Rec'd 11/6/1978

0107
Cd
Plant

Plant and Soil 52. 393—402 (1979) Ms. 3915

ORGANIC MATTER INFLUENCES PHYTOTOXICITY OF CADMIUM TO SOYBEANS

by RICHARD C. STRICKLAND, WILLIAM R. CHANEY and ROBERT J. LAMOREAUX

Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana 47907

KEY WORDS

Cadmium Organic matter Soybeans

SUMMARY

Soybeans (*Glycine max* L. var. Williams) were grown for six weeks in a greenhouse in quartz sand containing 0, 0.5, 1, 2, 4 or 8% (w/w) sterilized peat moss. The cation exchange capacities of the organic matter-sand (OM-S) mixtures ranged from 0.01 to 8.88 meq/100 g dry weight. Imposed on each OM-S mixture was a treatment of 0, 1.25, 2.5, 5.0, 10.0 or 20.0 ppm Cd applied as $CdCl_2 \cdot 2\frac{1}{2}H_2O$. Height growth was measured weekly and at harvest plants were separated into leaves, stems and roots for dry weight and tissue Cd determinations. For plants grown in sand alone, height growth and dry matter accumulation in all tissues were reduced and Cd content was increased. These effects were correlated with increasing Cd concentration in the rooting medium. Inhibitions in growth by Cd were reduced by addition of organic matter; the amount of alleviation was dependent on both the level of organic matter and the cadmium treatment. In the 0, 0.5 and 1% OM-S mixtures, Cd content in the various tissues was correlated with metal treatment. Tissue levels were markedly reduced for Cd treatments in the 2, 4 and 8% OM-S mixtures, although there was a positive correlation between tissue Cd and the 1.25 and 2.5 Cd treatments. The order of Cd accumulation in the tissues was roots $\gg$ stems $>$ leaves.

INTRODUCTION

This study was part of a large interdepartmental project to study the environmental flow of cadmium and other trace elements in a relatively undisturbed site in the industrialized area of East Chicago, Indiana, U.S.A., and to determine the effects on plants of heavy metals which impact the site[13]. Soils at the site are deep, medium to fine sands with little profile development, contain 2–3% organic matter in the surface 3–4 inches, have a cation exchange capacity of approximately 12 milliequivalents per 100 grams in the A horizon, and contain an average of 10 ppm Cd in the upper 2.5 cm of the soil[11,13].

It is well established from numerous greenhouse pot studies that Cd has a

9259

marked effect on growth and development of a wide variety of plants grown in sand culture with incorporated $CdCl_2$. Reductions in growth of both top and root systems are very dramatic and usually result from concentrations of Cd one-half to one-quarter of that monitored at the East Chicago site[2,4,9,14]. However, plants grown in soil from the East Chicago site apparently are affected very little. Possible explanations for this lack of toxicity of Cd on the East Chicago Site are: 1) the presence of Cd in an insoluble form (*i.e.*, CdO, $Cd(OH)_2$, $CdCO_3$) or 2) the binding of Cd to cation exchange sites with a concomitant reduced availability. We have unpublished results which show that CdO affects growth of soybeans noticeably less than the highly soluble $CdCl_2$ and $CdSO_4$. The objective of the study reported here was to investigate the second possibility above and to determine if organic matter binds Cd, thereby reducing its detrimental impact on plant growth.

METHODS

Soil mixtures containing 0, 0.5, 1, 2, 4 or 8 percent organic matter (OM) were prepared (w/w basis) from sterilized shredded peat moss (passed through a 2 mm soil sieve) and white silica sand. Each organic matter-sand (OM-S) mixture for each of the five replications was prepared individually. The OM and sand were placed in a polyethylene bag and thoroughly mixed before placing the bag inside the 18 cm diameter pots (1400 cm^3). The polyethelene bag served as a pot liner. Cation exchange capacity (CEC) and quantities of exchangeable Ca^{2+}, Na^+ and K^+ for the various OM-S mixtures were determined by the Purdue University Soil Testing Laboratory using ammonium acetate extraction and subsequent atomic absorption analysis. The extractable acid fraction of exchange capacity was determined using the $BaCl_2$ and triethanolamine method[1]. The laboratory also determined pH from a 1 : 1 OM-S and water mixture. Table 1 summarizes the chemical and physical properties of the OM-S mixtures.

Imposed on each OM-S combination was a treatment of 0, 1.25, 2.5, 5, 10 or 20 ppm Cd applied as $CdCl_2 \cdot 2\frac{1}{2}H_2O$ based on the weight of the sand; 2400 g used in pots containing no organic matter. Cadmium treatments were applied to the surface of pots in sufficient deionized water to just saturate the soil, and after treatments all OM-S mixtures were allowed to equilibrate with the added Cd for 24 hours before planting.

Table 1. Physical and chemical characteristics of organic matter and sand mixtures

% Organic matter (w/w)	Water pH	Base sat.	Milliequivalents /100 g dry weight					
			Ca	Mg	K	Na	Ext. acid	CEC (sum)
0	6.4	100	0.01	0.0	0.0	0.0	0.0	0.01
0.5	5.9	93.9	0.25	0.09	0.01	0.01	0.02	0.38
1	5.9	91.7	0.60	0.16	0.01	0.05	0.08	0.90
2	5.7	67.4	0.85	0.18	0.01	0.00	0.50	1.54
4	5.6	68.9	1.95	0.41	0.01	0.07	1.10	3.54
8	5.2	61.8	4.46	0.95	0.01	0.07	3.39	8.88

Soybeans (*Glycine max* L. var. Williams) were planted three/pot and after emergence thinned to one/pot. Plants were fertilized weekly with half-strength Hoagland's solution and watered as needed with deionized water. Each pot had holes punched through the plastic bag and was placed on 25 cm^2 of aluminum foil fashioned to collect any leachate. While some of the leachate undoubtedly evaporated, the majority was reabsorbed into the pot. Occasionally plants were watered from below to solubilize any cadmium and nutrients for reabsorption.

Height was measured weekly to the nearest half centimeter beginning one week after seedling emergence. Six weeks after planting or after five measurement periods, plants were harvested and separated into leaves, roots and stems.

Roots were thoroughly rinsed with tap water to remove adhering sand and/or organic matter. Tissues were dried at 70°C prior to dry weight determination and then ground in a Wiley mill to pass a 20 mesh screen. Leaves, stems or roots for all replications of each OM-S and Cd combination were pooled for Cd analysis by atomic absorption following nitric acid digestion. There was no replications of the Cd analyses. Pooling of the replicates was necessary to provide enough tissue for analysis in some of the treatments.

The experimental design of this study was a randomized complete block with five blocks each containing 36 different OM-S and Cd combinations. Growth and dry weight data were examined by analysis of variance with blocks, OM concentration and Cd treatment considered fixed effects. Main effects of Cd and OM were examined using all the data while significant interactions between OM and Cd were more clearly investigated, because of the large number of treatment combinations, by holding one factor constant and examining variation in the other factor.

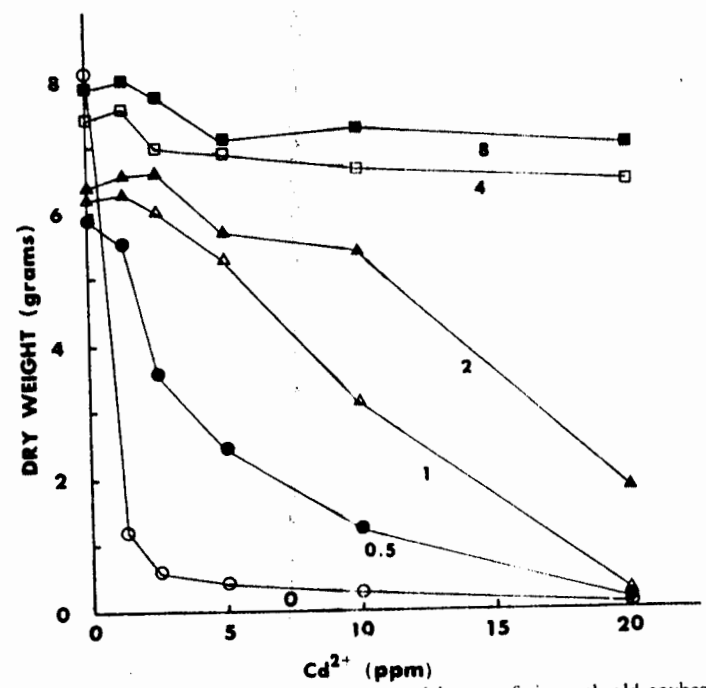

Fig. 1. Combined dry weight (g) of roots, stems and leaves of six-week-old soybean seedlings grown in organic matter-sand mixtures treated with various concentrations of Cd. Each point is the mean of five replications. Percent organic matter (OM) in the rooting medium is shown on the graph.

RESULTS

Dry weight of leaf, stem and root tissues were all markedly reduced by even the lowest concentration of Cd applied to pure sand. Since reductions in dry weight of the roots, stems and leaves were proportionally similar even though they differed in absolute amounts, only the summation or the total dry weight of the seedlings is shown in Fig. 1. The drastic reduction in dry weight of the seedlings at 1.25 ppm Cd was significantly lacking in seedlings grown with 0.5% or greater amounts of OM. The relationship between OM content and toxicity of Cd was very quantitative. For example, when 20 ppm Cd was incorporated into the rooting medium, 2% OM only slightly ameliorated the detrimental impact on growth whereas 4% OM completely eliminated the effect of Cd. However, 2% OM was effective in eliminating the growth impact of 10 ppm of applied Cd.

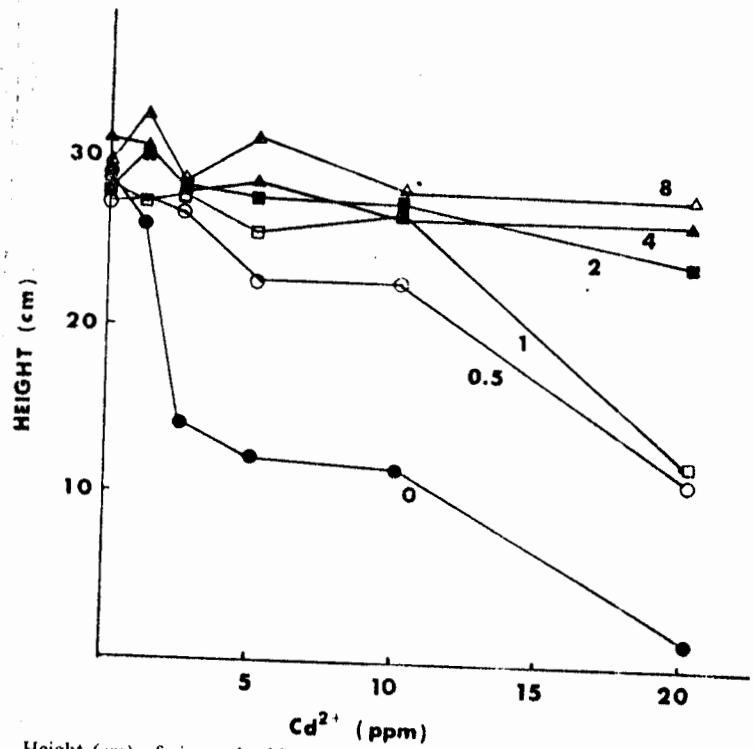

Fig. 2. Height (cm) of six-week-old soybean seedlings grown in OM-S mixtures treated with various concentrations of Cd. Each point is the mean of five replications. Percent OM in the rooting medium is shown on the graph.

Relationships established after the first week between seedling height, Cd treatment and OM content of the rooting mixtures were retained throughout the experiment and were similar after six weeks of growth. Height of seedlings was affected by Cd, but the reduction was mitigated by OM (Fig. 2). For example, the markedly reduced height growth of six-week-old seedlings grown in pure sand with 2.5 ppm Cd was not evident in seedlings grown in sand and 0.5% or higher OM. The detrimental effect of 20 ppm Cd was overcome by 2% OM.

Analysis of variance showed that height and dry weight of all components (leaf, stem, root and total) were significantly ($P < 0.01$) influenced by both OM and Cd. The main effect of OM becomes even more evident if the data are averaged over all Cd treatments for OM effects (Table 2). The reverse could also be done; average over all OM treatments for Cd effects. It is clear from Table 2 that height growth and dry weight accumulation in root, stem and leaf tissues of six-week-old soybeans were all improved by increasing amounts of OM in the rooting medium. The interaction between OM and Cd also was highly significant ($P < 0.01$).

Tissue concentrations of Cd (μg/g dry weight) for each Cd-OM treatment are shown in Figures 3–5. A gradient of concentration was evident with roots accumulating the greatest amount of Cd and leaves the least with stems intermediate. Concentrations of Cd in roots were about an order of magnitude greater than that in stems and leaves. No determinations of Cd content were made for seedlings grown in the 20 ppm Cd – 0% OM treatment because the seedlings were so small that enough tissue was not available for analysis. Increasing amounts of OM markedly reduced the concentration of Cd in the tissues. Two percent OM appeared to be a threshold level for the concentrations of Cd applied in this study since tissue concentration in seedlings grown in

Table 2. Organic matter effects over all cadmium treatments*

% Organic matter	Height (cm)	Dry weight (g)			
		Leaf	Stem	Roots	Total
0	15.6 a	.84 a	.35 a	.65 a	1.81 a
0.5	22.9 b	1.47 b	.77 b	.92 b	3.17 b
1	24.5 c	2.15 c	1.04 c	1.39 c	4.58 c
2	27.7 d	2.59 d	1.20 d	1.67 d	5.46 d
4	28.5 d	3.40 e	1.51 e	2.02 e	6.94 e
8	29.8 e	3.84 f	1.68 f	2.00 e	7.51 f

* Each mean based on 30 observations. Means within columns followed by different letters are significantly different ($P < 0.05$) by the Newman-Keuls sequential range test.

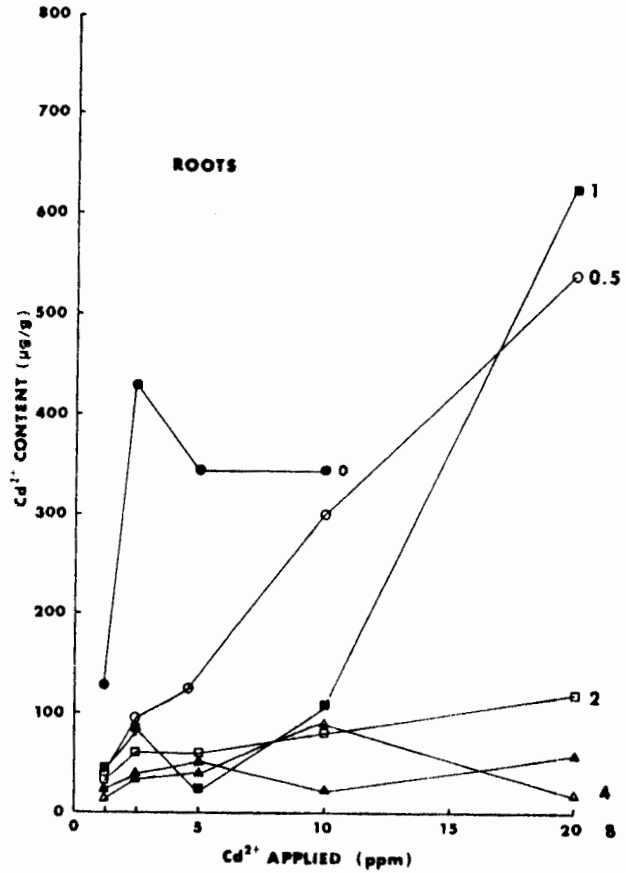

Fig. 3. Cd^{2+} content (µg/g) in roots of six-week-old soybean seedlings grown in organic matter-sand mixtures treated with various concentrations of Cd. Percent organic matter (OM) in the rooting medium is shown on the graph.

ppm Cd were similar to that in untreated seedlings if 2% OM was present in the rooting medium. Four and 8% OM further reduced tissue Cd content. The aberrant concentrations measured in root tissue of seedlings grown in pure sand with 5 and 10 ppm Cd (Fig. 3) can only be explained as experimental error. It is very interesting and noteworthy that if Cd in the various tissues over all the treatments is expressed in terms of absolute amounts of Cd (µg) rather than µg/g of tissue, the differences evident in Figures 3–5 are minimized or even reversed. For example, in treatments with 0% OM and 10 ppm Cd, leaves contained

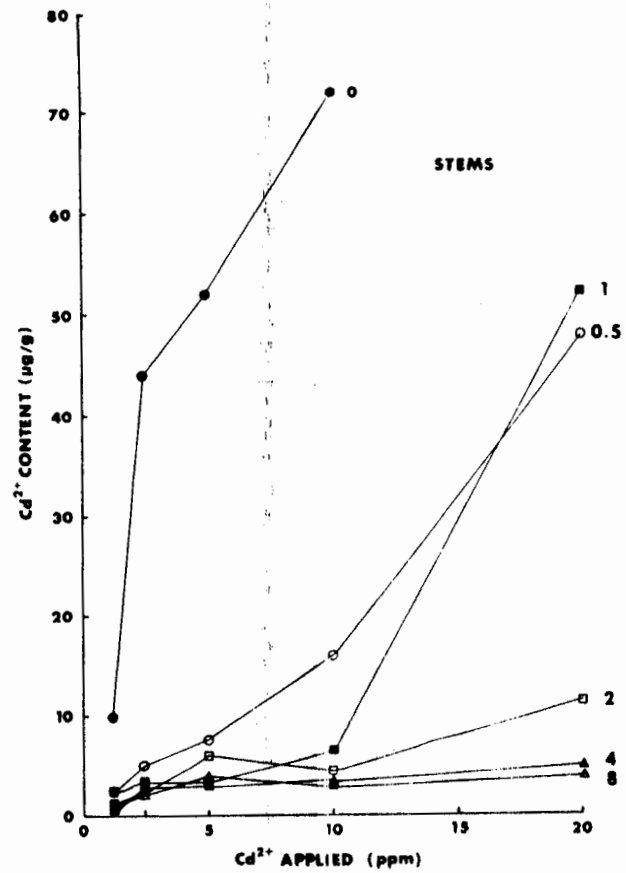

Fig. 4. Cd^{2+} content (µg/g) in stems of six-week-old soybean seedlings grown in organic matter-sand mixtures treated with various concentrations of Cd. Percent organic matter (OM) in the rooting medium is shown on the graph.

approximately 2 µg Cd whereas leaves from seedlings grown in 10 ppm Cd with 8% OM contained approximately 6.8 µg Cd. Obviously the greater biomass of leaf tissue diluted the absorbed metal and a toxic concentration was not attained in the seedlings. The greater biomass of tissue accumulated only in those treatments which apparently moderated the availability of applied Cd by binding it in some manner in the rooting medium. Determination of cation exchange capacity of the 0, 0.5, 1, 2, 4 and 8% OM–sand mixtures showed 0.01, 0.38, 0.90, 1.54, 3.54 and 8.88 milliequivalents per 100 grams, respectively (Table 1).

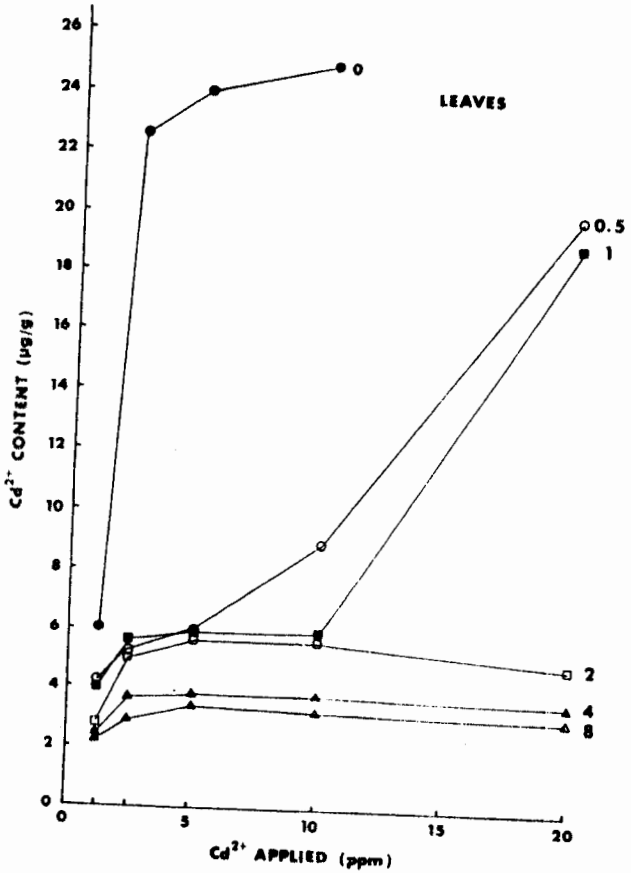

Fig. 5. Cd^{2+} content (µg/g) in leaves of six-week-old soybean seedlings grown in organic matter-sand mixtures treated with various concentrations of Cd. Percent organic matter (OM) in the rooting medium is shown on the graph.

Mitigation of availability of applied Cd to seedling roots and the dramatic effect of OM on growth possibly were due to the binding of Cd to the exchange sites. Theoretically, because of its high molecular weight and valence, cadmium should have a strong affinity for cation exchange sites on the peat.

DISCUSSION

A similar gradient of decreasing Cd content from roots to leaves found in this study also has been reported for seedlings of honeylocust[8] and red oak[10].

Haghiri[4] applied radioactive cadmium to soybean leaves and determined its translocation into other tissue. The percent of applied label in plant parts decreased in the following order; stems > leaves > pods > beans. Since walls of vessels and tracheids are negatively charged, they may act much as cation exchange sites in soils and this phenomenon may explain the gradient in concentration observed. An exception to this pattern of distribution has been reported in radish which shows greater accumulation of Cd in the leaves than in the roots[7]. A similar accumulation of Cd in leaves has been shown for quaking aspen[13].

This study demonstrates the importance of soil organic matter in reducing the growth impact of Cd. The apparent complexing and slow release of Cd to root systems alleviates its toxicity even though the absolute amount absorbed may be greater in the larger and more vigorous plants. This is probably a result of dilution of the absorbed Cd in a large biomass of plant tissue. Severe stunting of growth and toxicity occurs when the Cd in the rooting medium is readily available in the soil solution or when the concentration exceeds the complexing capacity of the soil. Furthermore it seems reasonable that toxicity may occur when the capacity of cell walls or other inert structures to absorb or accumulate Cd is exceeded. Cutler and Rains[3] demonstrated a strong, irreversible binding of Cd by barley roots and suggested that the binding occurred at cell wall exchange sites. They suggested that such binding may reduce phytotoxicity.

Results of this study suggest that the minimal impact on plant growth of 10 ppm Cd in the upper layer of soils in the East Chicago area is partially due at least to the organic matter content (2–3%) of the soil with its associated cation exchange capacity (12 meq/100 g). Similar results have been reported for soils at other sites. John *et al.*[6] showed Cd content of lettuce and radish to be correlated with amounts of exchangeable Cd in the soil rather than to the total amount of Cd added to the soil. Cadmium content of oat shoots also was found to be decreased by increasing the cation exchange capacity of the soil[5]. In a recent study Miller *et al.*[12] reported that Cd uptake by soybeans was correlated with the ratio of cadmium added to the cadmium sorptive capacity of soils.

ACKNOWLEDGEMENTS

This work was supported by the Purdue Agricultural Experiment Station (Journal Paper Number 7476) and a grant (No. GI 35106) from the National Science Foundation – RANN. We gratefully acknowledge the technical assistance of Elizabeth C. Schall.

Received 6 November 1978

REFERENCES

1 Black, C. A. (Ed.) 1965 Methods of Soil Analysis. Pt. 2. Chemical and Microbiologica Properties. Am. Soc. Agron., Inc., Madison, Wisconsin, 1572 p.

2 Chaney, W. R., Strickland. R. C. and Lamoreaux, R. J. 1977 Phytotoxicity of cadmiun inhibited by lime. Plant and Soil **47**, 275–278.

3 Cutler, J. M. and Rains, D. W. 1974 Characterization of cadmium uptake by plant tissue Plant Physiol. **54**, 67–71.

4 Haghiri, F. 1973 Cadmium uptake by plants. J. Environ. Qual. **2**, 93–96.

5 Haghiri, F. 1974 Plant uptake of cadmium as influenced by cation exchange capacity, organic matter, zinc, and soil temperature. J. Environ. Qual. **3**, 180–183.

6 John, M. K., VanLaerhoven, C. J., and Chuah, H. H. 1972 Factors affecting plant uptake and phytotoxicity of cadmium added to soils. Environ. Sci. Technol. **6**, 1005–1009.

7 Lagerwerff, J. W. 1971 Uptake of cadmium, lead, and zinc by radish from soil and air. Soil Sci. **111**, 129–133.

8 Lamoreaux, R. J., Strickland, R. C., and Chaney, W. R. 1978 The plastochron index as applied to a cadmium toxicity study. Environ. Pollut. **16**, 311–317.

9 Lamoreaux, R. J. and Chaney, W. R. 1977 Growth and water movement in silver maple seedlings affected by cadmium. J. Environ. Qual. **6**, 201–205.

10 Levings, M. K. 1977 Effects of cadmium chloride on growth and pigments in *Glycine max* L., *Quercus rubra* L., *Acer saccharinum* L., and *Cucumis sativus* L. M.S. Thesis. Department of Forestry and Natural Resources. Purdue University, West Lafayette, Indiana, 73p.

11 Miles, L. F. 1978 Effects of cadmium and other heavy metals on native plant species: Experimentation and Modeling. Ph.D. Thesis. Department of Forestry and Natural Resources. Purdue University, West Lafayette, Indiana, 231p.

12 Miller, J. E., Hassett, J. J. and Koeppe, D. E. 1976 Uptake of cadmium by soybeans as influenced by soil cation exchange capacity, pH and available phosphorus. J. Environ. Qual. **5**, 157–160.

13 Parker, G. R., McFee, W. W. and Kelly, J. M. 1978 Metal distribution in a forested ecosystem in urban northwestern Indiana. J. Environ. Qual. **7**, 337–342.

14 Turner, M. A. 1973 Effect of cadmium treatment on cadmium and zinc uptake by selected vegetable species. J. Environ. Qual. **2**, 118–119.