

Chemical Fractionation of Trace Elements in Biosolid-Amended Soils and Correlation with Trace Elements in Crop Tissue

Amy L. Shober

University of Florida, IFAS, Gulf Coast Research and Education Center,
Wimauma, Florida, USA

Richard C. Stehouwer and Kirsten E. MacNeal

Department of Crop and Soil Sciences, Pennsylvania State University,
Pennsylvania, USA

Abstract: A previous study indicated that agricultural biosolid applications increased the concentration of EPA₃₀₅₀-digestible trace elements in soils on Pennsylvania production farms but could not indicate potential trace-element environmental availability. This study was conducted to determine if biosolid application had altered the distribution of trace-elements among operationally defined soil fractions and the relationship of trace element concentrations in soil and crop tissues. Biosolid-amended and unamended soils from production farms in Pennsylvania were extracted using a modified Bureau Communautaire de Référence (BCR) sequential fractionation technique and analyzed for chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). Trace-element concentrations in crop tissues (soybean silage, sudangrass, corn grain, alfalfa hay, and orchardgrass hay) from the same farms were also determined. Fractionation results indicated that the proportion of Cr, Cu, Ni, Pb, and Zn that is potentially bioavailable is quite small in unamended soils. Biosolid applications significantly ($P \leq 0.1$) increased concentrations of Cu in all soil fractions (average increase over unamended soil = 1.14, 8.27, 6.04, and 5.84 mg kg⁻¹ for the exchangeable, reducible, oxidizable, and residual fractions, respectively), Ni (0.41, 1.65 mg kg⁻¹ for the reducible and residual fractions, respectively), Pb (5.12 and 1.49 mg kg⁻¹ for the reducible and residual fractions, respectively), and Zn (8.28, 7.12, 4.44, and 8.98 mg kg⁻¹ for the exchangeable, reducible, oxidizable, and residual fractions,

Received 26 July 2005, Accepted 16 March 2006

Address correspondence to A. L. Shober, University of Florida, IFAS, Gulf Coast Research and Education Center, 14625 CR 672, Wimauma, FL 33598, USA. E-mail: ashober@ufl.edu

respectively) but did not significantly increase Cr in any soil fraction. Concentrations of Cu in all soil fractions were significantly ($P \leq 0.01$) correlated with concentrations of Cu in orchardgrass tissue ($r = 0.70, 0.66, 0.76,$ and 0.69 for the exchangeable, reducible, oxidizable, and residual soil fractions, respectively). Concentrations of exchangeable and reducible Zn were significantly correlated with Zn in sudangrass tissue ($r = 0.81$ and 0.67), and reducible Zn was significantly correlated with Zn concentrations in orchardgrass tissue ($r = 0.65$). Application of biosolids had little effect on bioavailability of Cr, Ni, or Pb, whereas higher loadings of Cu and Zn led to a shift toward the more labile soil fractions. Loadings of Cu and Zn were much smaller than cumulative loadings permitted under U.S. Environmental Protection Agency (USEPA) Part 503 regulations. Chemical soil fractionation was able to detect increases in labile soil Cu and Zn that relate to increased phytoavailability.

Keywords: Metals, municipal wastewater treatment residuals, sequential extraction, sewage sludge

INTRODUCTION

In the United States, more than half of the approximately 5.6 million dry tons of sewage sludge used or disposed of annually is land applied, and agricultural utilization of biosolids occurs in every region of the country (NRC 2002). Nevertheless, concerns that agricultural utilization of biosolids could have negative effects on environmental, human, and animal health due to the accumulation of trace elements remain prevalent among the general public and continue as a matter of scientific debate. A study was recently completed to assess the effects of agronomic biosolid application on nutrient and trace-element loads in soils and crops in Pennsylvania (Shober, Stehouwer, and MacNeal 2003). Application of biosolids led to small increases in surface soil concentrations (0–10 cm) of Environmental Protection Agency (EPA)₃₀₅₀-digestible chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). However, these trace metals can occur in several different forms in biosolids and in soils, each with varying degrees of environmental availability. Therefore EPA₃₀₅₀-digestible-metal-concentrations provide little indication of potential environmental availability of these metals in biosolid-amended soils. Total trace-element concentrations in the soil, as determined by strong acid digestions, have also been proven to be a poor indicator of trace-element concentrations in crop tissue grown on that soil (Qian et al. 1996; Sims and Kline 1991).

Sequential chemical fractionation techniques separate trace elements into different operationally defined soil fractions by subjecting the soil to a series of chemical reagents, each more destructive or using a different chemical reaction than the previous one (Zufiaurre et al. 1998; Tessier, Campbell, and Bisson 1979; Morabito 1995). Many studies of biosolid-amended soils have used various published fractionation procedures to

partition trace elements into the following soil fractions: (1) exchangeable trace elements; (2) trace elements bound to carbonates (carbonate); (3) trace elements bound to iron (Fe) and manganese (Mn) oxides and hydroxides (reducible); (4) trace elements bound to organic matter and sulfides (oxidizable); and (5) trace elements in the mineral lattice of silicates or nonreducible oxides or hydroxides (residual) (McLaren and Crawford 1973; Sims and Kline 1991; Morabito 1995; Tessier, Campbell, and Bisson 1979; Emmerich et al. 1982). The soil fractions are, ideally, indicative of the potential bioavailability or mobility of the trace elements with bioavailability and mobility decreasing in the order of the sequential extraction steps. Researchers have used a variety of extraction procedures, leading to difficulties when attempting to compare the results of studies done by different researchers. In the early 1990s, the Bureau Communautaire de Référence (BCR) of the European Union determined that it would be beneficial to take steps toward creating an acceptable standard sequential extraction procedure (Quevauviller, Rauret, and Griepink, 1993). The BCR recommended the use of a three-step method that separates elements into carbonate, reducible, and oxidizable fractions as a standard method (Quevauviller 1998). Continuing studies indicated that the procedure needed some modification including requiring specific shaking procedures and centrifuge speeds (Rauret et al. 1999).

Numerous fractionation studies of biosolid-amended soils have been conducted in field experiments where factors such as soil type, biosolid composition and application rates, and uniformity of spreading are carefully controlled. Many of these studies have demonstrated that trace elements are predominantly associated with operational soil fractions that are considered to be relatively unavailable for crop uptake (Canet et al. 1998; Chang et al. 1984; Emmerich et al. 1982; Sloan et al. 1997; Sposito, Lund, and Chang 1982). Studies also have reported significant correlations between the amounts of trace elements in chemical soil fractions and the amounts in plant tissues (Canet et al. 1998; Qian et al. 1996; Zhang, Shan, and Li 1998). Although many factors contribute to plant uptake and translocation of trace-elements, correlations between trace-element soil fractions and trace element concentrations in crop tissue indicate that the operationally defined chemical fractions do relate to phytoavailability. Therefore, it is of interest to examine the correlation of trace elements in soils fractions with concentrations of trace elements in crop tissue. Although these carefully controlled studies offer insight regarding trace-element distribution in long-or short-term studies, there is concern that controlled plot experiments do not give an accurate picture of commercial-scale agricultural biosolid application where variability in biosolid quality, application rates, and uniformity of spreading may be large. Therefore, the objectives were to determine (i) if biosolid application at working production farms in Pennsylvania has altered the distribution of trace elements among operationally defined soil fractions (exchangeable, reducible, oxidizable, and residual) and (ii) if

trace-element concentrations in the soil fractions are correlated with trace-element concentrations in crop tissue.

MATERIALS AND METHODS

In a previous study, paired fields were sampled at 18 Pennsylvania farms to assess the effects of long-term agricultural biosolid applications on soil quality (Shober, Stehouwer, and Macneal 2003). At each farm, a biosolid-amended field was paired with an unamended field. Paired fields had the same soil series and similar management history. The pairing of fields was necessary because time-zero background samples (or data) were not available for most of the treated fields. The assumption was that prior to the use of biosolids, the trace-element concentrations were similar in both members of a pair.

For this study, five representative farms from the soil-quality study were selected for which corresponding soil and crop samples were available for two consecutive years. The soils at these farms included a Duffield silt loam (fine-loamy, mixed, active, mesic Ultic Hapludalfs), Glenelg loam (fine-loamy, mixed, semiactive, mesic Typic Hapludults), Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalfs), Hublersburg gravelly silt loam (clayey, illitic, mesic Typic Hapludults), and Volusia channery silt loam (fine-loamy, mixed, active, mesic Areic Fragiaquepts). Soil samples were collected from five locations in each field in 1999 and 2000. All soil-sampling locations were identified and mapped using a global positioning system with differential correction (allowing for submeter accuracy) so that soil samples could be taken from the same locations in consecutive years. Soil samples were collected from the 0- to 10-cm depth with a 5-cm-diameter bucket auger, air dried, and ground to pass a 2-mm sieve. Soil samples were analyzed for (i) total and inorganic carbon by combustion (Nelson and Sommers 1996); (ii) organic carbon by subtracting the amount of inorganic carbon from the amount of total carbon; (iii) soil pH (1:10 soil-to-water ratio); and (iv) total digestible elements by EPA₃₀₅₀ hot plate acid digestion (USEPA 1986). Each soil sample also was analyzed using a modified BCR sequential chemical fractionation for trace elements: (i) 0.11 M acetic acid, 16 h of reaction time for exchangeable and weak acid soluble elements (exchangeable); (ii) 0.5 M hydroxylamine hydrochloride, 16 h of reaction time for trace elements associated with Fe and Mn oxides (reducible); (iii) 30% hydrogen peroxide (H₂O₂), 2 h of reaction time at 85 ± 2°C, followed by 0.05 M ammonium acetate, 16 h of reaction time for trace elements associated with organic matter or sulfides (oxidizable); (iv) nitric acid (HNO₃) and hydrochloric acid (HCl), hot-acid digestion for residual trace elements (residual) (Quevauviller 1998; Rauret et al. 1999). After each extraction, the sample was centrifuged and washed with deionized water. Modifications were made to step 3 of the BCR sequential extraction as follows. The level of solution in the tube was marked after the addition of H₂O₂. At the conclusion of the heating step, the tube was cooled, and the

volume of liquid was restored to the graduation on the tube with deionized water. Assuming the complete reaction of H_2O_2 with the soil, the reaction yields approximately 15 mL of water in the tube. The ammonium acetate solution concentration was changed from 1.0 M to 1.67 M, and 22.5 mL of 1.67 M ammonium acetate were added to the tube, yielding 37.5 mL of 1 M ammonium acetate solution in the centrifuge tube (as prescribed in the original procedure; Rauret et al. 1999). This change was necessary because of the refluxing of the solution in the tube that prevented evaporation of the liquid as required in the extraction procedure. All soil extracts and digests were analyzed for Cr, Cu, Ni, Pb, and Zn concentration by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Soltanpour et al. 1996) with the exception of the H_2O_2 -ammonium acetate extracts for Cu and Zn, which were analyzed using flame emission atomic absorption spectrophotometry (Wright and Stuczynski 1996). Trace-element concentrations in all sequential extraction fractions were summed, and that value was compared to the EPA₃₀₅₀-digestible trace elements to assess if comparable amounts of each element were being extracted by both methods (USEPA 1986).

Crop tissue samples from soybean (*Glycine max* Merr.), alfalfa (*Medicago sativa* L.), corn grain (*Zea mays* L.), orchardgrass (*Dactylis glomerata* L.), and sudangrass (*Sorghum bicolor* L.) were collected at each of the five soil sampling locations in each biosolid-amended and unamended field. The tissue collected was representative of the tissue that would be harvested at each farm (i.e., corn for grain, soybean for silage). Crop samples were dried to a constant mass at 70°C and ground to pass a 2-mm sieve. A dry-ash method was used to destroy organic matter in the crop tissue samples (Dahlquist and Knoll 1978). Dilute nitric acid was then added to dissolve the ash, and the resulting solutions were analyzed for Cu, Ni, Pb, and Zn using ICP-AES. Crop tissue concentrations were obtained from a previous study that did not examine tissue Cr concentrations. Crop tissues were no longer available, preventing analysis of Cr in this study.

Data Analysis

A split-plot design with time as a repeated measure was used for analysis of treatment effects on the concentration of trace elements in each soil fraction using PROC GLM (SAS Institute Inc.; Cary, NC). Two error strata were used to account for treatment effects and the repeated measures factor (time). The error term from the farm \times treatment interaction (Ea) was used to test for the effect of biosolid treatment, and residual error (Eb) was used to test for treatment \times time interaction. If the ratio of Ea/Eb was not significant at $P \leq 0.25$, the error terms from the two strata were pooled. The residual error term (Eb) was used to test for treatment effects when Ea/Eb was not significant at $P \leq 0.05$ but was significant at $P \geq 0.25$. Data were evaluated to determine overall treatment effects on the soil fractions for each element

with results considered significant at probability level of 0.10 (Littell et al. 1996). Crop tissue samples were grouped according to crop type and matched with soil samples from the corresponding sampling location. Because the crop and soil trace-element concentrations were not normally distributed, the nonparametric Spearman's rank correlation was used to test for significant correlation between crop tissue and soil fraction concentrations at a probability level of 0.01. A two-tailed t test was also used to determine significant increases in crop tissue trace-element concentrations due to the application of biosolids at a probability level of 0.05. For the purpose of conducting statistical analysis, a value of half the detection limit was substituted when trace-element concentrations were less than the method detection limit. Relationships significant at the 0.1, 0.01, and 0.001 probability levels are marked in the tables as *, **, and ***, respectively, unless otherwise noted.

RESULTS AND DISCUSSION

Soil and Site Characteristics

On average, biosolid-amended soils received 54 Mg ha^{-1} of biosolids cumulatively over several years (Table 1). Application of biosolids to the soil did not result in significant differences in cation-exchange capacity (CEC), organic carbon (C), or pH compared to unamended soils. Biosolid-amended and unamended soils at each farm had pH values that were slightly acidic and typical for agricultural soils in Pennsylvania (Table 1). Chemical properties were comparable for all soils at all five farms, with the exception of organic C composition, which was higher in the Volusia soil than in the other soils. Agricultural crops grown at each farm were often different in consecutive years as a consequence of crop rotations on individual fields (Table 1).

Biosolid Effects on Fractionation of Trace Elements

The trace-element concentrations by sum of sequential extraction fractions were comparable to the EPA₃₀₅₀-digestible concentrations of each element (Table 2). Average recoveries by the sequential fractionation were 97, 101, 114, 96, and 110% of EPA₃₀₅₀-digestible Cr, Cu, Pb, Ni, and Zn, respectively, suggesting that both methods extract similar concentrations of trace elements from soil. These results are similar to other studies where the sum of fractions was within $\pm 10\%$ (Qian et al. 1996; Hickey and Kittrick 1984) and $\pm 20\%$ (Sloan et al. 1997) of the total element concentration by hot-acid digestions. These results indicate good agreement between the chemical fractionation and EPA₃₀₅₀-digestion methods and that the cumulative error in the sequential fractionation procedure was reasonably low. Loss of solid material in the decanted supernatant, efficiency of individual extractants, and sampling and analytical variability may have contributed to any differences between

Table 1. Cumulative biosolid applications and selected chemical properties of soils used in the fractionation study

Soil	Cumulative biosolids applied (Mg ha ⁻¹)	Years of biosolid application	Soil property ^a			Crop grown
			CEC (cmol/kg)	pH	Organic C (%)	
Duffield	0		11.4 ± 0.7	6.7 ± 0.1	2.0 ± 0.1	Corn grain (1999), alfalfa hay (2000)
	40.3	1986, 1989, 1997, 2000	7.7 ± 1.3	6.4 ± 0.3	1.4 ± 0.4	Corn grain (1999), alfalfa hay (2000)
Glenelg	0		8.7 ± 0.7	6.2 ± 0.2	2.0 ± 0.1	Soybean (1999), corn grain (2000)
	64.3	1988–1993, 1995–97	17.4 ± 0.3	7.2 ± 0.2	2.9 ± 0.1	Soybean (1999), corn grain (2000)
Hagerstown	0		11.0 ± 1.4	7.0 ± 0.3	1.7 ± 0.2	Sorghum, sudangrass, and corn silage
	47	1980–81, 1984, 1990–91, 1996–98	12.1 ± 2.1	6.6 ± 0.5	2.1 ± 0.2	Sorghum, sudangrass, and corn silage
Hublersburg	0		11.1 ± 0.0	6.5 ± 0.1	2.4 ± 0.1	Corn grain
	20.8	1991–96, 1997–2000	13.8 ± 0.0	6.7 ± 0.0	2.6 ± 0.3	Corn grain
Volusia	0		10.5 ± 0.5	6.1 ± 0.1	4.4 ± 0.4	Orchardgrass hay
	99.7	1982–95, 1997–2000	16.0 ± 1.2	6.8 ± 0.1	5.7 ± 0.5	Orchardgrass hay

^aMean of measurement on composite soil samples from two consecutive years.

Table 2. Distribution of Cr, Cu, Pb, Ni, and Zn (mg kg^{-1}) in operationally defined fractions of biosolid-amended and unamended soils.

Soil	Unamended						Biosolids					
	Carbonate	Reducible	Oxidizable	Residual	Sum	EPA ₃₀₅₀	Carbonate	Reducible	Oxidizable	Residual	Sum	EPA ₃₀₅₀
Cu												
Duffield	BD ^a	1.15	BD	12.30	13.45	15.42	BD	2.37	BD	13.68	16.05	19.35
Glenelg	0.20	1.02	1.22	16.19	18.63	19.60	2.53	18.09	11.65	25.00	57.27	59.65
Hagerstown	0.45	3.92	1.76	11.78	17.91	18.99	2.03	10.59	3.49	21.23	37.34	41.25
Hublersburg	BD	1.80	1.83	12.34	15.97	19.06	BD	5.02	4.37	15.92	25.31	33.27
Volusia	BD	0.49	BD	7.27	7.76	10.14	1.69	14.34	14.60	13.88	44.51	48.40
Cr												
Duffield	BD	0.33	2.81	20.87	24.01	25.93	BD	0.48	2.88	20.72	24.08	27.99
Glenelg	0.12	BD	3.30	21.73	25.15	27.42	0.17	3.06	9.95	24.62	37.80	40.07
Hagerstown	BD	BD	3.33	14.26	17.59	19.58	BD	0.23	2.89	14.44	17.56	19.69
Hublersburg	BD	0.19	3.52	16.73	20.44	24.11	BD	BD	6.32	20.94	27.26	28.34
Volusia	BD	0.3	2.62	9.96	12.88	10.14	BD	0.71	2.62	9.65	12.98	17.42

Element Fractionation in Biosolid-Amended Soils												
pB												
Duffield	BD	11.48	BD	3.20	14.68	16.72	BD	20.04	BD	5.77	25.81	15.78
Glenelg	0.46	12.78	BD	10.18	23.42	24.76	BD	15.87	BD	11.15	27.02	27.92
Hagerstown	BD	19.39	BD	7.93	27.32	28.37	BD	22.42	BD	11.25	33.67	32.95
Hublersburg	BD	15.53	BD	3.91	19.44	31.68	BD	31.72	BD	10.39	42.11	49.48
Volusia	BD	13.25	BD	3.52	16.77	18.97	BD	19.83	BD	5.27	25.10	27.04
Ni												
Duffield	0.58	0.80	BD	16.23	17.61	17.94	0.64	0.74	BD	16.77	18.15	20.18
Glenelg	0.68	BD	BD	7.50	8.18	10.21	1.22	1.14	BD	8.84	11.20	14.35
Hagerstown	1.20	0.91	BD	12.18	14.29	15.74	1.52	1.03	2.28	16.15	20.98	21.07
Hublersburg	0.70	0.87	BD	13.02	14.59	16.66	0.81	1.69	BD	16.11	18.61	22.18
Volusia	1.32	1.19	BD	10.98	13.49	15.47	1.06	1.48	BD	10.59	13.13	15.79
Zn												
Duffield	4.56	4.15	3.00	57.24	68.95	62.53	5.41	4.77	3.56	57.61	71.35	68.15
Glenelg	3.56	2.06	2.19	36.97	44.78	43.44	22.19	16.86	10.88	54.24	104.2	102.9
Hagerstown	6.81	6.68	3.79	58.32	75.60	77.04	13.32	11.22	5.95	76.90	107.4	108.3
Hublersburg	6.15	5.52	3.84	54.65	70.16	73.61	8.20	8.53	4.30	62.15	83.18	115.7
Volusia	2.54	2.85	5.68	69.72	80.79	80.69	16.61	16.11	15.05	72.80	120.6	123.7

^aBD indicates values was less than the instrument detection limit.

Table 3. Mean trace-metal concentration (mg kg^{-1}) in soil fractions in unamended soils and mean concentration increase (Δ) in biosolid-amended soils

Soil fraction	Cr		Cu		Ni		Pb		Zn	
	Unamended	Δ	Unamended	Δ	Unamended	Δ	Unamended	Δ	Unamended	Δ
Exchangeable	<0.12 ^a	NC ^b	0.10	1.14*	0.87	0.16	0.26	NC	4.70	8.28*
Residual	0.21	0.71	1.68	8.27*	0.79	0.41*	15.41	5.12*	4.23	7.12*
Oxidizable	<5.23	1.72 ^c	1.30	6.04*	<2.10	NC	<6.65	NC	3.92	4.44*
Residual	16.61	1.29	11.91	5.84*	11.91	1.65*	6.80	1.49*	55.04	8.98*
Sum of fractions	<22.17	3.72	14.99	22.59*	<15.67	2.22*	<29.12	6.82*	67.89	28.83*
EPA 3050	22.46	4.07	16.35	22.59*	15.05	3.22*	23.79	5.54*	66.59	30.58*

^aIndicates detection limit for specific trace element.

^bNC: Could not be calculated because results were less than the detection limit for treated and/or unamended soil.

^cCalculated by subtracting the concentration in treated soils from value of $0.5 \times$ detection limit.

*Indicates significant increase in trace element compared to unamended soil at the 0.1 probability level.

element concentrations by sum of fractions and EPA₃₀₅₀-digestible trace-element concentration. The concentration by sum of sequential fractions and the concentration of EPA₃₀₅₀-digestible Cu, Ni, Pb, and Zn were higher in biosolid-amended soils than in unamended soils (Tables 2 and 3).

Based on average concentrations in unamended soils on all five farms, the largest amounts of Cr, Cu, Ni, and Zn (0.80–0.82 of total) were extracted by hot-acid (HCl and HNO₃) digestion, indicating that these elements were associated predominantly with the most recalcitrant, residual fraction (Table 2). Compared to the other elements, only a small amount of Pb (0.26 of total) in unamended soils was residual. Soil Pb was concentrated mainly in the reducible soil fraction, which is considered to be more labile than the residual fraction. Relatively small amounts of Cu, Ni, and Zn (<0.07 of total) were extracted by acetic acid. Concentrations of exchangeable Cr and Pb were less than the method detection limit. These data suggest that these elements did not exist as labile or exchangeable forms in unamended soils. Thus, the fractionation results indicate that the proportion of Cr, Cu, Ni, Pb, and Zn that is potentially bioavailable is quite small in unamended soils.

In addition to the increases in EPA₃₀₅₀-digestible elements in the soil, the application of biosolids also increased concentrations of Cu, Ni, Pb, and Zn in two or more soil fractions but did not significantly increase Cr in any soil fraction (Table 3). Biosolid application increased reducible and residual Ni; however, the increases were relatively small when compared to the increases in other trace elements. Trends indicate that application of biosolids led to small shifts in the distribution of Cr and Ni among the soil fractions (Table 2). The Glenelg soil had the largest redistribution of soil Cr and Ni from the residual fraction to the reducible and oxidizable fractions, corresponding with the heaviest cumulative loading of these elements (Table 4). These results suggest that there has been little change in bioavailability of Cr or Ni due to the application of biosolids and agree with other studies that have reported that Cr (Hickey and Kittrick 1984; McGrath and Cegarra 1992; Mester et al. 1998; PerezCid, Lavilla, and Bendicho 1996) and Ni (Canet et al. 1998; Emmerich et al. 1982; Hickey and Kittrick 1984) were associated predominantly with the residual soil fraction. The reducible fraction

Table 4. Cumulative loadings of biosolid trace elements (kg ha⁻¹) for each soil

Site	Cr	Cu	Pb	Ni	Zn
Duffield	NR ^a	9.20	1.05	0.40	16.35
Glenelg	7.89	36.43	5.31	2.88	47.14
Hagerstown	NR	37.79	2.56	5.37	80.74
Hublersburg	0.37	9.72	1.73	0.63	15.25
Volusia	NR	59.00	13.83	2.51	76.92

^aNR indicates cumulative Cr loading was not reported.

represents the largest soil fraction of Pb in biosolid-amended soils. Trends indicate that the overall distribution of Pb among the soil fractions was similar in biosolid-amended and unamended soils (Table 2). Previous studies also have reported Pb in biosolid-amended soils to be associated primarily with the reducible soil fraction (Fe and Mn oxides) (Canet et al. 1998; McGrath and Cegarra 1992; Sposito, Lund, and Chang 1982). These results indicated that soil Pb is a result of anthropogenic activities, rather than the weathering of Pb-rich parent material. In addition to Pb added to the soil in biosolids, atmospheric deposition of Pb due to the long-term use of leaded gasoline, prior to the U.S. leaded-gasoline bans in the 1970s, may explain enrichment of the reducible soil fraction.

On a mass basis, land application of biosolids resulted in the addition of much more Cu and Zn than Pb, Cr, or Ni to these soils (Table 4), but the added Cu and Zn were not distributed proportionately among soil fractions. Consequently, proportionate increases in exchangeable, reducible, and oxidizable Cu and Zn were much larger than for the other metals. Trends indicate that the distribution of Cu and Zn in biosolids-amended soils is shifting from the recalcitrant, residual fraction to more labile fractions (Tables 2 and 3). Despite this increase, the amount of exchangeable Cu remained low on a mass basis as well as fractional basis (0.03 of total). In contrast, increases in exchangeable Zn were much larger on both a mass basis and fractional basis (0.13 of total). Previous fractionation studies of biosolid-amended soils also have reported that the majority of Zn was associated with the exchangeable and residual soil fractions (Canet et al. 1998; Emmerich et al. 1982; Sloan et al. 1997). The results for the effects of biosolid application on Cu distribution, however, were somewhat different than many studies have reported. The largest increases in reducible Cu of biosolid-amended soils were observed; however, other studies have observed large increases in Cu associated with organic matter (oxidizable) (Canet et al. 1998; Chang et al. 1984; Emmerich et al. 1982; McGrath and Cegarra 1992; PerezCid, Lavilla, and Bendicho 1996; Sposito, Lund, and Chang 1982). The difference in results occurred despite similar concentrations of organic C levels in the soils and the soils examined in these other studies. The low concentrations of oxidizable Cu, as well as the other trace metals in the study, likely is due to the order in which the fractions were extracted. The BCR procedure extracts elements associated with hydrous oxides before extracting those associated with organic matter; however, the sequence was performed in reverse order in the other studies. Treatment of the sample with H₂O₂ prior to removal of elements associated with Mn and Fe oxides could cause the concentration of trace elements associated with organic matter to be elevated because oxidation by-products (i.e., oxalate ions) can attack and release elements bound to Mn oxides (Pickering 1986). Incomplete oxidation of organic matter by the peroxide solution might have been another cause of low concentrations of oxidizable Cu (Pickering 1986). Another explanation for the low concentrations of Cu associated with organic matter could be

due to liming of the soils to maintain pH above 6.0 as required by the USEPA Part 503 regulations (USEPA 1993). Sims (1986) observed that increasing the pH of three mid-Atlantic coastal plains soils from pH 4.8 to 7.0 by liming resulted in the decrease in Cu associated with organic matter and a corresponding increase in the amount of Cu associated with Fe and Mn oxides.

Comparison of Cu and Zn loadings (Table 4) with the distribution of Cu and Zn in each soil (Table 2) clearly shows that the larger loadings of Cu and Zn in the Volusia, Glenelg, and Hagerstown soils led to the greatest changes in distribution of these elements in the soil. However, even the largest loadings of Cu and Zn in this study represent only a fraction of Cu and Zn loadings permitted under the USEPA Part 503 biosolids regulations, 1500 kg Cu ha⁻¹ and 2800 kg Zn ha⁻¹ (USEPA 1993). The data provide no indication if the shift in Cu and Zn toward more labile soil fractions observed at these low loadings would continue or if added Cu and Zn would stabilize toward more recalcitrant fractions as loadings continue.

Correlation of Trace Elements in Soil Fractions and Crop Tissues

All crop tissue samples were near or less than the instrument detection limit for Pb, and thus crop tissue concentrations could not be correlated with Pb in the soils. The extremely low tissue concentration of Pb indicated minimal uptake and translocation of Pb into the crop tissue. There were no differences in the concentrations of Cu, Ni, or Zn in the tissue of crops grown on biosolid-amended soils compared to the tissue of crops grown on unamended soils. Crop tissue Ni concentrations were not correlated with Ni in any soil fraction; however, concentrations of Cu in all soil fractions were significantly correlated with concentrations of Cu in orchardgrass tissue (Table 5). Concentrations of exchangeable and reducible Zn were significantly correlated with Zn in sudangrass tissue. In addition, reducible and EPA₃₀₅₀-digestible Zn were significantly correlated with Zn concentrations in orchardgrass tissue. These correlations occurred at the two sites with largest loadings of Cu and Zn (Table 5), suggesting that at higher cumulative trace-element biosolid loadings, enrichment of more labile soil fractions led to increased crop uptake of Cu and Zn from biosolid-amended soils. Concentrations of Cu and Zn in soybean, alfalfa, and corn grain tissue were not correlated with Cu or Zn in any soil fraction; however, Cu and Zn loadings to the soils at the farms where these crops were grown were low, ranging from a fraction of 0.16 up to 0.64 of the highest Cu and Zn loadings. The study examined the concentrations of trace elements in the harvestable crop tissue; however, several trace elements may be taken up and accumulate in the root system with only limited translocation into aboveground tissues (Qian et al. 1996). A study of wheat root and shoot tissues in biosolid-amended soils reported substantially larger amounts of Pb, Cu, and Ni in the roots of wheat (*Triticum* spp.) than in shoots (Qian et al. 1996). Copper

Table 5. Spearman's rank sum correlation coefficients for concentrations of trace elements in crop tissues and soil fractions

Fraction	Sorghum, sudangrass and corn silage (n = 20)	Corn grain (n = 42)	Alfalfa hay (n = 8)	Orchard- grass hay (n = 20)	Soybean silage (n = 10)
Cu					
Exchangeable	-0.08	-0.08	-0.41	0.70***	0.02
Reducible	0.07	0.03	-0.12	0.66**	-0.01
Oxidizable	0.51	0.24	-0.26	0.76***	0.05
Residual	0.05	-0.01	-0.36	0.69***	-0.02
EPA ₃₀₅₀	0.10	0.09	-0.17	0.71***	0.09
Ni					
Exchangeable	-0.47	0.07	-0.05	0.42	0.07
Reducible	-0.38	0.27	0.08	0.26	-0.10
Oxidizable	— ^a	—	—	—	—
Residual	0.05	0.20	0.58	-0.22	0.07
EPA ₃₀₅₀	0.13	0.29	0.59	0.18	-0.56
Zn					
Exchangeable	0.81***	0.21	-0.05	0.56	-0.30
Reducible	0.67***	0.31	-0.02	0.65**	-0.40
Oxidizable	0.52	0.26	0.11	0.51	-0.29
Residual	0.26	0.17	0.02	0.32	-0.29
EPA ₃₀₅₀	0.41	0.17	0.29	0.61**	-0.50

^aCorrelation could not be performed because all soil values were less than the detection limit.

**Indicates significance at the 0.01 probability level.

*** Indicates significance at the 0.001 probability level.

was the only element with substantial concentrations in the shoots (Qian et al. 1996). Thus, lack of correlation between trace-element concentrations in harvested shoot tissue and concentrations in chemical soil fractions does not necessarily mean the operationally defined soil fractions are not indicative of trace-element phytoavailability.

Tissue concentrations of Cu and Zn were within the normal concentration range for soybean, alfalfa, and corn for all tissue samples grown in biosolid-amended and unamended soils (Jones, Wolf, and Mills 1991), again suggesting little change in the phytoavailability of Cu and Zn following low cumulative trace-element loading from biosolid applications. These results were expected because the prior crop and soil-quality study reported no effect of biosolid application on concentrations of trace elements in crop tissue samples (Shober, Stehouwer, and MacNeal 2003). However, application of biosolids at high loadings of Cu and Zn in resulted in significant

Table 6. Recommended and measured concentrations (mg kg^{-1}) of Cu and Zn in crop tissue

Element	Optimum ^a			Measured	
	Low	Sufficient	High	No biosolids	Biosolids
Orchardgrass ^a					
Cu	<3	3–5	>5	2.1–7.6	5.0–42.7*
Zn	<20	20–50	>50	16.8–33.0	23.4–149.9*
Sudangrass ^b					
Cu	<2	2–7	>7	6.0–8.8	7.0–9.1
Zn	<15	15–30	>30	30.0–40.0	36.5–71.0*
Soybean ^c					
Cu	5–9	10–30	31–50	9.5–10.9	9.3–12.8
Zn	10–20	21–50	51–75	28.3–48.7	27.2–33.8*

*Crop tissue grown on biosolid-treated fields had significantly ($P \leq 0.01$) higher concentration of trace elements than crop tissue grown on unamended soils.

^aOptimum values are for top of plant 3–4 weeks between clippings. Whole plants cut for hay production were sampled for analysis (Jones, Wolf, and Mills 1991).

^bOptimum values are for sorghum (*S. bicolor*) for third leaf below head at bloom stage. Whole plants cut for silage production were sampled for analysis (Jones, Wolf, and Mills 1991).

^cOptimum values are for most recent fully developed leaf prior to pod set. Whole plants cut for silage production were sampled for analysis (Jones, Wolf, and Mills 1991).

increases in concentrations of tissue Zn in alfalfa, sudangrass, and soybean and Cu and Zn in orchardgrass to levels greater than what would be considered sufficient for optimum crop growth (Table 6) (Jones, Wolf, and Mills 1991). Despite these increases in tissue concentration, there was no negative effect of biosolid application on crop yields (data not shown), and no visible signs of Cu or Zn toxicity were observed.

CONCLUSIONS

Commercial-scale agronomic biosolid utilization has increased Cu, Ni, Pb, and Zn concentrations in one or more operationally defined soil fractions. Application of biosolids led to minimal changes in the overall distribution of Cr, Ni, and Pb among soil fractions. Application of biosolids also did not increase concentrations of these metals in the most labile soil fractions. Thus, at the relatively low loadings of these trace metals to soils, biosolid application appears to have had little effect on trace-element bioavailability. In contrast, application of biosolids resulted in a shift in the overall distribution of Cu and Zn toward the more labile soil fractions due to larger increases in Cu and Zn concentrations in those fractions than in the more recalcitrant

fractions. The differing responses for these metals generally can be attributed to the much larger soil loadings of Cu and Zn than Cr, Ni, and Pb when biosolids were land applied. Nevertheless, loadings of Cu and Zn were still much smaller than cumulative loadings permitted under USEPA Part 503 regulations. These data cannot predict if the observed shift of Cu and Zn toward more labile soil fractions will continue as cumulative loadings increase.

Operationally defined soil fractions (as determined by chemical fractionation) were generally poorly correlated with crop accumulation, particularly for Ni and soils receiving lower cumulative loadings of Cu ($<38 \text{ kg ha}^{-1}$) and Zn ($<48 \text{ kg ha}^{-1}$). However, at sites with larger biosolids loadings of Cu (59 kg ha^{-1}) and Zn ($77\text{--}80 \text{ kg ha}^{-1}$), Cu and Zn concentrations in the tissues of sudangrass and orchardgrass were higher when crops were grown on biosolid-amended soils and were correlated with Cu and Zn concentrations in the soil fractions. These results demonstrate that chemical soil fractionation can detect increases in labile Cu and Zn that relate to increased phytoavailability. Because plant roots may take up trace metals with little translocation to shoots, additional research in this area should investigate the relationships between trace-element correlations in crop roots and soil fractions.

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