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Nickel in Plants

II. DISTRIBUTION AND CHEMICAL FORM IN SOYBEAN PLANTS[1]

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ABSTRACT

The gross tissue distribution, intracellular fate, and chemical behavior of Ni^{2+} in soybean plants (*Glycine max* cv. Williams) were investigated. Following root absorption, Ni was highly mobile in the plant, with leaves being the major sink in the shoots for Ni during vegetative growth. A senescence >70% of the Ni present in the shoot was remobilized to seeds. Fractionation of root and leaf tissues showed >90% of the Ni to be associated with the soluble fraction of tissues; ultrafiltration of the solubles showed >77% of the Ni to be associated with the 10,000 to 500 molecular weight components of both roots and leaves. Chemical characterization of the soluble components (10,000 to 500 and >500 molecular weight) by thin layer chromatography and electrophoresis resolved a number of Ni-containing organic complexes. Major Ni-containing components formed in the root are transported in the xylem stream, and undergo partial modification on deposition in leaves. Nickel accumulated in seeds is primarily associated with the cotyledons. Chemical fractionation of cotyledon components showed 80% of the Ni to be associated with the soluble whey fraction, while 70% of this fraction was composed of Ni-containing components with molecular weight <10,000.

Plants are known to accumulate Ni readily (9), and unlike most nonessential elements, Ni is mobile in plants and accumulated in seeds (9). The unusual behavior of Ni in plants may be a result of its behavior as an analog of Cu and Zn as shown in root transport studies (3). If Ni behaves as an analog (functional or nonfunctional) of essential nutrients in plants, the metabolic potential may exist to alter its chemical form and thereby alter its potential for uptake and toxicity to successive trophic levels. Studies on the toxicological aspects of Ni in animal systems (5, 6) suggest that organonickel compounds and complexes are more toxic than inorganic forms of Ni.

Relatively few studies have been concerned with the form of metals in plant tissues. Investigations of the form of cations such as Fe, Cu, Zn, and Mn in leaf tissues of agronomic species (2, 8) have shown Fe, Cu, and Zn to be present as anionic complexes, while Mn is apparently uncomplexed or present as chemically unstable complexes. Studies on the chemical form of Ni in plant tissues have been confined to leaves of accumulator species (7, 10, 15). These show Ni to exist as low mol wt cationic complexes. Investigations have dealt with the chemical form of trace elements in xylem (1, 12, 13) and phloem exudates (16). These show that ions such as Ca, Fe, and Ni are transported within the xylem as organic complexes while Mn and Zn appear to have complexed forms in the phloem. Tiffin (14) has shown that Ni transported in the xylem fluid of crop plants was present as an anionic complex that had similar electrophoretic behavior in five species. In addition, Tiffin demonstrated that the form of Ni in exudate was dependent on metal concentration in xylem fluid. In tomato, a cationic Ni complex was found in the xylem in addition to the anionic form when physiological levels (<3 μM) in the xylem were exceeded. This latter observation indicates a need for care in defining the fate and chemical form of metals in plants, since the plant-available concentration of trace elements in the environment will generally be low.

Although studies have dealt with specific aspects of the behavior of Ni in plants, none have fully described the fate of Ni following root absorption, vegetative growth, and senescence. The present study describes the over-all behavior of Ni^{2+} in soybean plants by evaluating its gross distribution and fate, and its chemical behavior following root absorption, xylem transport, and deposition in leaves and seeds.

MATERIALS AND METHODS

Plant Culture. Seeds of *Glycine max* cv. Williams were germinated and plants were grown hydroponically as previously described (3). Thirty-two-day-old plants were employed for analysis of Ni distribution and form in leaves and roots, and for collection and characterization of xylem exudate. Plants at early seed filling (94 days old) were used to evaluate distribution and remobilization of Ni during vegetative growth and senescence, and to provide seeds for study of the fate of Ni^{2+} absorbed during seed development.

Nickel Uptake. For evaluation of Ni distribution and remobilization, individual plants were transferred to 1-liter containers containing 1.0 μM $^{63}NiCl_2$ (0.10 μCi of $^{63}Ni^{2+}/\mu$g of Ni^{2+}) in nutrient solution (pH 5.8). Following 24 hr of uptake, roots were rinsed of sorbed or exchangeable Ni^{2+} and placed into fresh nutrient solution for an additional 24 hr or 21 days. Leaf and root tissues employed in fractionation and characterization studies were obtained from 32-day-old plants grown in nutrient solution (pH 5.8) containing 10 μM $^{63}NiCl_2$ (0.50 μCi of $^{63}Ni^{2+}/\mu$g of Ni^{2+}). After 48 hr, plants were transferred to unlabeled nutrient solution for an additional 24 hr.

Collection of Xylem Exudate. Plants were placed on 10 μM $NiCl_2$ (0.05 μCi of $^{63}Ni^{2+}/\mu$g of Ni^{2+}) in nutrient solution for 2 hr to allow time for Ni to enter the xylem, and were decapitated below the primary leaf node. The stem was then tightly fitted with a short piece (2 cm) of gum rubber tubing, the open end of which was fitted with a 1-mm (o.d.) polyethylene tube. Exudate was

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collected in a cooled vial (4 C) for 4 hr, assayed, and ^{63}Ni-containing components characterized.

Fractionation of Leaf and Root Tissues. Leaf and root tissues were cut into ~5 mm sections, placed into 0.2 M ammonium acetate buffer at pH 6.9 (7.5 ml/g tissue), and homogenized three times for 45 sec, using a Sorvall Omni-Mixer[2] (setting 10). All procedures were performed at 4 C. The homogenate was filtered through a single layer of 20-μm nylon filtration cloth and liquid 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 less than 5% of the total material recovered, was combined with the first filtrate and centrifuged at 25,000g for 15 min. The resultant supernatant solution, referred to as the soluble fraction, was employed in subsequent characterization studies as described below. The insoluble pellet fraction (organelles) was resuspended, recentrifuged, and analyzed for radioactivity.

For comparative purposes, control tissues were carried through both the tissue fractionation and characterization procedures. These were produced by adding a level of $^{63}Ni^{2+}$ (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 hr for equilibration, and carrying these through the entire fractionation procedure. This provided a basis for comparing the relative affinity of Ni^{2+} 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 membranes resulted in fractionation of the soluble components into >10,000, <10,000, and <500 equivalent mol wt fractions (based on globular proteins). From the ^{63}Ni activity contained in retentate and filtrate, and their respective volumes, the distribution of Ni 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, dehulled, and embryos separated from cotyledons. Dehulled seeds, ground to 40 mesh, were fractionated by a modification of the method of Rackis *et al.* (11). Freeze-dried, dehulled seeds (3 g) were extracted twice with 15 ml of *n*-hexane for 2 hr at room temperature. The solutions were centrifuged at 20,000g 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 of H_2O (pH 7.0) for 3 hr 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 20,000g for 10 min. The pellet (soy curd) was washed once, recentrifuged, and the wash combined with the original supernatant solution (soy whey). The soy curd was resuspended, neutralized to pH 7.0 with KOH, forming soy proteinate. The soy whey was further fractionated by ultrafiltration using Amicon 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.

Characterization of Ni Form. Plant fractions containing sufficient ^{63}Ni activity were characterized using one-dimensional TLC followed by TLE[3] perpendicular to the solvent front of chromatography (fingerprint analyses). Chromatographic and electrophoretic separations were performed using precoated cellulose plates (Brinkmann, CEL-300-10 UV254). The TLC solvent system was ethanol-25% ammonium hydroxide-H_2O (726:182:92, v/v/v). Electrophoresis was performed using a pyridine-acetic acid-H_2O buffer (100:3.5:1897:, v/v/v) (pH 6.9) 400 v for 30 min. Components containing ^{63}Ni were visualized using autoradiography.

Radioanalysis of ^{63}Ni. Tissues containing Ni were digested in 10 M HNO_3 and the ^{63}Ni analyzed by liquid scintillation spectrometry (3). Aqueous extracts containing solubles were assayed directly, with appropriate quench correction.

RESULTS AND DISCUSSION

Distribution and Remobilization of Ni in Intact Plants. The concentration and distribution of Ni in plants are dependent on many factors (9) including plant species. Nickel, unlike many nonessential trace elements, is accumulated in both leaves and seeds (4). This would suggest that it persists in the plant as a chemically stable, soluble species, which like some nutrilites, may be remobilized from vegetative tissues to seeds. The fate of Ni in maturing soybean plants was determined by providing a 24-hr pulse of ^{63}Ni to 94-day-old plants, with subsequent evaluation of its distribution and tissue concentration after an additional 24 hr and 21 days (Table I). Twenty-four hr following feeding, 85% of the Ni accumulated by the plants (~7.0 μg) was retained by the roots, with the remaining Ni distributed in shoot tissues. After 21 days, there was a marked reduction in Ni content of roots and a concomitant increase in seed content (38%). As the 94-day-old plants were at an early stage of seed filling and the 115-day-old plants (21st day) were at physiological maturity (yellowing of pods) with little increase in vegetative growth, the change in tissue concentrations of Ni over the 21-day period suggests a remobilization of Ni from vegetative tissues to seeds. Since this cultivar is an indeterminant type, it is difficult to show the progression of events occurring with respect to Ni concentration in developing, mature, and senescencing leaves over the 21-day period on a whole plant basis. Analysis of Ni in leaves at various stages of leaf development shows a gradual increase in Ni concentration during growth followed by a subsequent decrease during the senescence process (unpublished data).

Table I. Distribution of nickel, with time following 24-hr uptake from 1.0 µM $NiCl_2$ by 94-day-old soybean plants.

Tissue	Distribution Following $^{63}Ni^{2+}$ Pulse: 24 hr (%)[1]	21 day (%)[1]	Change in Tissue Concentration (ng Ni/g dry wt tissue)	Change in Dry Weight (g)
Roots	85.3 ± 1.7	50.7 ± 2.3	-126.1	+ 0.31
Stems	5.2 ± 0.2	3.7 ± 0.6	- 7.7	- 0.40
Leaves	6.2 ± 0.5	4.3 ± 1.0	- 6.8	+ 3.18
Pods	1.8 ± 0.1	2.5 ± 0.6	- 1.1	+ 2.53
Seeds	1.5 ± 0.3	38.8 ± 1.1	+ 65.2	+24.92

[1]Mean ± standard error for two replicate samples.

Table II. Distribution of nickel in various tissue fractions following homogenization and centrifugation.

Thirty-two-day-old soybean plants supplied with a 24-hr pulse of 10µM $NiCl_2$, tissues were fractionated after an additional 24 hr.

Fraction	Treated Plant (%)[1]	Control Plant
Roots		
Insoluble Residue[2]	7.6 ± 1.4	5.3
Soluble Fraction[3]	91.9 ± 1.1	93.7
Pellet Fraction[4]	0.5 ± 0.3	1.0
Leaves		
Insoluble Residue[2]	5.9 ± 0.1	1.5
Soluble Fraction[3]	93.5 ± 0.1	95.7
Pellet Fraction[5]	0.6 ± 0.3	2.8

[1]Mean ± standard error of two replicate samples for treated plants, single replicate for control.
[2]Primarily cell wall material.
[3]Soluble cytoplasm and readily exchangeable compounds.
[4]Primarily chloroplasts and mitochondria.
[5]Primarily mitochondria and vesicles.

[2] Brand names are used to assist the reader in replicating the experiment, and use does not constitute endorsement by Battelle Memorial Institute.
[3] Abbreviation: TLE: thin layer electrophoresis.

The mobility of Ni in plants, and its potential for remobilization would indicate that the plant has the metabolic capability to handle Ni, possibly as a functional or nonfunctional analog of Cu or Zn (3). If so, the metabolic potential may exist to alter the chemical form of Ni in plants substantially and thereby alter its potential for toxicity to successive trophic levels.

Fractionation of Ni-containing Leaf and Root Tissues. The distribution of Ni contained within root and leaf tissue was determined using 32-day-old soybean plants grown for 24 hr in solutions containing 10 μM $NiCl_2$ (0.59 μg ^{59}Ni/g). Tissues were fractionated after an additional 24 hr had elapsed (Table II). Of the 210 μg of Ni absorbed, 94% was associated with the roots and 4.5% was contained in leaves. Following fractionation of leaves and roots, >90% of the Ni was associated with the soluble fractions; with <1% associated with organelles, and <8% of the Ni contained in the insoluble residue. This suggests that Ni may exist primarily as inorganic Ni^{2+} or as soluble organic complexes. The gross distribution of Ni in control plants approximated that of the treated plants (Table II), indicating similar over-all affinities of these fractions for Ni irrespective of the presence or absence of metabolic activity or route of entry. Similar over-all distributions have been shown following fractionation of leaves of Ni accumulator species (7) and leaves of several tree species growing on high Ni content soils (15), except that 5 to 10% of tissue Ni was associated with organelles in both of these studies. Differences in the content of the organelle fraction may represent species differences or a kinetic effect with respect to metabolic incorporation.

The soluble fractions of both treated and control tissues were further fractionated by ultrafiltration using membranes with nominal equivalent mol wt exclusions of 500 and 10,000. The <500 mol wt fraction contained primarily inorganic Ni^{2+} or very low mol wt complexes, since the UM-05 membrane has a high rejection factor for linear carbon compounds with mol wt of 165 to 500. The mol wt distribution serves only to demonstrate gross differences in distribution of Ni-containing components. On this basis, approximately 8% of the extractable Ni in roots and 2% in leaves may exist as inorganic Ni^{2+} (<500 mol wt fraction) with the remainder existing as complexed forms of Ni (Table III). Control leaf tissues show similar mol wt distributions while the distribution differs for root components. The low concentration of Ni in <500 mol wt fraction of control tissues indicates that Ni^{2+} is readily complexed by cellular metabolites. While it could be argued that similarities between treated and control tissues may result from a chemical redistribution of Ni^{2+} during fractionation and would not be characteristic of *in vivo* behavior, primary emphasis was on stable Ni complexes, which at least for the soluble fraction could be resolved by comparative chromatographic analysis of root and leaf fractions with xylem transport forms.

Characterization of Ni Form in Roots, Leaves, and Xylem Water. Nickel-containing components in the <500 and <10,000 mol wt fractions of roots were characterized using TLC and TLE procedures. In addition, xylem exudate was collected from 32-day-old plants grown under a similar set of experimental conditions. Insufficient activity was present for analysis of the <500 mol wt fraction of leaf tissue.

A comparison of fingerprints for the <500 mol wt extracts of treated and control roots (Fig. 1) shows at least two components (C, G, and possibly F) to be common to both treated and control fractions. An additional major Ni-containing component (I) and several minor components were peculiar to the treated samples. Inorganic Ni was not detected (N denotes position of Ni^{2+}) in either the treated or control samples, suggesting that the concentration of complexing ligands in plant roots was sufficient to complex the Ni^{2+} rapidly and effectively.

Inasmuch as >75% of the extractable Ni in roots and leaves was associated with the <10,000 mol wt fraction, an attempt was made to determine whether Ni-containing components in the roots have a metabolic role. This was accomplished by a comparative analysis of specific Ni-containing components in roots (storage and transport), xylem exudate (transport), and leaves (storage and/or metabolism). A comparison of these plant fractions (Fig. 2, plates a, c, and d) shows three (components A, B, and C) of the four major Ni-containing components of the root to be in xylem exudate, thereby representing transport forms of Ni. As components A, B, and C were transferred to leaves, there appeared to be a reduction in the content of component B, an apparent loss of component C, with a concomitant production of a new component, D. The comparative behavior of treated leaf, root, and exudate fractions would indicate that redistribution of Ni^{2+} during fractionation

Table III. Distribution of nickel in the soluble fraction of homogenates following ultrafiltration

Tissue homogenates obtained from 32-day-old soybean plants grown on 10μM $NiCl_2$ for 24 hr.

Molecular Weight Fraction	Treated Plant [1]	Control Plant
	---------- % ----------	
Root		
>10,000	15.3 ± 4.2	43.2
10,000-500	77.1 ± 5.4	53.3
<500	7.6 ± 1.2	3.5
Leaves		
>10,000	18.6 ± 2.8	20.9
10,000-500	79.0 ± 2.9	74.5
<500	2.4 ± 0.2	4.5

[1] Mean ± standard error of two replicate samples for treated plant, single replicate for control.

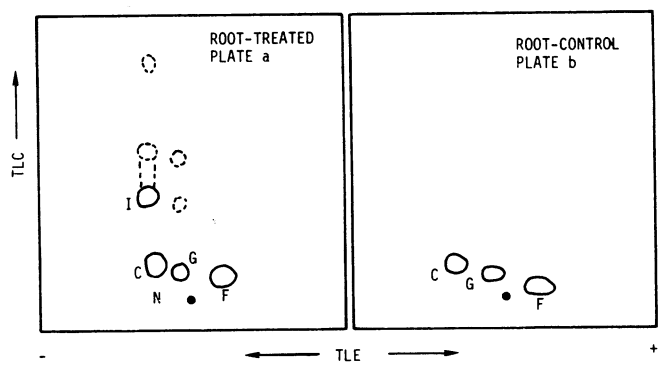

FIG. 1. Fingerprint of Ni-containing components in <500 mol wt fraction of treated and control root tissues. Common letters denote components having similar chromatographic and electrophoretic behavior.

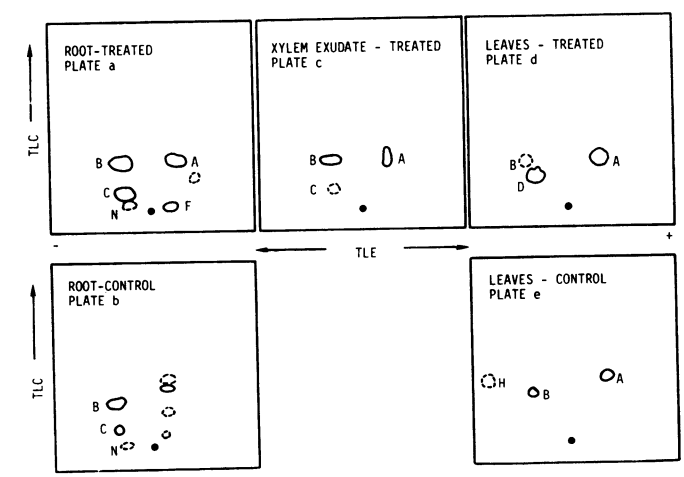

FIG. 2. Fingerprint of Ni-containing components in the <10,000 mol wt fraction of treated and control roots and leaves, and xylem exudates. Common letters denote components having similar chromatographic and electrophoretic behavior.

may not represent a serious problem at least for the major Ni^{2+}-containing components identified (A, B, C, and D). A comparison of treated tissues and their respective controls (plates a, b, d, and e) indicates that major components A and F were not present in the <10,000 mol wt fraction of control roots, while component D was absent in control leaves.

While component F was present in the <500 mol wt fraction of treated and control roots (Fig. 1), its absence in the <10,000 mol wt fraction of control roots may result from concentration being below detection limits. The comparative behavior of Ni complexes in root, leaf, and xylem exudate suggests that with the exception of component D (Fig. 2, plate d), these ligands are normal components of the plant and not produced in response to Ni^{2+}. Component D appears to be a secondary metabolic product or storage form peculiar to the treated leaves. A comparison of chromatographic data for roots, xylem exudate, and leaves (Fig. 2, plates a, c, and d) provides a basis for understanding the metabolic behavior of specific Ni ligands formed following root absorption, their role in xylem transport, and their ultimate fate in leaves. Although inorganic Ni^{2+} was not detected in the <500 mol wt fraction of roots (Fig. 1), small quantities were detected in root samples of the <10,000 mol wt fraction. This inorganic Ni may result from dissociation of weaker complexes of larger mol wt compounds (not present in the <500 mol wt fraction), during chromatography and/or electrophoresis.

The present study demonstrates the presence of both cationic and anionic Ni complexes in leaves and roots (Figs. 1 and 2); this contrasts with the work of Kelley *et al.* (7) and Timperly *et al.* (15) which indicated that Ni^{2+} existed in cationic complexes in Ni-accumulating species. This suggests that the chemical fate of Ni^{2+} in plants may be species dependent. Tiffin (14) has demonstrated the presence of a single anionic Ni complex in the exudates of tomato, cucumber, corn, carrot, and peanut when Ni^{2+} concentrations in exudates are within physiological limits for the plant. When Ni^{2+} concentrations exceeded 1 to 3 μM for tomato and corn, a minor cationic complex began to appear in addition to the anionic form, suggesting limited concentration of the anionic carrier. The cationic complex was not present in carrot, cucumber, or peanut when Ni concentrations were increased. In the present study, both tissues and exudates were obtained from plants using feeding solutions of 10 μM Ni, which are well within the physiological range for root absorption (3). The concentration of Ni in xylem exudate of soybean was 3.9 μM, well below the 11 to 14 μM Ni reported by Tiffin (14) to induce formation of substantial amounts of the cationic Ni complex in corn exudate. This suggests that both cationic and anionic Ni complexes were formed in soybean following root absorption, with similar complexes being transported in the xylem to the leaves, and subsequent modification of the cationic form in the leaves.

Distribution of Ni in Seed Components. Seeds produced in the distribution and remobilization study were fractionated to determine the fate of Ni using fractionation procedures routinely employed in the commercial processing of soy products. The highest Ni concentration was found in the embryo with the cotyledons and the hull comparable on a dry weight basis (Table IV). The seed components, consisting of hull, embryo, and cotyledons, contained 8.5, 4.0, and 87.5% of the total Ni in the seed (81 μg), respectively. Solvent extraction of cotyledon material with *n*-hexane (soy oil) shows 95% of the Ni to be associated with the defatted flakes. Extraction of the defatted flakes with water, followed by acid precipitation, shows the protein fraction to contain 7% of the Ni. The soy whey, consisting of acid-nonprecipitable protein, sugars, amino acids, phenolics, and other minor constituents, contains 82% of the Ni.

Since the soy whey contained the major fraction of Ni in the seed, a mol wt fractionation was performed to determine the extent of Ni complexation in the soluble whey fraction. Ultrafiltration of the whey fraction (Table V) showed only 18% of Ni to be associated with the <500 mol wt fraction. Nickel in this fraction may be in the form of inorganic Ni^{2+} or very low mol wt complexes (<250). These data, on the distribution of Ni in seed fractions, as in the case of root and leaf tissues, indicate that Ni persists in the plant primarily as complexed organic forms.

Table IV. Distribution of nickel in seed components and soy products from soybean plants grown hydroponically.

	Distribution of Nickel	
	µg/g dry wt	%
Seed Component		
Seed hull	2.21	8.5 ± 3.1
Embryo	3.95	4.0 ± 0.2
Cotyledon	2.48	87.5 ± 3.0
Cotyledon Fraction[1]		
Soy oil	0.54	4.6 ± 1.0
Defatted flakes	3.23	95.4 ± 1.1
Soy proteinate	0.59	7.6 ± 1.0
Residue	0.54	5.5 ± 1.9
Soy whey	10.32	82.3 ± 3.9

[1]Soy products expressed as % of cotyledon content; proteinate, residue and soy whey expressed as % of defatted flakes.

Table V. Distribution of nickel in soy whey following ultrafiltration.

Molecular Weight Fraction	Distribution %[1]
>10,000	29.0 ± 0.3
10,000-1,000	28.0 ± 3.9
1,000-500	25.5 ± 0.4
<500	17.5 ± 3.9

[1]Mean ± standard error for two replicate samples.

CONCLUSIONS

The behavior of specific Ni-containing components with respect to storage forms in roots and leaves, and their presence as transport forms would suggest that the plant has metabolic mechanisms for maintaining the solubility and mobility of inorganic ions. The behavior of Ni^{2+} may be regulated by mechanisms utilized for nutrient ions such as Cu^{2+} and Zn^{2+}, since Ni^{2+} has been shown to be a competitor for root transport sites employed for both Cu^{2+} and Zn^{2+} (3).

While a knowledge of the chemical behavior of nonessential elements such as Ni^{2+} in plants is necessary in understanding its involvement in the metabolic processes of plants, there are indirect implications with respect to man. If plants possess the potential for metal modification and can alter the bioavailability of metals along the food web, there are implications with respect to health affects. Although the chemical behavior of Ni following root absorption of inorganic forms of Ni^{2+} by soybean plants has been evaluated, many questions remain. In the environment, the plant root is undoubtedly confronted with other than inorganic forms of metals. How does the plant respond to a complexed Ni species resulting from microbial activity? Do these ligands dissociate prior to uptake or are they absorbed intact? If absorbed intact, the chemical form of Ni in the plant will likely differ from that resulting from uptake of the inorganic ion.

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