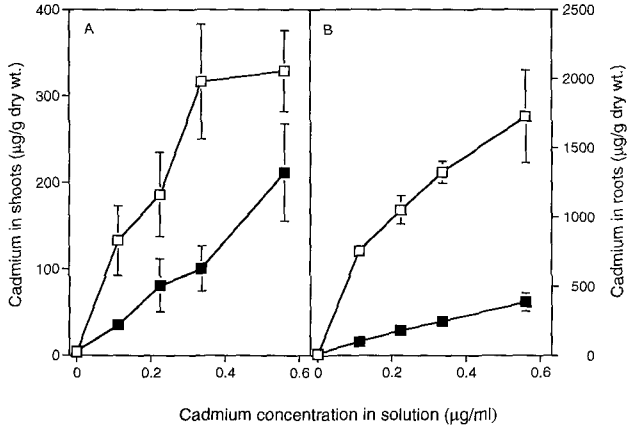

Figure 1. Cd accumulation in shoots (A) and roots (B) of *B. juncea* (□) and *T. caerulescens* (■) exposed to various Cd concentrations in the nutrient solution for 7 d. Data represent the means ± SD of six plants.

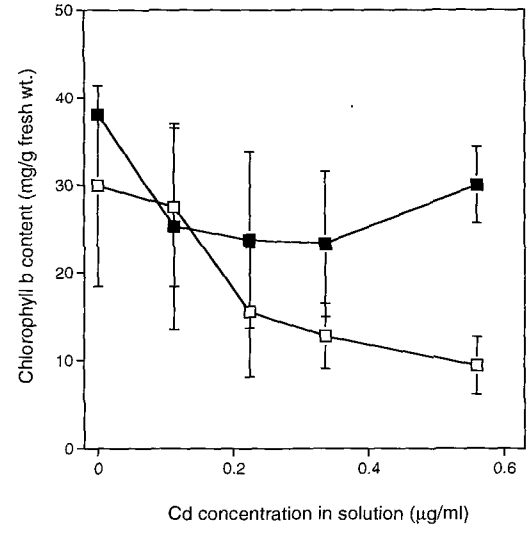

Figure 2. Chl content of partially expanded (leaf 3) (□) and fully expanded (leaf 5) (■) leaves of *B. juncea* exposed to various Cd concentrations in the nutrient solution for 7 d. Data represent the means ± SD of four individual leaves from different plants.

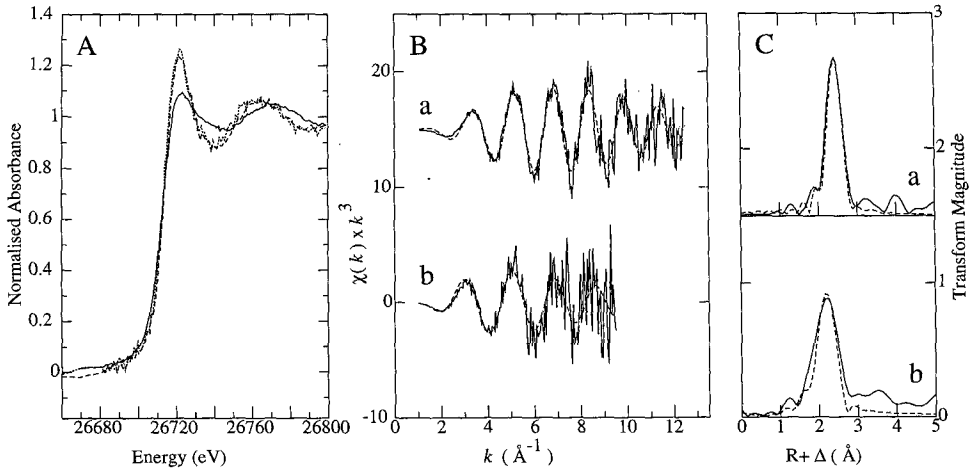

Figure 3. Cd K-absorption near-edge spectra (A) of *B. juncea* whole-root tissue (solid line) and xylem sap after 10 h and 7 d of exposure (dotted and dashed lines, respectively). Cd K-edge EXAFS (B) and Fourier transforms (C) of root tissue (a) and xylem sap (b) from *B. juncea* exposed to 0.6 μg/mL Cd for 7 d. The solid lines show the k^3-weighted EXAFS data, and the dashed lines show the results of the fits as shown in Table I. The EXAFS xylem sap data are the sums of the data from 10 h and 7 d of exposure. R+ Δ, Radial distance from the absorbing atom; *k*, photoelectron wavenumber.

both 10-h and 7-d treatments, indicating that the same species were present throughout. However, roots appeared to contain a different Cd species from that of the xylem sap. The Cd K-edge EXAFS spectra of *B. juncea* root tissue and xylem sap are shown in Figure 3B, together with the results of EXAFS curve-fitting analysis and the corresponding Fourier transforms (Fig. 3C). The numerical results of the curve fitting are given in Table II. The Fourier transforms of both samples have one main peak, indicating that the EXAFS is dominated by one prominent interaction. EXAFS curve-fitting analysis can readily distinguish backscatterers of very different atomic numbers, such as S and O, because of their differing phase and amplitude functions. It can be used to obtain bond lengths typically to ±0.02 Å, depending on the photoelectron wavenumber and other factors. It also can be used to obtain an estimate of the coordination number to ±25%. In the case of the root tissue, the EXAFS fits the Cd-S interactions, with an interatomic distance of 2.53 Å. This most likely represents Cd-S_4 coordination. Similar Cd-S coordination was also observed in shoots (data not shown). In contrast, the xylem sap fits either oxygen or nitrogen coordination at a somewhat shorter distance of 2.3 Å and probably represents Cd coordinated with six ligands. The poorer signal:noise ratio and shorter *k*-range obtained from the xylem sample was due to the low concentration of Cd (approximately 13 μM) in this sample.

Table II. *Results of EXAFS curve-fitting analysis*

Coordination number (N), interatomic distances (R) (Å), Debye-Waller factors (σ^2) (Å^2), and extent of data (k_{max}) (Å^{-1}). The estimated error (3 times the estimated SD) is shown in parentheses for the fitted values. Coordination numbers were adjusted to the nearest best integer and then fixed for the final fit. The E_o offset was −13.5 electron volt for both samples. The goodness of fit is defined as $\Sigma[(\chi_{obsd.} - \chi_{calcd.})^2 k^6]/n$, where *n* is the number of points in the spectrum and $\chi_{obsd.}$ and $\chi_{calcd.}$ are the observed and calculated points, respectively.

Sample	Type	N	R	σ^2	k_{max}	Goodness of Fit
Whole-root tissue	Cd-S	4	2.53(1)	0.005 (1)	12.5	1.31
Xylem sap	Cd-O or N	6	2.3 (2)	0.008 (3)	9.5	3.11

Cd Concentration in Xylem Sap of *B. juncea*

B. juncea seedlings were exposed for 10 h to different concentrations of Cd in the nutrient solution. Thereafter, the plants were decapitated and the xylem sap was collected. The concentration of Cd in the xylem sap followed a biphasic curve with relation to the Cd concentration in solution (Fig. 4). The concentration of Cd in the xylem sap increased rapidly and reached saturation at a Cd concentration of 0.3 μg/mL in

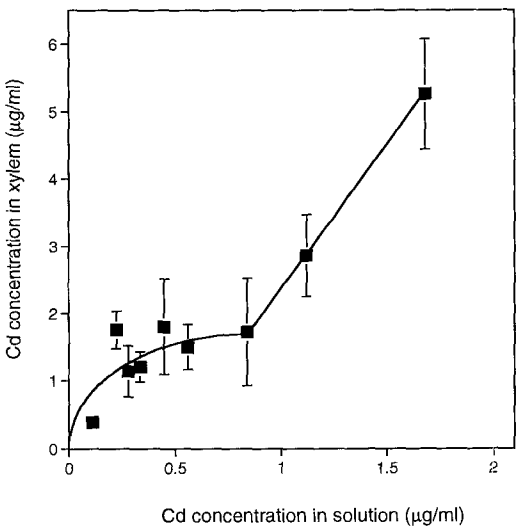

Figure 4. Concentration of Cd in the xylem sap of *B. juncea* exposed to various concentrations of Cd for 10 h. Cd concentrations were measured using ^{109}Cd and verified using graphite furnace atomic absorption spectroscopy. Data represent the means ± SD of three plants.

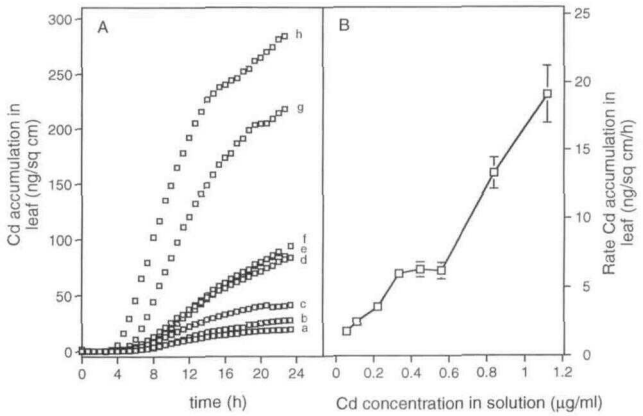

Figure 5. Accumulation of Cd in intact leaves of *B. juncea* exposed to various Cd concentrations in the nutrient solution. A, Accumulation of Cd with time in leaf 3 (counting the youngest leaf as 1) at 0.06 (a), 0.11 (b), 0.22 (c), 0.34 (d), 0.45 (e), 0.56 (f), 0.84 (g), and 1.12 (h) μg/mL Cd in the nutrient solution. Each curve represents a mean of four individual uptake experiments. B, Initial rates of Cd accumulation in leaf 3 (calculated from data in A) versus Cd concentration in the nutrient solution. Data represent the means ± SE of four initial rates.

the nutrient solution. The Cd concentration in the xylem sap then remained relatively constant until the concentration of Cd in the nutrient solution reached 0.8 μg/mL. Above this concentration, Cd concentration in the xylem sap increased sharply with a linear correspondence to the Cd concentration in the solution. Xylem sap from plants exposed to 0.6 μg/mL Cd for either 10 h and 7 d contained 2 and 1.7 μg/mL Cd, respectively.

Kinetics of Cd Accumulation in *B. juncea* Leaves

Using the Cd probe described in "Materials and Methods," we were able to measure the rate of Cd accumulation in the intact leaves of *B. juncea*. Cd accumulated rapidly within the third leaf, first appearing 4 to 6 h after exposure (Fig. 5A). After this initial lag phase, Cd accumulated linearly with time for a further 6 to 8 h. Thereafter, the rate of Cd accumulation diminished gradually. The rate of Cd accumulation during the linear period displayed biphasic kinetics with relation to the initial Cd concentration in the nutrient solution (Fig. 5B). The rate of Cd accumulation initially increased with increasing Cd concentration but reached saturation at 0.3 μg/mL Cd. Accumulation rates remained stable until 0.6 μg/mL Cd and then increased sharply thereafter. Cd accumulation rates (ng cm^{-2} h^{-1}) correlated well with measured Cd content of leaves. For example, the Cd accumulation rate in leaf 3 was 2.5 ng cm^{-2} h^{-1} (Fig. 5B), and based on a leaf area of 68.4 cm^2 and a leaf dry weight of 174 mg, the calculated Cd content of this leaf after 24 h of exposure was 23 μg/g dry weight. This correlates well with a Cd content of 40.3 μg/g dry weight observed for the same leaf number in a separate experiment (Table I).

Remarkably, in leaves of *B. juncea* exposed to 0.1 μg/mL Cd for 24 h, trichomes (hairs) on the leaf surface contained 556 μg/g dry weight Cd compared to 17 μg/g dry weight in leaves. This represents a 43-fold accumulation of Cd within the trichomes. A careful comparison of the distribution of Cd and trichomes in these leaves (Fig. 6) clearly shows that Cd preferentially accumulates in trichomes.

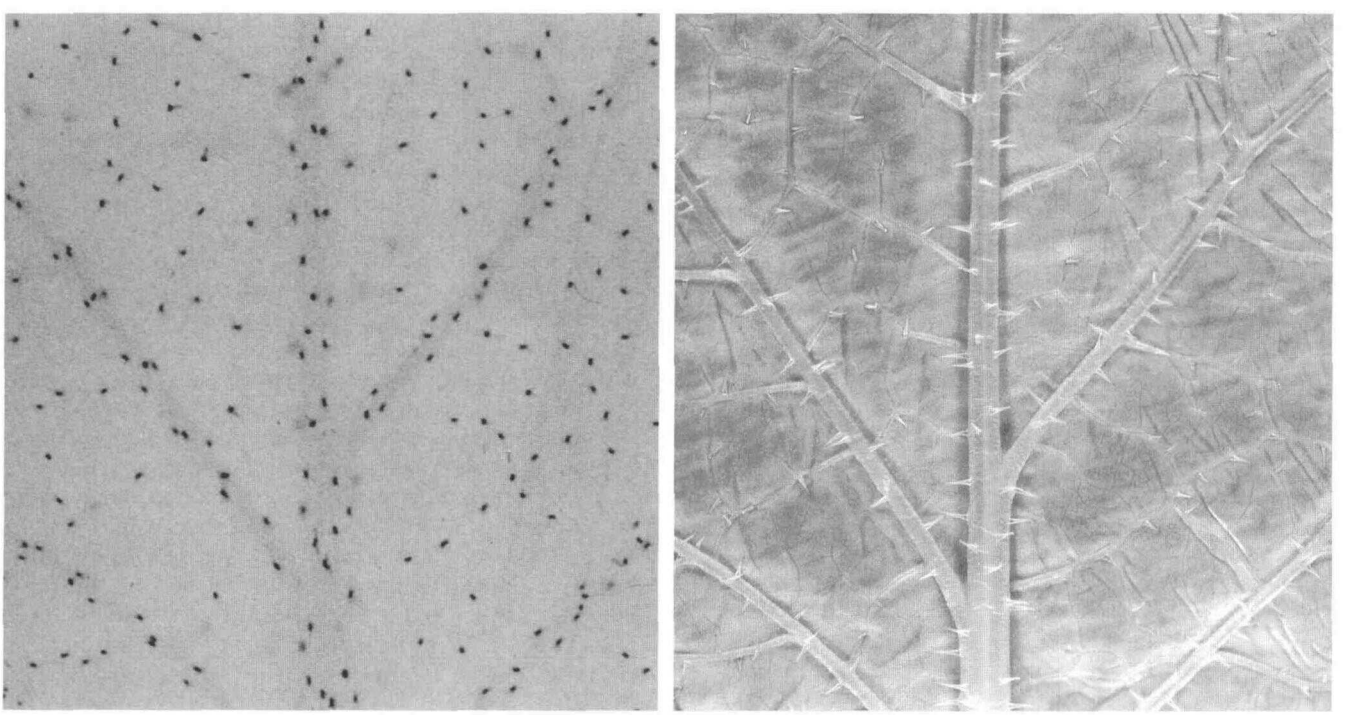

Figure 6. Autoradiograph (left) and photograph (right) of a leaf from *B. juncea* exposed to 0.1 μg/mL Cd (containing 10 μCi/μmol ^{109}Cd) for 24 h.

Effect of ABA on Cd Accumulation

Exposure of *B. juncea* to 100 μM ABA in the nutrient solution for 24 h caused an increase in the stomatal diffusive resistance from <0.1 to between 2 and 5 s/cm. Preliminary experiments indicated that this ABA concentration caused almost complete stomatal closure. Under the conditions of increased stomatal diffusive resistance and, hence, reduced transpiration, Cd accumulation into leaves was dramatically reduced (Fig. 7). However, ABA had no effect on Cd removal from the nutrient solution by roots (data not shown). To eliminate any possibility of ABA binding to Cd in solution and thereby reducing Cd uptake, these experiments were performed after ABA had been removed from the nutrient solution.

DISCUSSION

One of the important factors affecting the success of phytoremediation of Cd-polluted soils is the availability of high biomass plants with the ability to concentrate Cd to high levels within their shoots. Several reports suggest that *T. caerulescens* is capable of accumulating high levels of Cd in shoots from soil or hydroponic solution (Baker et al., 1994; Brown et al., 1994, 1995). Unfortunately, *T. caerulescens* has a low biomass when compared to crop plants. *T. caerulescens* is, however, a member of the Brassicaceae family, which is known to contain many high biomass crop plant species, including *B. juncea*, which has been reported to accumulate Cd as well as other toxic metals (Speiser et al., 1992; Kumar et al., 1995). In a direct comparison of *B. juncea* and *T. caerulescens* in hydroponic solution containing Cd, *T. caerulescens* accumulated less Cd in both roots and shoots than *B. juncea* (Fig. 1). Cd concentrations used were based on estimates of Cd concentrations in soil solution from Cd-contaminated sites (Salt and Wagner, 1993). Mild toxicity symptoms were observed in *B. juncea* but not in *T. caerulescens* when grown in nutrient solutions with >0.1 μg/mL Cd. Cd toxicity in *B. juncea* produced chlorosis in the young leaves (Fig. 2), and our observation that Cd preferentially accumulates within these leaves (Table I) probably explains the localization of this chlorosis. The higher resistance of *T. caerulescens* to Cd may explain why this plant has been reported to be able to accumulate high levels of Cd within its shoots from substrates containing relatively high concentrations of Cd (Baker et al., 1994; Brown et al., 1994, 1995).

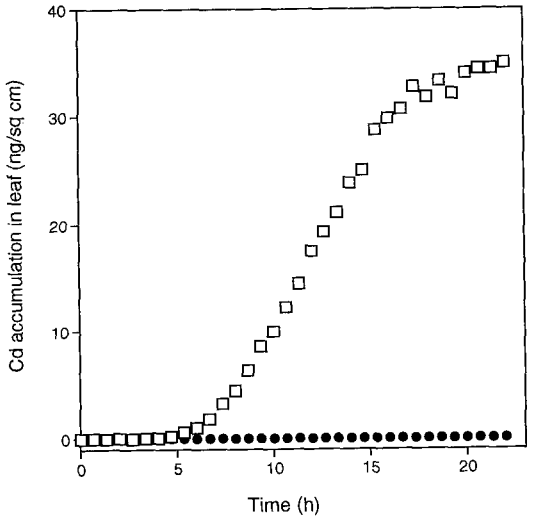

Figure 7. Cd accumulation in an intact leaf of *B. juncea* exposed to 0.1 μg/mL Cd in the nutrient solution using plants that had been previously exposed to 100 μM ABA for 24 h (●) or control plants (□).

After 7 d of exposure to 0.6 μg/mL Cd, roots of *B. juncea* contained about 6-fold higher Cd than shoots, with the majority of this Cd bound to S ligands with a probable coordination of Cd-S_4 and a bond length of 2.53 Å (Fig. 3). S-rich PCs accumulate in roots of *B. juncea* upon exposure to Cd and, as reported earlier, may be involved in Cd binding in *B. juncea* (Speiser et al., 1992). The Cd K-edge EXAFS of the purified Cd-PC complex (Strasdeit et al., 1991) showed Cd-S interactions similar to those observed in *B. juncea* root tissue (Fig. 3), with a probable coordination of Cd-S_4 and a bond length of 2.52 Å. Therefore, it seems PCs may be involved in binding a significant amount of Cd in the intact roots of *B. juncea* exposed to 0.6 μg/mL Cd for 7 d. However, this may not be the case for short-term Cd exposure or exposure to lower levels of Cd, for which cell wall and organic acids may play a larger role in Cd binding (Wagner, 1994). If a thiol:Cd ratio of between 1 and 2 for the Cd-PC complex in plants (De Knecht et al., 1994; Salt and Rauser, 1995) is assumed, it would require 15 to 30 μmol/g dry weight total thiols to chelate the 15 μmol/g dry weight Cd (Fig. 1) found in these roots. Roots of *B. juncea* exposed to this level of Cd contained 26 μmol/g dry weight PC thiols, which would be sufficient to bind the majority of Cd detected in roots.

Translocation of Cd in the xylem sap appears to be independent of PC production in the roots. After 7 d of exposure to Cd, when PCs appear to bind most of the Cd in the root, the concentration of Cd in the xylem sap remained the same as that seen in plants exposed to Cd for only 10 h, before the production of any significant amount of PCs. Prolonged exposure to Cd also had no effect on the speciation of Cd in the xylem sap. Cd K-edge EXAFS of xylem sap isolated from plants exposed to 0.6 μg/mL Cd for 10 h or 7 d showed Cd interactions with O or N, with a probable coordination of 6 and a bond length of 2.30 Å. PCs, therefore, appear to play no direct role in the transport of Cd to the shoot.

Total Cd accumulation in shoots of *B. juncea* appeared to saturate at 0.3 μg/mL Cd in the nutrient solution (Fig. 1), and this saturation was observed in the rate of Cd accumulation into leaves, as measured with the Cd probe (Fig. 5). This saturation probably reflects the presence of rate-limiting step(s) in the pathway of Cd uptake and accumulation into the shoot. The fact that the concentration of Cd in the xylem sap also saturates at 0.3 μg/mL Cd in the nutrient solution (Fig. 4) suggests that these rate-limiting step(s) occur somewhere between Cd uptake into the root and Cd loading into the xylem. When the concentration of Cd in the nutrient solution exceeded 0.6 μg/mL, the accumulation rate of Cd in the shoot increased dramatically (Fig. 5), and this was also reflected in an increase in the Cd concentration in the xylem sap (Fig. 4). The rapid increase

in Cd accumulation above 0.6 μg/mL Cd in the nutrient solution probably reflects the breakdown of some physiological barrier in the root because of the toxic effects of Cd. This kind of biphasic accumulation has also been reported for Pb (Kumar et al., 1995).

Exposure of *B. juncea* to ABA caused a large increase in the stomatal diffusive resistance of leaves, and this was associated with a dramatic reduction in the accumulation of Cd in the leaves (Fig. 7), suggesting that Cd accumulation in leaves is driven mainly by mass flow due to transpiration. This type of transpiration-driven transport has also been observed for several other ions, including Ca, B, Si (Marschner, 1986, and refs. therein), and Cl (Greenway, 1965). However, reduced translocation of Cd to the shoot had no effect on the uptake of Cd from the solution, and therefore root uptake and shoot accumulation of Cd appear to be independent processes. This ABA effect has also been observed in rice (Rubio et al., 1994). However, this study did not correlate ABA treatment with reduced transpiration.

When in the leaf, Cd accumulated preferentially in the trichomes on the leaf surface (Fig. 6). The storage of Cd in trichomes may represent a detoxification mechanism, since trichomes represent a tissue external to the leaf. There are reports that trichomes accumulate other toxic metals, including Mn (Blamey et al., 1986) and Pb (Martell, 1974). Expression of a gene encoding a type 2 metallothionein (a metal-binding protein) is localized in trichomes of bean plants (Foley and Singh, 1994).

Cd accumulation in shoots of *B. juncea* appears to be limited by several factors, including the transpiration rate and, possibly, root uptake, radial transport, and xylem loading. A better understanding of these root transport processes should facilitate the production of plants with an increased ability to accumulate Cd within their shoots.

ACKNOWLEDGMENTS

The authors wish to extend their appreciation to Ted Kruse for his advice concerning setting up the Cd probe system, Alan Baker for his kind gift of the *Thlaspi* seeds, Graham George for his help and advice with the EXAFS, Tom Leustek for his help with the photography, and Andres Alvarado and Robin Torquati for their technical assistance. We are particularly grateful to Harry Moto for analytical support of this work.

Received June 21, 1995; accepted September 14, 1995.

LITERATURE CITED

Baker AJM, Reeves RD, Hajar, ASM (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). New Phytol **127:** 61–68

Banuelos GS, Meek DW (1990) Accumulation of selenium in plants grown on selenium-treated soil. J Environ Qual **19:** 772–777

Bhattacharyya MH, Whelton BD, Stern PH, Peterson DP (1988) Cadmium accelerates bone loss in ovariectomized mice and fetal rat limb bones in culture. Proc Natl Acad Sci USA **85:** 8761–8765

Blamey FPC, Joyce DC, Edwards DG, Asher CJ (1986) Role of trichomes in sunflower tolerance to manganese toxicity. Plant Soil **91:** 171–180

Brown SL, Chaney RL, Angle JS, Baker AJM (1994) Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc- and cadmium-contaminated soil. J Environ Qual **23:** 1151–1157

Brown SL, Chaney RL, Angle JS, Baker AJM (1995) Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. Soil Sci Soc Am J **59:** 125–133

Buchet JP, Lauwerys R, Roels H, Bernard A, Bruaux P, Claeys F, Ducoffre G, De Plaen P, Staessen J, Amery A, Lijnen P, Thijs L, Rondia D, Sartor F, Saint Remy A, Nick L (1990) Renal effects of cadmium body burden of the general population. Lancet **336:** 699–702

De Knecht JA, Van Dillen M, Koevoets PLM, Verkleij JAC, Ernst WHO (1994) Phytochelatins in cadmium-sensitive and cadmium-tolerant *Silene vulgaris*. Chain length distribution and sulfide incorporation. Plant Physiol **104:** 255–261

Foley RC, Singh KB (1994) Isolation of a *Vicia faba* metallothionein-like gene: expression in foliar trichomes. Plant Mol Biol **26:** 435–444

Greenway H (1965) Plant responses to saline substrates. IV. Chloride uptake by *Hordeum vulgare* as affected by inhibitors, transpiration, and nutrients in the medium. Aust J Biol Sci **18:** 249–268

Grill E, Winnacker E-L, Zenk MH (1985) Phytochelatins: the principle heavy-metal complexing peptides in higher plants. Science **230:** 674–676

Koningsberger DC, Prins R (1988) X-Ray Absorption. Principles, Applications and Techniques of EXAFS, SEXAFS and XANES. John Wiley and Sons, New York

Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextration—the use of plants to remove heavy metals from soils. Environ Sci Technol **29:** 1232–1238

Marschner H (1986) Mineral Nutrition of Higher Plants. Academic Press, London, pp 77–82

Martell EA (1974) Radioactivity of tobacco trichomes and insoluble cigarette smoke particles. Nature **249:** 215–217

Mustre de Leon J, Rehr JJ, Zabinsky SL, Albers RC (1991) *Ab initio* curved-wave x-ray-absorption fine structure. Physiol Rev **B44:** 4146–4156

Nriago JO (1979) Global inventory of natural and anthropogenic emissions of trace metals in the atmosphere. Nature **279:** 409–411

Petit CM, Van de Geijn SC (1978) In vivo measurements of cadmium ($^{115\ m}$Cd) transport and accumulation in the stems of intact tomato plants (*Lycopersicon esculentum*, Mill.). I. Long distance transport and local accumulation. Planta **138:** 137–143

Rauser WE (1991) Cadmium-binding peptides from plants. Methods Enzymol **205:** 319–333

Rehr JJ, Mustre de Leon J, Zabinsky SL, Albers RC (1991) Theoretical x-ray absorption fine structure standards. J Am Chem Soc **113:** 5135–5140

Rubio MI, Escrig I, Martinez-Cortina C, López-Benet FJ, Sanz A (1994) Cadmium and nickel accumulation in rice plants. Effects on mineral nutrition and possible interactions of abscisic and gibberellic acids. Plant Growth Regul **14:** 151–157

Ryan JA, Pahren HR, Lucas JB (1982) Controlling cadmium in the human food chain: a review and rationale based on health effects. Environ Res **28:** 251–302

Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology **13:** 468–474

Salt DE, Rauser WE (1995) MgATP-dependent transport of phytochelatins across the tonoplast of oat roots. Plant Physiol **107:** 1293–1301

Salt DE, Wagner GJ (1993) Cadmium transport across tonoplast of vesicles from oat roots. Evidence for a Cd/H antiport activity. J Biol Chem **268:** 12297–12302

Speiser DM, Abrahamson SL, Banuelos G, Ow DW (1992) *Brassica juncea* produces a phytochelatin-cadmium-sulfide complex. Plant Physiol **99:** 817–821

Strain HH, Cope BT, Svec WA (1971) Analytical procedures for the isolation, identification, estimation and investigation of the chlorophylls. Methods Enzymol **23:** 452–476

Strasdeit H, Duhme A-K, Kneer R, Zenk MH, Hermes C, Nolting H-F (1991) Evidence for discrete $Cd(SCys)_4$ units in cadmium phytochelatin complex from EXAFS spectroscope. J Chem Soc Chem Commun **16:** 1129–1130

Wagner GJ (1994) Accumulation of cadmium in crop plants and its consequences to human health. Adv Agron **51:** 173–212