Bull. Environ. Contam. Toxicol. (1995) 54:120–127

Environmental Contamination and Toxicology

Heavy Metal Concentrations in Earthworms Following Long-Term Nutrient Enrichment

S. R. Brewer, G. W. Barrett*

Department of Zoology, Miami University, Oxford, Ohio 45056, USA

Received: 28 February 1994/Accepted: 20 June 1994

Although several studies have examined the effects of long-term nutrient enrichment (commercial fertilizer and/or municipal sludge) on old-field community dynamics (Maly and Barrett 1984; Kruse and Barrett 1985; Levine et al. 1989), few studies have examined mechanisms of community or ecosystem recovery or the feasibility of restoration following long-term enrichment. Whereas municipal sewage sludge is relatively high in nitrogen and phosphorous necessary for plant growth and reproductive success, sludge also contains heavy metals, posing a risk of accumulation through the food chain.

Metal accumulation in old-fields treated with municipal sludge have been observed in the producer (Bingham et al. 1975; Dowdy and Larson 1975; Stucky and Newman 1977; Levine et al. 1989; Anderson et al. 1982), primary consumer (Chaney et al. 1978; Williams et al. 1978), secondary consumer (Martin and Coughtrey 1975; Ireland 1977; Brueske and Barrett 1991), and detritivore (Kruse and Barrett 1985; Levine et al. 1989) trophic levels. Detritivores, especially earthworms, have been shown to be excellent indicators regarding the long-term ecological magnification of heavy metals within old-field communities. Earthworms (*Lumbricus rubellus* and *Eisenia foetida*), for example, have been shown to accumulate significant levels of cadmium, copper, lead, and zinc from soils contaminated with sewage sludge (Hartenstein et al. 1980; Kruse and Barrett 1985; Levine et al. 1989). Further, Brueske and Barrett (1991) observed a significant accumulation of Cd, Cu, Pb, and Zn in the least shrew (*Cryptotis parva*) fed earthworms from sludge-amended soils.

Additional studies are needed because earthworms (a) represent an important food source for amphibians, reptiles, birds and mammals, thereby increasing the risk of biological magnification through the food web (Ireland 1977; Williams et al. 1978; Brueske and Barrett 1991); (b) appear to function as an environmental sink for heavy metals in old-field

**Present address:* Institute of Ecology, University of Georgia, Athens, GA 30602, USA
Correspondence to: G. W. Barrett

communities (Kruse and Barrett 1985), and (c) provide an ideal indicator species to monitor the long-term fate of metals in communities following sludge application (Levine et al. 1989). The purpose of this study was to examine changes in the annual accumulation of heavy metals in earthworms collected from old-field communities during ecosystem restoration.

MATERIALS AND METHODS

This long-term investigation was conducted at the Miami University Ecology Research Center located near Oxford, Butler County, Ohio. Eight 0.1-ha. plots were planted with winter wheat (*Triticum aestivum* var. Ranger) in 1977 and was not harvested the following year. Three plots were treated monthly (May - September) for eleven years (1978 - 1988) with Milorganite, an aerobically digested municipal sewage sludge (6-2-0, N-P-K). Sludge was applied at an annual rate of 8960 kg ha^{-1} yr^{-1}. Three plots were treated simultaneously with a commercial urea-phosphate fertilizer to provide an equivalent nutrient subsidy. Two plots remained untreated to serve as controls.

Sludge and fertilizer application was discontinued in 1989 following eleven years of nutrient enrichment. At this time each former nutrient-enriched plot was manipulated to evaluate biological and/or chemical mechanisms of community recovery. Former fertilizer- and sludge-treated plots were each subdivided into four equivalent subplots that were manipulated as follows: one subplot was tilled to disturb the seed bank and reintroduce secondary succession, one subplot was limed to increase soil pH values to control levels, one subplot was both tilled and limed to evaluate possible interaction effects, and the remaining subplot was unmanipulated. Former control plots were also subdivided into four equal subplots - two subplots were tilled and two were left unmanipulated to serve as long-term (16-yr) controls.

Earthworm populations (*Lumbricus rubellus* and *Eisenia foetida*) were sampled during May and October (1989 - 1993) to assess relative population abundance. Three 0.2 m^3 samples of soil were randomly selected in each subplot on each sample date and then sifted to count the number of earthworms present. Approximately 10 earthworms, mainly *Lumbricus rubellus*, were collected from each site for heavy metal analysis. Worms were cleaned to remove soil particles and placed in petri dishes in environmental chambers at 15°C for 24 hr to eliminate soil from their alimentary tracts. Earthworms were then frozen until heavy metal analysis at a later date.

Earthworms were oven-dried at 80°C for 48 hr prior to metal analysis. Samples were weighed to 0.5 g and repeatedly digested in concentrated nitric acid over heat to remove organic matter. Samples were next dissolved in a 10% nitric acid solution and filtered to remove particulate matter. Samples were then analyzed for HNO_2-extractable Cd, Cu, Pb, and Zn using flame atomic absorption spectroscopy (IL 157 AA/AE Flame Spectrophotometer; see Levine et al. 1989 for details).

Soil samples (N = 72) were also collected during May and October (1989 - 1993). Three cores (each 10 cm in depth) were collected from three random sites in each subplot, air-dried for seven days, pulvarized to a fine powder, and frozen until heavy metal analysis at a later date.

Soil samples were oven-dried at 80°C for 72 hr prior to metal analysis. Samples (1.0 g each) were repeatedly digested in concentrated nitric acid over heat to remove organic matter. Samples were then dissolved in a 10% nitric acid solution and filtered to remove particulate matter. Samples were then analyzed for HNO_2-extractable Cd, Cu, Pb, and Zn as previously described.

Differences in mean Cd, Cu, Pb, and Zn concentrations among long-term treatments, subplot manipulations, and years were analyzed using analysis of variance (ANOVA). Treatment means were separated using Duncan's New Multiple Range test. Significance was established at the $P \leq 0.05$ level of probability.

RESULTS AND DISCUSSION

Interestingly, short-term subplot manipulations (tilling and/or liming) had no significant effect on heavy metal concentrations in soil or in earthworm populations. Heavy metal concentrations in soils and earthworms, therefore, were pooled per plot and analyzed by former long-term treatment. Significantly greater ($P \leq 0.05$) concentrations of Cd, Cu, Pb, and Zn were observed in soil collected from former sludge treatments compared to control or fertilizer treatments throughout the study (Figs. 1 and 2). All heavy metal concentrations in sludge-amended soils tended to decrease during the period of restoration.

Concentrations of Cd and Pb were significantly greater in earthworms collected from sludge treatments compared to control or fertilizer treatments throughout the 5-year study (Figs. 1 and 2), but concentration values tended to decrease, especially in 1993. Copper and zinc concentrations, however, tended to increase in earthworms collected from sludge-treated plots, especially during 1993. Increased copper and zinc concentrations in 1993 compared to 1992 may be a result of a cycling of organic matter and trace elements in the old-field community. This is suggested by a decrease in soil organic matter content and increased soil nitrate levels in enriched treatments during 1993

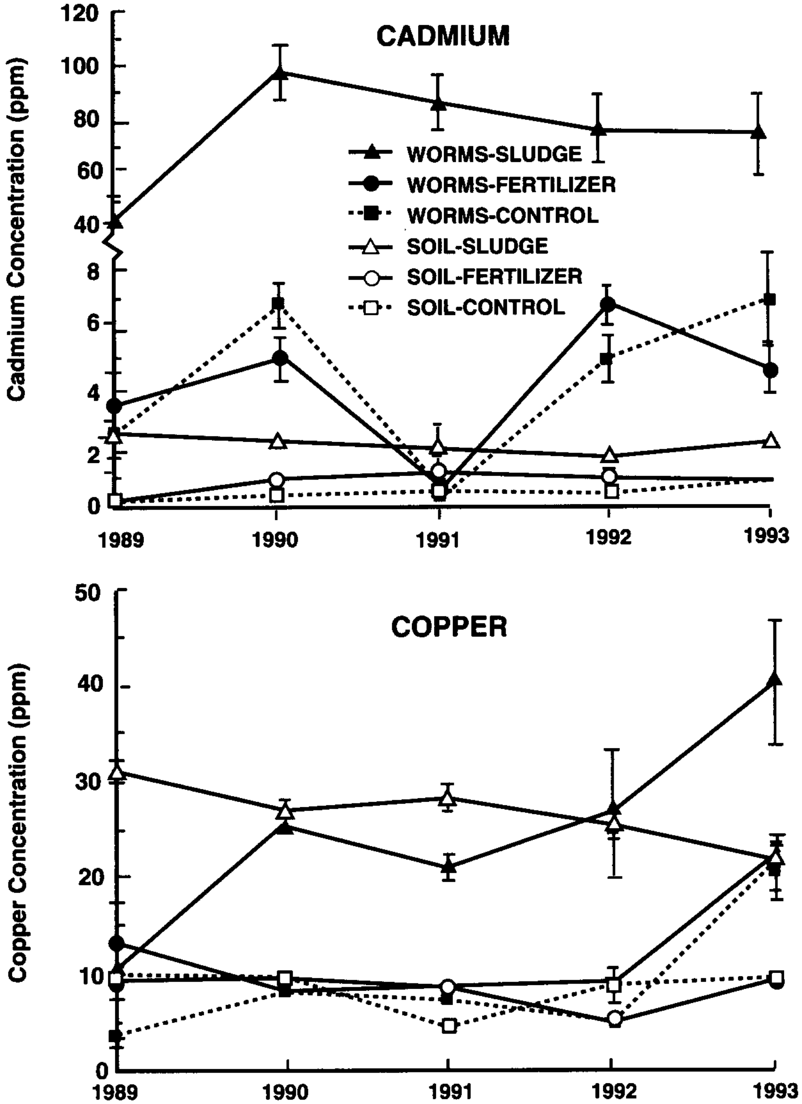

Figure 1. Mean concentrations (ppm) ± SE of cadmium and copper in earthworms and in soil collected from sludge-treated, fertilizer-treated, and control plots (1989-1993).

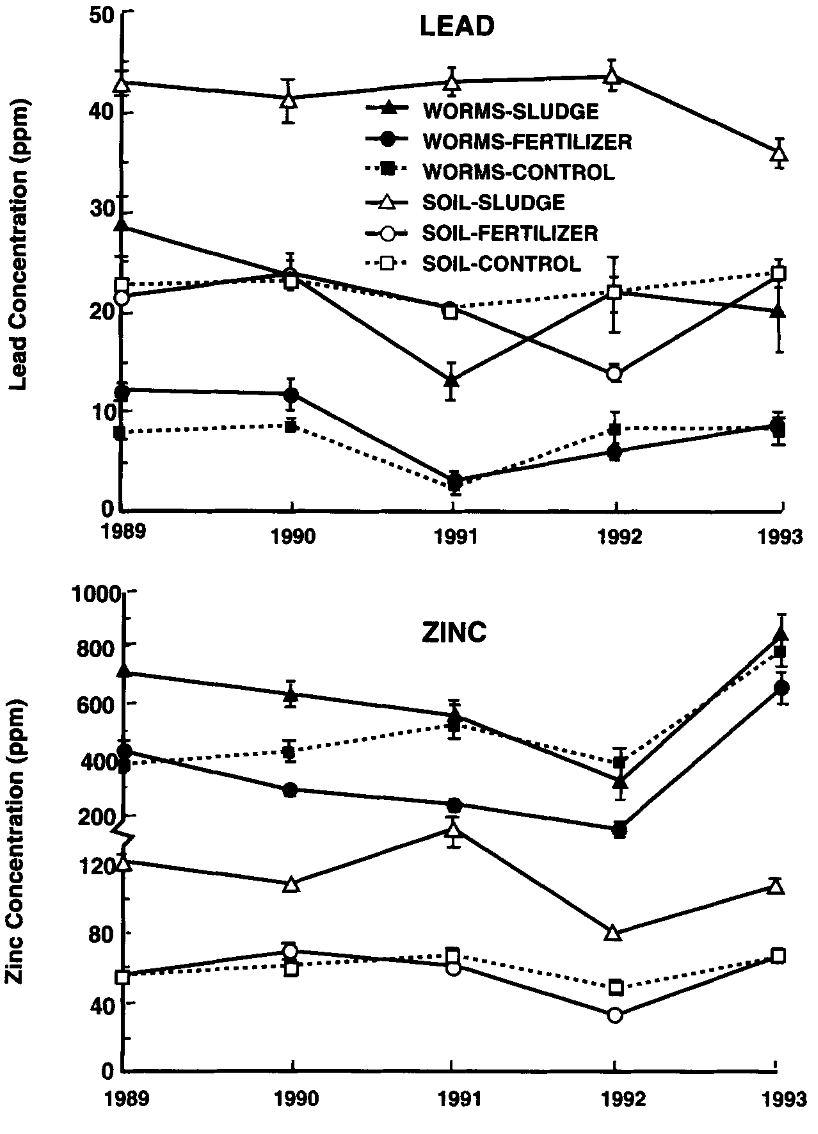

Figure 2. Mean concentrations (ppm) ±SE of lead and zinc in earthworms and in soil collected from sludge-treated, fertilizer-treated, and control plots (1989-1993).

compared to 1992 (Brewer and Barrett, in preparation). Decreasing soil organic matter content and increasing soil nitrate concentrations suggest that organic matter has been decomposed and inorganic elements may be available in the soil for uptake into the food web. Heavy metals have been shown to adsorb to and bind organic matter particles in soil, removing the metals from solutions and rendering them unavailable to higher trophic levels (Kelling et al. 1977, Petruzzelli et al. 1977). Decreased organic matter content in the soil during 1993 (Brewer and Barrett in preparation) may have resulted in higher concentrations of metals such as Cu and Zn present in free ionic form in solution, therefore available for uptake by earthworms as well as the plant community. Kruse and Barrett (1985) reported that earthworms collected from these sludge-amended treatments in 1981 accumulated Cd, Cu, Pb and Zn to significantly higher levels than those collected from control or fertilizer treatments. Zinc concentrations in earthworms from sludge treatments increased to a threshold ($\bar{x}$ = 1218 ppm) during June and declined thereafter, suggesting that a maximum tissue Zn level may have been reached (Kruse and Barrett 1985). The soil in sludge-amended treatments during 1981 also contained significantly higher levels of all four metals than control or fertilizer treatments throughout the growing season. Cd, Cu, and Zn also accumulated to greater concentrations in earthworms compared to soil collected from sludge treatments during 1981. Cd concentrations in earthworms ($\bar{x}$ = 136.0 ppm) was magnified 105 times above soil concentrations ($\bar{x}$ = 1.3 ppm). Cu concentrated to a mean 20.8 ppm in earthworms compared to 16.9 ppm in soil (1.2X magnification), and Zn to 1087.0 in earthworms compared to 136.9 ppm in soil (8.0X). Lead, however, only concentrated to a mean 8.8 ppm in earthworms compared to 23.1 ppm in soil collected from sludge treatments in 1981.

Levine et al. (1989) found that earthworms collected from these sludge-treated plots in 1986 and 1987 again accumulated Cd, Cu, Pb, and Zn to significantly higher levels than those collected from control or fertilizer-treated plots. Whereas Cd concentrations in earthworms collected from sludge-treated plots peaked at 135.6 ppm in 1981, then declined thereafter, ranging from 42.8 ppm in 1989 to 96.4 ppm in 1990. Zinc concentrations in earthworms peaked at 1087.0 ppm in 1981 and decreased to 315.0 ppm in 1992 before increasing to 831.4 ppm in 1993. Copper concentrations varied only slightly, ranging from 10.3 ppm in 1989 to 39.8 ppm in 1993. Lead concentrations rose from 8.8 ppm in 1981 to a peak of 28.5 ppm in 1989, then declined thereafter.

Cd, Cu, Pb, and Zn in soil collected from sludge treatments during the 12-yr period ranged from 1.3 ppm to 2.7 ppm, 16.9 ppm to 36.0 ppm, 23.1 ppm to 48.0 ppm, and 81.0 ppm to 140.5 ppm, respectively. Levine et al. (1989) reported significant bioconcentration of Cd and Zn in earthworms above levels in soil. For example, earthworms concentrated Cd as high as 32 times above soil levels in sludge treatments in 1987 (Levine et al. 1989). However, copper concentrations in earthworms were similar to those found in soil whereas mean lead concentrations in worms were actually lower than those reported for soil.

Copper and zinc concentrations in earthworms fluctuated significantly in all treatments during this study. These fluctuations were attributed to (a) natural cycling of these essential metals within the old-field community, (b) changes in plant community composition (i.e., nutrient-enriched plots dominated by annuals during early restoration, then by perennials during the fifth year of restoration), and (c) changes in soil organic matter as a result of decomposition and possible release of metals into the food web. Increased Zn and Cu concentrations in earthworms in 1993 may also be a result of a lag period between sewage sludge application and release of heavy metals from the organic layer (Levine et al. 1989). Although Cd and Pb concentrations in earthworms decreased in 1993, earthworms bioconcentrated Cd and Zn above levels found in soil. For example, earthworms concentrated Cd as high as 46 times above soil levels in 1991 and 1992 and Zn as high as 7.7 times above soil levels in former sludge-treated plots in 1993. This suggests that heavy metals in the detritus layer are being ingested by detritivores and likely become available to higher trophic levels. Similar to the results reported in Levine et al. (1989), Pb concentrations in earthworms were below those found in soil and Cu concentrations were equivalent in soil and earthworms.

Since earthworms represent an important food source for primary and secondary consumers (Ireland 1977; Kruse 1985; Brueske and Barrett 1991), and because they serve as an ideal indicator species in old-field ecosystems (Hartenstein et al 1980; Kruse and Barrett 1985), earthworms appear to represent an important taxonomic group to monitor regarding both heavy metal toxicity and rates of community recovery following long-term nutrient enrichment.

Acknowledgements. We thank Mary Benninger-Truax, Brett Dietz, Christopher Brueske, Mike Lucas, John Peles, and the crew of the Miami University Ecology Research Center for laboratory and field assistance. This research was supported by EPA Grant R-815033-01-1 and NSF Grant BSR-8818086 awarded to G. W. Barrett and Sigma Xi Grant-in-Aid of Research ZA1892 awarded to S. R. Brewer.

REFERENCES

Anderson TJ, Barrett GW, Clark CS, Elia VJ, and Majeti VA (1982) Metal concentrations in tissues of meadow voles from sludge-treated fields.

J Environ Qual 11: 272-277.
Bingham FT, Page AL, Mahler RJ, Ganje TJ (1975) Growth and cadmium accumulation of plants grown on a soil treated with cadmium enriched sewage sludge. J Environ Qual 4: 207-211.
Brueske CC, Barrett GW (1991) Dietary heavy metal uptake by the Least Shrew, *Cryptotis parva*. Bull Environ Contam Toxicol 47: 845-849.
Chaney RI, Stoewsand GS, Furr AK, Bache CA, Lisk DJ (1978) Elemental content of tissues of guinea pigs fed Swiss chard grown on municipal sewage sludge-amended soil. J Agric Food Chem 26: 994-997.
Dowdy RH, Larson WE (1975) Metal uptake by barley seedlings grown on soils amended with sewage sludge. J Environ Qual 4: 229-233.
Hartenstein R, Neuhauser EF, and Bicklehaupt DH (1980) Accumulation of heavy metals in the earthworm *Eisenia foetida*. J Environ Qual 9: 23-26.
Ireland MP (1977) Lead retention in toads *Xenopus laevis* fed increasing levels of lead-contaminated earthworms. Environ Pollut 12: 85-92.
Kelling KA, Keeney DR, Walsh LM, and Ryan JA (1977) A field study of the agricultural use of sewage sludge. III. Effects on uptake and extractability of sludge-borne metals. J Environ Qual 6: 352-358.
Kruse EA and Barrett GW (1985) Effects of municipal sludge and fertilizer on heavy metal accumulation in earthworms. Environ Pollut 38: 235-244.
Levine MB, Hall AT, Barrett GW, and Taylor DH (1989) Heavy metal concentrations during ten years of sludge treatment to an old-field community. J Environ Qual 18: 411-418.
Magnuson JJ (1990) Long-term ecological research and the invisible present. BioScience 40: 342-357.
Maly MS and Barrett GW (1984) Effects of two types of nutrient enrichment on the structure and function of contrasting old-field communities. Am Midl Nat 111: 342-357.
Martin MH and Coughtrey PJ (1975) Preliminary investigation of the levels of cadmium in a contaminated environment. Chemosphere 4: 155-160.
Petruzzelli G, Guidi G, and Lubrano L (1977) Cadmium occurrence in soil organic matter and its availability to wheat seedlings. Water Air Soil Pollut 8: 393-399.
Stucky DJ and Newman TS (1977) Effect of dried anaerobically digested sewage sludge on yield and elemental accumulation in tall fescue and alfalfa. J Environ Qual 6: 271-274.
Williams PH, Shenk JS, and Baker DE (1978) Cadmium accumulation by meadow voles (*Microtus pennsylvanicus*) from crops grown on sludge treated soil. J Environ Qual 7: 450-454.