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Cadmium Distribution and Chemical Fate in Soybean Plants[1]

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ABSTRACT

The distribution and chemical behavior of Cd^{2+} in tissues and its chemical form in xylem water of soybean plants (cv. Williams) were investigated. Following root absorption, Cd is strongly retained by roots, with only 2% of the accumulated Cd being transported to leaves; as much as 8% was transported to seeds during seed filling. *In vivo* xylem exudates contained two anionic Cd complexes in addition to inorganic forms of Cd. Once accumulated in root and leaf tissues, Cd rapidly equilibrated between the insoluble, soluble, and organelle fractions. Of the solubles, which contain 50% of the Cd, >50% was associated with components of >10,000 molecular weight, and <8% was associated with <500 molecular weight components. Cadmium accumulated in soybean seeds was primarily associated with cotyledons. Fractionation of seeds showed the soy proteinate and soy whey to contain 32 and 50% of the accumulated Cd, respectively.

Cadmium may enter surface soils in fertilizer and sewage sludge amendments, deposition of particulates from fossil fuel combustion, disposal of industrial wastes, and possibly in the future development and large scale production of photovoltaic systems. In the past, research on the behavior of Cd in plants has been focused on phytotoxicity; however, emphasis recently has been directed towards health implications (10). It is well established that the chemical form of elements, following plant incorporation from soil, may affect both gastrointestinal absorption and retention in consuming animals (13, 14, 21). Similarly, the source of biologically incorporated Cd appears to affect both tissue content in consuming animals and its retention (18).

Although much of the work on Cd metabolism has centered around animal systems, the behavior of Cd in plants increasingly is being examined. Cutler and Rains (8) demonstrated that the uptake of Cd by excised barley roots, using Cd concentrations of 9 to 178 μM, was primarily a function of exchange adsorption, irreversible adsorption, and diffusion involving nonmetabolic binding to organic materials of a cell wall. Unfortunately, since the Cd^{2+} activity normally encountered in soils of pH 6 to 6.5 is restricted to ~0.1 μM due to solubility controls (19), use of excessively high Cd concentrations can seriously affect interpretation of both uptake mechanisms and metabolic fate studies. In fact, Cd uptake studies, both with higher plants (6) and algae (11), employing Cd concentrations <1 μM, show absorption isotherms consistent with metabolically mediated transport. In metabolic studies, the proportion of Cd found in root residues compared with that found in metabolic compartments decreases when the concentration of Cd supplied to roots is decreased from 0.9 to 0.009 μM (9). Several studies have evaluated the chemical fate of Cd found in soluble fractions of roots and leaves (2, 9, 26). These have shown Cd to be associated with protein having mol wt of 3,000 to 10,000 in roots (2, 9, 26) and 700 to 5,000 in leaves (2, 26). The soluble Cd containing fractions of rice roots was found to be high in cysteine (9), and it is suggested that sulfhydryl groups may play a role in fixation of Cd as they do in animal systems (2, 9, 16).

This investigation was directed toward further definition of the behavior of Cd in soybean plants following root absorption. Particular emphasis is placed on Cd distribution in plant tissues and form in xylem water, roots, leaves, and seeds, as related to plant metabolism and ultimate form in soy products.

MATERIALS AND METHODS

Plant Culture. Seeds of *Glycine max* cv. Williams were germinated and plants grown hydroponically, as previously described (4). Plants, 33 days old, were used for determination of Cd distribution and form in leaves and roots and for collection and characterization of xylem exudate. Plants at early seed filling (90 days old) were employed to evaluate the distribution and remobilization of Cd during vegetative growth and senescence and to provide seeds for Cd distribution analysis.

Cadmium Uptake. For evaluation of Cd distribution and mobilization, individual plants were grown in 2-liter polyethylene containers containing 1.0 μM $^{109}CdCl_2$ (0.088 μCi $^{109}Cd^{2+}$ per μg Cd^{2+}) in pH 5.8 nutrient solution. Roots were rinsed for 30 min in 0.5 mM $CaCl_2$ solution containing 10 μM $CdCl_2$ to remove sorbed or exchangeable Cd after 24 h of uptake and placed in fresh nutrient solution for an additional 21 days. Leaf and root tissues employed in fractionation and characterization studies were obtained from 33-day-old plants grown in 1-liter containers of nutrient solution containing 1.0 μM $^{109}CdCl_2$. After 24 h of uptake, plant roots were rinsed and plants transferred to fresh solution for 1, 3, or 19 days.

Xylem Exudate Collection and Characterization. Plants 37 days old, were placed on 1.0 μM $CdCl_2$ (0.45 μCi of ^{109}Cd per μg Cd) in nutrient solution for 2 h to allow time for Cd to enter the xylem and decapitated below the primary leaf node. The stem was fitted with a short piece (2 cm) of gum rubber tubing, the open end of which was fitted with a 1-mm polyethylene tube. *In vivo* exudate containing root-adsorbed ^{109}Cd was collected in a cooled vial (4 C) for 0 to 2, 2 to 4, and 4 to 24 h. Control exudates (0–2 h) were collected in a similar manner, and $^{109}CdCl_2$ (constant radioactivity) was added to provide Cd concentrations from 0.001 to 5 μM. Components containing ^{109}Cd were characterized by thin layer electrophoresis using precoated cellulose plates (Brinkmann; CEL-300-10 UV 254). Electrophoresis was performed in 0.1 M Hepes buffer (pH 7.55) at 400 v for 30 min. Components containing ^{109}Cd were visualized by autoradiography.

Control exudates (0–2 h) were collected, components (organic acids, amino acids, neutrals) separated by ion exchange (1), organic carbon content determined for each fraction using carbon analyzer 0524B-NR (Oceanography International, College Station, TX), organic acids determined by gas chromatography (20), and amino acids determined using amino acid analyzer MM-70

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(Glenco Scientific, Houston, TX). Components comprising >90% of the organic acid and amino acid fractions were individually added in a 20-fold M excess to $^{109}CdCl_2$ solutions (pH 6.2), equilibrated for 2 h, and subjected to electrophoresis, and the extent of complexation was determined following autoradiography, as previously described.

Fractionation of Leaf and Root Tissues. Leaf and root tissues (5 g) were cut into ~5-mm sections, placed into 20 mM ammonium acetate buffer at pH 6.9 (7.5 ml/g tissue), and homogenized 3 times for 45 s using a Sorvall Omni-Mixer, setting 10 (DuPont Instruments, Newtown, CT). All procedures were performed at 4 C. The homogenate was filtered through a single layer of 20-μm nylon filtration cloth, and liquid was expressed from the tissue residue. The tissue residue was resuspended in buffer and homogenized to assure recovery of soluble material and organelles (primarily chloroplasts and/or mitochondria). The second filtrate, containing <5% of the total material recovered, was combined with the first filtrate and centrifuged at 25,000*g* for 15 min. The resultant supernatant solution, referred to as the soluble fraction, was employed in subsequent characterization studies, as described below. The pellet fraction (organelles) was resuspended, recentrifuged, and analyzed for radioactivity.

For comparative purposes, control tissues were carried through both the tissue fractionation and tissue characterization procedures. These were produced by adding a concentration of ^{109}Cd (constant specific radioactivity), equivalent to that found in the root or shoot of the treated plant, to the homogenization buffer containing control tissue; homogenizing; allowing 2 h for equilibration; and carrying these through the entire fractionation procedure. This provided a basis for comparing the relative affinity of Cd for specific ligands.

The supernatant fraction, containing the soluble leaf and root components, was further fractionated using Amicon ultrafiltration cells. Sequential filtration of the soluble fraction through UM 10 and UM 05 Diaflo membranes (Amicon Corp., Lexington, MA) resulted in fractionation of the soluble components into >10,000, <10,000, and <500 equivalent mol wt fractions (based on globular proteins). From the ^{109}Cd radioactivity contained in retentate (soluble fraction enriched in higher mol wt components based on rejection coefficients) and filtrate and their respective volumes, the distribution of Cd in the >10,000, 10,000 to 500, and <500 mol wt fractions was calculated.

Fractionation of Seed Components. Seeds collected from mature soybean plants (redistribution study) were freeze-dried and dehulled, and the embryos were separated from cotyledons. Dehulled seeds, ground to 40 mesh, were fractionated by a modification of the method of Raskis *et al.* (17). Freeze-dried, dehulled seeds (3 g) were extracted twice with 15 ml *n*-hexane for 2 h at room temperature. The solutions were centrifugated at 20,000*g* to obtain the lipid fraction (supernatant) and defatted flakes. Hexane was removed from the latter by vacuum evaporation; the defatted flakes were extracted twice with 15 ml H_2O (pH 7.0) for 3 h each and centrifuged to yield the insoluble residue and defatted soy milk. The soy milk was acidified with 0.01 N HCl to pH 4.5 and centrifuged at 20,000*g* for 10 min. The pellet (soy curd) was washed once and recentrifuged, and the wash was combined with the original supernatant solution (soy whey). The soy curd was resuspended and neutralized to pH 7.0 with KOH, forming soy proteinate. The soy whey was further fractionated by ultrafiltration using Diaflo UM 10, DM 5, and UM 05 membrane filters; this yielded equivalent mol wt fractions of >10,000, 10,000 to 1,000, 1,000 to 500, and <500, respectively.

RESULTS AND DISCUSSION

Distribution of Cd following Root Absorption. A number of studies have described the retention of Cd by roots and its subsequent partitioning to shoots based on plant species and Cd concentration (12, 15, 26). Although these demonstrate a generally reduced transfer between roots and shoots, adequate details have not been provided with respect to time and concentration in specific tissues to allow for evaluation of Cd form and distribution in the maturing plant. The distribution of ^{109}Cd in soybean plants 48 h and 21 days following a 24-h pulse of ^{109}Cd is shown in Table I. Following ^{109}Cd uptake, 92% of the Cd (175 μg) was still retained by the roots at 48 h, with the majority of the shoot Cd associated with the stem tissue (~7%). After 21 days, plants were at physiological maturity (yellowing of pods), with little increase noted in vegetative growth over the 21-day period. Therefore, any change in Cd concentration in specific tissues during this period should have resulted from redistribution of Cd. At maturity, 84% of the Cd (162 μg) was still retained by the roots, with an increase in Cd content of leaves, pods, and, particularly, seeds (15 μg). The change in Cd concentration of tissues between 48-h and 21-day treatments showed that a fraction of the Cd retained in roots and stems is redistributed to other tissues.

An interesting aspect of the distribution data was the comparatively large increase in both Cd percentage (7.8%) and concentration (0.035 to 0.37 μg/g) in soybean seeds after 21 days. Inasmuch as it is unlikely that evapotranspiration by pods and seeds could have caused the disproportionate concentration of Cd in these tissues, it seems that Cd was redistributed (*via* phloem) to seeds as the plants matured. Based on the apparent immobility of Cd (*i.e.* its retention in roots and accumulation in stem tissues), which has been shown to be a function of ion exchange phenomena (25), the observed redistribution of Cd to seeds at maturity suggests a change in Cd solubility. This might be most readily accomplished by the formation of soluble organic complexes of Cd, similar to these shown for Fe (22) and Ni (5) prior to, or following, transfer of Cd to the xylem.

Cd Form in Xylem Exudates. To determine the chemical behavior of xylem mobile Cd, xylem exudates were collected over a 24-h period and analyzed for Cd concentration, and Cd form (inorganic complexes and/or organic complexes) was determined by electrophoresis (Fig. 1). The concentration of Cd decreased from 0.045 to 0.02 μM over the 24-h period for the *in vivo* exudates. A comparison of the electrophoretic behavior of Cd in *in vivo* exudates and inorganic $^{109}CdCl_2$ showed ~50% of the Cd to be cationic (component a) or electrically neutral (component b) and probably represent Cd^{2+} and $Cd(OH)_2$, respectively. Component c, a slow-moving anionic component, contained ~35% of Cd, while component d accounted for ~15% of the activity. Tailing of the latter indicated that this Cd component was unstable under the electrophoretic conditions employed. The decrease in component c and d between 4 to 24 h may suggest a decrease in complexing ligand with time following decapitation or a decrease of Cd available for transfer to the shoot.

To determine whether exudates have the capacity to complex Cd^{2+} added *in vitro*, control 0- to 2-h exudates were spiked with 0.001 to 5.0 μM $^{109}CdCl_2$ at constant radioactivity. At all concentrations, the inorganic components (a and b) were present, and component c was absent. Component d was present only at the lower Cd concentrations. A new anionic component (e), with higher electrophoretic mobility, was present in *in vitro*, but not in *in vivo*, exudates. Since ^{109}Cd was held constant over these concentrations, it is apparent that component d forms at Cd concentrations below 0.05 μM (d), while component e forms below 0.5 μM. Neither were observed at 5μM (d).

The inability to form component c *in vitro* precluded direct characterization of the chemical nature of this Cd complex. To determine whether the *in vivo* Cd complexes could be produced in the presence of xylem constituents, control exudates were fractionated to provide organic acid, amino acid, and neutral fractions. These showed 64% of the organic carbon to be as amino acids, 25% as organic acids, and 11% as neutrals. Malic, citric, succinic,

Table I. *Distribution and Concentration of Cadmium with Time following 24-h Uptake from 1.0 μM $CdCl_2$ by 90-Day-Old Soybean Plants*

Tissue	Distribution following $^{109}Cd^{2+}$ Pulse[a]		Tissue Concentration		Dry Weight at 21 Days
	48 h	21 Days	48 h	21 Days	
	μg Cd		*μg Cd/g dry wt tissue*		*g*
Roots	175.10 ± 8.73	162.50 ± 4.44	15.41 ± 0.81	10.35 ± 0.60	15.7 ± 0.2
Stems	12.90 ± 3.60	8.69 ± 2.32	0.68 ± 0.10	0.46 ± 0.04	18.9 ± 2.7
Leaves	1.14 ± 0.38	3.42 ± 1.16	0.072 ± 0.013	0.13 ± 0.05	26.3 ± 4.6
Pods	0.38 ± 0.08	3.18 ± 0.58	0.045 ± 0.009	0.16 ± 0.05	19.9 ± 2.0
Seeds	0.19 ± 0.06	15.13 ± 0.77	0.035 ± 0.015	0.37 ± 0.06	40.9 ± 1.7
Abscissed leaves		0.18 ± 0.04		0.51 ± 0.010	3.6 ± 0.4
Total content	189.71 ± 9.21	193.10 ± 5.72			

[a] Mean of two replicates.

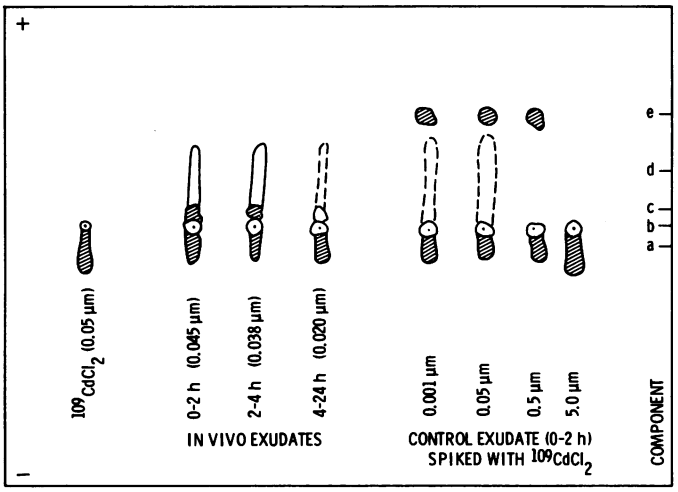

FIG. 1. Comparison of *in vivo* and *in vitro* behavior of Cd complexes in xylem exudates of soybean. Thirty-seven-day-old plants were placed on 1.0 μM $^{109}CdCl_2$ for 2 h prior to exudate collection. The Cd-containing components were visualized by autoradiography. Degree of shading denotes relative radioactivity: shaded > solid line > dashed line.

and adipic acids accounted for >93% of the organic acids detected. Amino acid analyses demonstrated the presence of 20 known amino acids (Asp, Thr, Ser, Asn, Gln, Pro, Gly, Ala, Val, Met, Cys, Tyr, Phe, Gaba, Orn, Lys, His, Arg, α-amino butyric acid, and β-amino isobutyric acid) and several unknown ninhydrin positive components. Each of the identified organic and amino acids were individually added to 0.5 μM $^{109}CdCl_2$ at 20-fold M excesses and subjected to electrophoresis. No discrete, stable Cd containing complexes were observed; in each case, only electrically neutral (component b) and unstable, tailing components (similar to component d; Fig. 1) were observed.

While the complexing ligand responsible for the anionic Cd complex found *in vivo* exudates (component c) was not identified, its presence is consistent with the observed behavior of numerous metal cations in plants. These studies have been reviewed by Tiffin (24), and demonstrate the presence of anionic complexes of Cu, Ni, and Zn in xylem exudates. In addition, the organic ligand stabilizing Fe for transport has been shown to be citrate (23), while citrate and malate stabilize Ca for transport (3). It is assumed that organic complexation, especially with essential trace metals, is necessary to prevent hydrolysis and precipitation, which is likely to occur in transport fluids due to comparatively high concentrations of phyroxyl ions, phosphates, and other components. Recent studies of White *et al.* (27–29) have described in detail the behavior and have demonstrated the importance of organic complexation in stabilizing divalent metal cations for transport in plant fluids.

Fractionation of Cd-Containing Root and Leaf Tissues. The distribution of Cd within soybean tissues was determined using 33-day-old hydroponically grown plants. Plants were provided with a 24-h pulse of 1.0 μM $^{109}CdCl_2$ and fractionated after additional 1-, 3-, and 19-day periods to determine the time required for metabolic equilibration of Cd (Table II). At day 1 following treatment, 82% of the Cd contained in roots (20.8 μg) was extractable and apparently soluble, with only 12% associated with the insoluble residue. At 3 and 19 days, Cd distributions stabilized, with ~40% of the Cd being associated with both the insoluble residue and soluble fraction and ~20% with the pellet fraction. The control tissues labeled with ^{109}Cd during homogenization exhibited a behavior similar to that of the 3- and 19-day treatments. The distribution of Cd in fractionated leaves was similar to that in roots, with 35%, 50%, and 15% of the ^{109}Cd being associated with the insoluble residue, solubles, and pellet fraction, respectively. Similar studies with rice (9) grown to maturity on 0.1 μg/g ^{109}Cd have shown distribution in the residue, soluble, and pellet fractions of root to be 75%, 15%, and 10%, respectively; as Cd concentrations are decreased to 0.005 μg/g, the proportion of Cd associated with solubles and the pellet fraction increased.

Studies with bushbean (26) have shown >70% of the Cd associated with leaves and roots to be as soluble components, with only 10% being associated with organelles. However, the latter study was performed using phytotoxic concentrations of Cd (4 μM), which can affect its distribution (9). Whatever the basis of differences, these data show a substantial association of Cd, both with extractable solubles and organelle fractions of soybean roots and leaves. Whether this represents nonspecific binding or metabolic incorporation/detoxification is not assessed here.

The soluble fractions of leaves and roots were further fractionated by ultrafiltration to determine the mol wt distributions of Cd (Table III). After 1 day of uptake and metabolism, <7% of the soluble Cd was found to be associated with low mol wt compounds (<500) in roots and leaves. A major fraction (71%) of the soluble Cd associated with roots was in the >10,000 mol wt fraction; in leaves, 38% of the Cd was associated with >10,000 mol wt compounds, while 56% of the Cd was in the intermediate 10,000 to 500 mol wt fraction. An apparent equilibrium in distribution occurred in both leaves and roots after 3 days, with 80 and 95% of the Cd found in the >10,000 mol wt fraction of leaves and roots, respectively, and <8% of the Cd existing as inorganic Cd or associated with <500 mol wt components. A similar analysis of new leaves, developed after the administered pulse of Cd, again showed >90% of the soluble Cd to be associated with >10,000 mol wt components, suggesting that mol wt distribution may be more specific than simple adsorption. These mol wt distribution data are consistent with that previously reported for rice (9), bushbean (26), and tomato (2). The distribution of Cd in control tissues, which were labeled during homogenization with a concen-

Table II. *Distribution of Cadmium in Various Tissue Fractions of Soybean following Homogenization and Centrifugation*

Soybean plants, 33 days old, were supplied with a 24-h pulse of 1.0 μM $^{109}CdCl_2$; tissues were fractionated after additional periods of 1, 3, and 19 days.

Fraction	Treatment Period[a]			Control Plant
	1 Day	3 Days	19 Days	
		%		
Roots				
Insoluble residue[b]	11.8 ± 0.2	47.5 ± 2.1	36.2 ± 1.8	39.7
Soluble fraction[c]	82.4 ± 1.0	32.0 ± 1.0	48.6 ± 2.7	38.2
Pellet fraction[d]	5.8 ± 0.7	20.5 ± 0.5	15.2 ± 0.3	22.1
Cd content[e]	20.83 ± 1.18	18.57 ± 0.42	8.87 ± 0.56	21.11
Leaves				
Insoluble residue[b]	14.9 ± 0.5	29.7 ± 2.1	37.1 ± 5.4	17.8
Soluble fraction[c]	78.1 ± 0.8	48.8 ± 3.3	47.6 ± 5.5	54.6
Pellet fraction[d]	7.0 ± 0.3	21.4 ± 0.4	15.3 ± 0.1	27.6
Cd content[e]	4.44 ± 0.20	5.02 ± 0.37	3.72 ± 0.22	6.05

[a] Mean of two replicate samples for treated plants; single replicate for controls.
[b] Primarily cell wall material.
[c] Soluble cytoplasm and readily exchangeable Cd.
[d] Primarily chloroplasts and/or mitochondria.
[e] μg Cd/5 g fresh weight root or leaf tissue fractionated.

Table III. *Distribution of Cadmium in the Soluble Fraction of Homogenate following Ultrafiltration*

Tissue homogenates obtained from 33-day-old soybean plants grown in 1.0 μM $^{109}CdCl_2$ for 24 h, followed by 1, 3, and 19 days for metabolism.

Mol Wt Fraction	Treatment Period[a]			Control Plant
	1 Day	3 Days	19 Days	
		%		
Root				
>10,000	71.3 ± 2.2	95.1 ± 4.1	96.4 ± 0.9	52.3
10,000 to 500	22.0 ± 5.4	3.4 ± 0.4	2.3 ± 0.3	1.7
<500	6.7 ± 0.4	1.4 ± 0.3	1.3 ± 0.4	46.1
Cd content[b]	17.16 ± 0.21	5.94 ± 0.19	4.31 ± 0.24	8.06
Old leaves				
>10,000	38.1 ± 4.7	84.9 ± 2.8	79.3 ± 1.6	58.2
10,000 to 500	56.0 ± 5.4	10.7 ± 1.1	12.4 ± 1.4	33.0
<500	5.9 ± 0.7	4.4 ± 0.6	8.3 ± 0.8	8.8
Cd content[b]	3.46 ± 0.04	2.45 ± 0.17	1.77 ± 0.21	3.30
New leaves[c]				
>10,000			90.6 ± 2.1	
10,000 to 500			5.3 ± 1.1	
<500			4.0 ± 0.7	
Cd content[b]			2.33 ± 0.24	

[a] Percentage of solubles from Table II.
[b] Cd content, μg Cd in solubles from 5 g leaves or roots.
[c] New leaves developed after initial Cd feeding period contained 5.54 μg Cd per 5 g fresh weight tissue, with 42.1% associated with solubles.

tration of Cd comparable to treatments followed by 2 h of equilibration, differed from treated tissues and was substantially different for roots and leaves. In the case of roots, Cd was evenly distributed between <500 and >10,000 mol wt components, while >90% of the Cd in control leaves was associated with >500 mol wt component.

Distribution of Cd in Soybean Seeds. Mature seeds produced in remobilization studies (Table I) were fractionated to determine Cd distributions. The concentrations of Cd in the seed hull, embryo, and cotyledons were similar, although, on a percentage basis, the embryo, hull, and cotyledons contained 2, 11, and 87% of the Cd, respectively (Table IV). This distribution between seed components is similar to that reported previously for Ni (5). Solvent fractionation of the cotyledon to remove soy oil showed >99% of the Cd to be associated with the defatted flakes. Subsequent extraction and fractionation of the defatted flakes showed the residue (nonwater soluble), soy proteinate (water-soluble and

Table IV. *Distribution of Cadmium in Seed Components and Soy Products from Soybean Plants Grown Hydroponically*

	Distribution of Cadmium[a]		
	μg Cd	*μg/g freeze dry wt*	%
Seed component[b]			
Seed hull	1.67 ± 0.24	0.44 ± 0.02	11.0 ± 1.6
Embryo	0.33 ± 0.016	0.30 ± 0.01	2.2 ± 0.05
Cotyledon	13.14 ± 0.27	0.36 ± 0.01	86.8 ± 1.8
Cotyledon fraction[c]			
Soy oil	0.005 ± 0.001	0.004 ± 0.001	0.4 ± 0.1
Defatted flakes	1.21 ± 0.01	0.38 ± 0.002	99.6 ± 0.1
Soy proteinate	0.36 ± 0.05	0.23 ± 0.05	32.5 ± 4.2
Residue	0.20 ± 0.01	0.13 ± 0.01	17.8 ± 0.1
Soy whey	0.55 ± 0.04	0.54 ± 0.02	49.6 ± 4.0

[a] Mean of two replicate samples.
[b] Forty-g seed separated into components.
[c] Soy oil and defatted flakes expressed as percentage of cotyledon content; proteinate, residue, and soy whey expressed as percentage of defatted flakes; 3 g cotyledon fractioned.

Table V. *Distribution of Cadmium in Soy Whey following Ultrafiltration*

Mol Wt Fraction	Distribution[a]	
	μg Cd	%
>10,000	0.120 ± 0.014	21.9 ± 2.6
10,000 to 1,000	0.073 ± 0.035	13.2 ± 6.4
1,000 to 500	0.175 ± 0.046	31.8 ± 8.4
<500	0.183 ± 0.003	33.2 ± 0.6

[a] Mean of two replicate samples of soy whey obtained from 3 g seed.

acid-precipitable fraction), and soy whey (water-soluble and not acid-precipitable) to contain 18, 32, and 50% of the Cd, respectively. In comparison, Casterline and Yip (7) have shown 80% of the Cd in soybean seeds to be bound to protein of >50,000 mol wt when dry seeds are homogenized and solubles subjected to gel permeation chromatography. In either case, substantially more Cd is associated with the acid-precipitable protein fraction (32%) than the 8% reported for Ni (5).

Further fractionation of the soy whey, which contains acid-nonprecipitable protein, sugars, amino acids, phenolics, and other minor constituents, was performed using ultrafiltration (Table V). Sixty-five percent of Cd was associated with components with mol wt of <1,000, while 22% was associated with acid-nonprecipitable protein of >10,000 mol wt. Whether the observed behavior of Cd is due to complexation by specific ligands or proteins and/or nonspecific binding to plant constituents is unclear. Whatever the mechanism, the observed change in Cd chemical form, especially in the seed fractions which are used as food supplements, may have a dramatic influence on Cd bioavailability to animals and its relative toxicity.

CONCLUSIONS

The distribution and chemical behavior of environmental pollutants, such as Cd in plants, are of increasing importance in understanding, projecting, and limiting the dietary intake of potentially harmful elements. In the present study, although Cd is shown to be relatively immobile in soybean plants, with the major fraction of Cd being retained within the root, a substantial fraction of the Cd associated with shoot tissues is remobilized to seed at maturity. The behavior of Cd in xylem exudates and tissues suggests a change in chemical form resulting from complexation with plant-produced ligands involved in transport and metabolism. Similarly, the distribution and form of Cd in soybean seeds and their food products suggests a change in the chemical behavior of Cd following root absorption and metabolism of inorganic Cd. This change in chemical form resulting from plant metabolism will undoubtedly affect the relative bioavailability of Cd with respect to gastrointestinal absorption.

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