Bull. Environ. Contam. Toxicol. (1996) 57:917-923

Bulletin of Environmental Contamination and Toxicology

Metal Uptake by Agricultural Plant Species Grown in Sludge-Amended Soil Following Ecosystem Restoration Practices

J. D. Peles,[1] S. R. Brewer,[2] G. W. Barrett[1]

[1]Institute of Ecology, University of Georgia, Athens, Georgia 30602-2202, USA
[2]Department of Zoology, Miami University, Oxford, Ohio 45056, USA

Received: 12 April 1996/Accepted: 26 August 1996

The disposal of municipal sewage sludge is an important environmental problem presently facing society. Because sludge is rich in plant nutrients such as nitrogen and phosphorous, land application as a fertilizer has been proposed as a cost-effective means of disposal (National Research Council 1996). This method of disposal, however, is frequently the subject of public health concern since municipal sludge may contain heavy metals that potentially could be introduced into the human food chain (National Research Council 1996).

Numerous investigations have documented heavy metal uptake by agricultural plant species grown in sludge-amended soil (e.g., Kelling et al. 1977; Chang et al. 1983; Heckman et al. 1987; Kiemenec et al. 1990). There exists, however, little information regarding the accumulation of metals by agricultural species following an extended period of remediation practices intended to reduce metal uptake. In addition, more information is needed regarding the allocation patterns of metals within plant structures of individual species. This is important because differential uptake by plant structures may influence the likelihood of heavy metals entering natural or domestic food chains.

This investigation examined metal concentrations in two agricultural species at a study site where ecosystem restoration practices (i.e., liming and tilling) were conducted for five years following 11 years of nutrient enrichment (i.e., application of sludge or fertilizer) (Brewer et al. 1994). At this site, increased metal uptake has previously been documented for old-field plant species and organisms at higher trophic levels collected from sludge-treated plots (e.g., Kruse and Barrett 1985; Levine et al. 1989; Brueske and Barrett 1991). Specifically, our purpose was (a) to examine the effects that restoration practices (liming and tilling) have had on concentrations of cadmium, copper, lead, and zinc in soybeans (*Glycine max*) and buckwheat (*Fagopyrum esculentum*) following long-term sludge application and (b) to compare differences in allocation of metals between plant structures within soybeans and buckwheat.

Correspondence to: G. W. Barrett

MATERIALS AND METHODS

This investigation was conducted at the Miami University Ecology Research Center located near Oxford, Butler County, Ohio (39°30'N, 84°44'W). The study area consisted of two 0.1-ha plots that had been used as part of a long-term investigation concerning the effects of nutrient enrichment (sludge or fertilizer application) on old-field succession (Brewer et al. 1994). From 1978-1988, one of these plots had been treated annually (8965 kg $ha^{-1} yr^{-1}$) with municipal sewage sludge (6-2-0, N-P-K) whereas the other plot received equivalent nutrient applications (1570 kg $ha^{-1} yr^{-1}$) of commercial fertilizer (34-11-0, N-P-K). The range of heavy metal concentrations in sludge applied at this site was Cd = 19.8 - 59.0 mg kg^{-1}, Cu = 320.0 - 380.6 mg kg^{-1}, Pb = 243.0 - 473.0 mg kg^{-1}, and Zn = 865.8 - 1281.0 mg kg^{-1} (Levine et al. 1989).

Restoration practices were initiated in 1989. Plots were subdivided into four equal subplots. One subplot was tilled, one limed, one tilled and limed, and one left undisturbed. Tilling was done once in May 1989 to disturb the seed bank and reinitiate secondary succession. Liming was repeated in April 1989, February 1990, and February 1991 to raise the mean soil pH value from approximately 4.4 to 5.5; the mean soil pH value for untreated control plots at this site was 5.5 (Brewer et al. 1994). Subplots were further manipulated in 1992 when one half of each subplot was tilled on 1 June and planted with soybean (*Glycine max*) on 3 June. The other half of each subplot was tilled on the same date and planted with buckwheat (*Fagopyrum esculentum*) on 26 June.

Three specimens each of soybean and buckwheat were collected from each subplot to determine concentrations of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn). Samples were collected at the time of seed set for each species. Each sample was separated into roots, stems, leaves, and seeds to determine mean allocation values of heavy metals within each species. Plants were frozen at -4°C until analyzed for heavy metals. Metals were extracted from each sample by a wet ash technique (see Levine et al. 1989 for details). Each sample was oven-dried at 80°C for 48 h, digested with nitric acid at 120°C, and analyzed for heavy metal content using an IL157 flame atomic absorption spectrophotometer.

Differences in mean Cd, Cu, Pb, and Zn concentrations among long-term (11-yr) treatments, restoration practice (liming or tilling), and plant component parts were analyzed using analysis of variance (ANOVA). Duncan's New Multiple Range Test was used for separation of means where appropriate. The level of significance for statistical analyses was established at $P < 0.05$.

RESULTS AND DISCUSSION

After analyzing the effects of short-term manipulations on heavy metal accumulation, it was found that tilling did not have a significant effect on any heavy metal within any plant species analyzed. Therefore, only results describing the effects of liming on agricultural plant species are presented.

Metal concentrations in soybeans at this site reflect the effects of 11 years of sludge application (Table 1). For example, Cd, Cu, and Zn concentrations were significantly greater in all soybean plant structures collected from unlimed sludge plots compared to unlimed fertilizer plots. Increased uptake of these metals by soybeans grown in sludge-amended plots also has been documented in previous investigations (Ham and Dowdy 1978; Heckman et al. 1987). Although Pb concentrations in leaves were significantly greater in unlimed sludge plots compared to unlimed fertilizer plots, no differences were observed between treatments for roots, seeds, and stems.

Within all sludge-amended plots, highest concentrations of Pb and Zn were found in the leaves of soybeans (Table 1). Cd accumulated in leaves collected within unlimed sludge-plots. The greatest concentrations of Cu were found in seeds. This finding has important implications because high concentrations in seeds increases the potential for introduction of Cu into the human food chain via livestock. This finding also has relevance since soybeans may be consumed by granivorous birds or small mammals (Warner et al. 1989).

Liming significantly reduced concentrations of Cd and Zn in all soybean plant parts (except Cd in roots and Zn in seeds) within sludge-amended plots (Table 1). However, liming had no significant effects on Cu and Pb concentration of soybeans within sludge-amended plots. Thus, identical restoration practices affected heavy metals in a different manner. Liming also significantly reduced the concentration of Zn in leaves collected from fertilizer-amended plots.

Metal concentrations in buckwheat also reflected long-term treatment (Table 2). For example, metal concentrations in buckwheat differed significantly between unlimed sludge plots and unlimed fertilizer plots regarding Cd (except seeds), Pb (except stems and seeds), and Zn (except seeds) concentrations. Concentrations of Cu in roots of buckwheat also differed significantly between long-term treatments. Within sludge-amended plots, Cd and Zn accumulated primarily in the leaves of buckwheat, Cu accumulated in roots, and Pb accumulated in leaves and roots.

In general, liming failed to decrease concentrations of any heavy metal in buckwheat plant structures grown in sludge-amended plots (Table 2). However, liming significantly reduced the concentration of Zn and Pb in stems collected from fertilizer-amended plots. Interestingly, liming increased the concentration of Cu in roots collected from fertilizer plots.

Although 11 years of nutrient enrichment had significantly reduced soil pH from approximately 5.5 to 4.4, liming restored pH to control levels by the third year of

Table 1. Mean metal concentrations (ppm) in soybean (Glycine max) plant structures collected from sludge- and fertilizer-treated plots. Different letters denote significant differences (ANOVA, $P < 0.05$).

Plant Structure	Fertilizer Lime	Fertilizer No Lime	Sludge Lime	Sludge No Lime
Cd				
Leaves	0.2 ± 0.1a	0.2 ± 0.1a	2.2 ± 0.3ab	11.2 ± 3.1d
Roots	0.0 ± 0.0a	0.0 ± 0.2a	1.2 ± 0.2ab	3.2 ± 1.1b
Seeds	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	6.4 ± 3.3c
Stems	0.0 ± 0.0a	0.0 ± 0.0a	0.6 ± 0.2a	5.8 ± 1.0c
Cu				
Leaves	5.2 ± 0.4a	6.0 ± 0.4a	9.0 ± 0.4b	10.1 ± 0.3b
Roots	4.5 ± 0.2a	5.6 ± 0.8a	12.6 ± 0.8c	12.7 ± 1.2c
Seeds	12.5 ± 0.7c	11.7 ± 0.8bc	18.6 ± 0.7d	18.9 ± 1.1d
Stems	4.6 ± 0.3a	5.2 ± 0.7a	9.3 ± 0.7b	8.2 ± 0.8b
Pb				
Leaves	4.9 ± 0.8d	3.8 ± 1.3c	5.3 ± 0.5d	6.3 ± 0.7d
Roots	0.7 ± 0.3ab	0.8 ± 0.5ab	1.5 ± 0.0ab	2.4 ± 0.6bc
Seeds	0.3 ± 0.3a	0.9 ± 0.8ab	0.4 ± 0.2a	0.3 ± 0.2a
Stems	0.1 ± 0.0a	0.8 ± 0.3ab	1.7 ± 0.4ab	2.2 ± 0.5bc
Zn				
Leaves	13.7 ± 2.2a	105.7 ± 16.2c	185.7 ± 14.7d	461.6 ± 21.0g
Roots	0.0 ± 0.0a	0.0 ± 0.0a	25.1 ± 12.8b	266.6 ± 23.2f
Seeds	38.0 ± 3.8b	44.8 ± 3.9b	114.1 ± 21.4c	151.2 ± 10.6cd
Stems	0.0 ± 0.0a	0.0 ± 0.0a	12.8 ± 2.8ab	224.1 ± 34.8e

restoration practices (Brewer et al. 1994). Because the bioavailability of metals that exist as cations in the soil increases with decreasing soil pH (Kiekens 1984), it was expected that restoration of pH would reduce metal uptake in soybeans and buckwheat. This hypothesis was supported by the fact that liming reduced metal concentrations in old-field species from sludge-amended plots at this site (Brewer et al. 1994). Liming also has been shown to reduce metal uptake in agricultural plant species (Lagerweff et al. 1977; Bingham et al. 1979), including soybeans (Heckman et al. 1987). However, results of the present investigation did not support the above hypothesis. These results suggest that the effectiveness of chemical restoration practices must be evaluated on an individual plant species basis.

Table 2. Mean metal concentrations (ppm) in buckwheat (Fagopyrum esculentum) plant structures collected from sludge- and fertiliier-treated plots. Different letters denote significant differences (ANOVA, $P < 0.05$).

Plant Structure	Fertilizer Lime	Fertilizer No Lime	Sludge Lime	Sludge No Lime
Cd				
Leaves	1.9 ± 0.3a	2.6 ± 0.4a	13.5 ± 3.1c	19.8 ± 6.3c
Roots	0.7 ± 0.1a	0.6 ± 0.1a	5.8 ± 1.4b	5.3 ± 1.4b
Seeds	1.4 ± 0.6a	1.3 ± 0.6a	0.6 ± 0.1a	0.9 ± 0.4a
Stems	1.1 ± 0.1a	1.1 ± 0.2a	4.9 ± 1.3b	4.3 ± 1.0b
Cu				
Leaves	7.9 ± 0.4bc	6.1 ± 0.8b	12.8 ± 0.9c	15.2 ± 2.7c
Roots	22.0 ± 6.6d	7.6 ± 0.4bc	22.4 ± 1.4d	23.3 ± 3.1d
Seeds	6.1 ± 0.6b	6.7 ± 0.7b	9.6 ± 0.7bc	9.6 ± 1.3bc
Stems	2.2 ± 0.2a	3.9 ± 1.2ab	5.3 ± 0.8ab	4.9 ± 0.6ab
Pb				
Leaves	13.3 ± 0.5cd	11.4 ± 1.8bc	16.5 ± 1.6d	19.5 ± 2.2de
Roots	11.0 ± 1.1bc	11.7 ± 1.5bc	20.5 ± 2.1de	21.6 ± 1.5e
Seeds	2.2 ± 0.5a	3.0 ± 0.9a	3.1 ± 0.6a	2.9 ± 0.8a
Stems	5.7 ± 1.0a	9.9 ± 2.2b	8.9 ± 1.3ab	8.3 ± 1.46ab
Zn				
Leaves	40.6 ± 3.8bc	68.9 ± 13.1c	176.2 ± 29.1d	196.6 ± 21.9d
Roots	24.2 ± 1.6b	28.6 ± 3.0b	64.9 ± 9.9c	74.8 ± 14.0c
Seeds	21.6 ± 2.8b	30.4 ± 2.1b	53.7 ± 7.7bc	62.8 ± 12.3bc
Stems	9.6 ± 1.8a	31.0 ± 7.2b	89.4 ± 19.3c	86.2 ± 19.2c

The bioavailability of metals in crop species is a complex process that involves the interaction of soil pH, cation exchange capacity, and organic matter content of the soil (Logan and Chaney 1983). In addition, metal uptake and allocation patterns among plants grown under similar conditions often varies depending on individual plant species (Berrow and Burridge 1991). Although the results of this study are not directly comparable, Cd uptake was generally greater in the agricultural plant species compared to *Ambrosia trifida* (an annual dicot), *Solidago canadensis* (a perennial dicot), and *Setaria faberii* (an annual monocot) collected from this study site (Brewer et al. 1994). Concentrations of Pb in buckwheat and Zn in soybeans were also greater compared to these old-field plant species. It is possible that fast-growing agricultural species, bred for increased yield (National Research Council

1989), concentrated greater amounts of heavy metals compared to old-field species during this “increased” growth process. Further investigations are needed to test this hypothesis and to better understand the potential for remediation of sludge-amended soil, especially as related to the use of sludge as commercial fertilizer.

Acknowledgments. We thank the research staff at the Miami University Ecology Research Center, especially M. F. Lucas for field assistance. We also thank M. Douglas for laboratory assistance. This study was supported by NSF Grant BSR-8818086 and EPA Grant R-8103033-01-0 awarded to G. W. Barrett and D. H. Taylor.

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