Fate of Biosolids Trace Metals in a Dryland Wheat Agroecosystem

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Biosolids land application for beneficial reuse applies varying amounts of trace metals to soils. Measuring plant-available or total soil metals is typically performed to ensure environmental protection, but these techniques do not quantify which soil phases play important roles in terms of metal release or attenuation. This study assessed the distribution of Cd, Cr, Cu, Mo, Ni, Pb, and Zn associated with soluble/exchangeable, specifically adsorbed/carbonate-bound, amorphous Mn hydroxyoxide-bound, amorphous Fe hydroxyoxide–bound, organically complexed, and residual inorganic phases. Biosolids were applied every 2 yr from 1982 to 2002 (except in 1998) at rates of 0, 6.7, 13.4, 26.8, and 40.3 dry Mg biosolids ha^{-1} to 3.6- by 17.1-m plots. In 2003, 0- to 20-cm and 20- to 60-cm soil depths were collected and subjected to 4 mol L^{-1} HNO$_3$ digestion and sequential extraction. Trace metals were concentrated in the 0- to 20-cm depth, with no significant observable downward movement using 4 mol L^{-1} HNO$_3$ or sequential extraction. The sequential extraction showed nearly all measurable Cd present in relatively mobile forms and Cr, Cu, Mo, Ni, Pb, and Zn present in more resistant phases. Biosolids application did not affect Cd or Cr fractionation but did increase relatively immobile Cu, Mo, and Zn phases and relatively mobile Cu, Ni, and Pb pools. The mobile phases have not contributed to significant downward metal movement. Long-term, repeated biosolids applications at rates considered several times greater than agronomic levels should not significantly contribute to downward metal transport and ground water contamination for soils under similar climatic conditions, agronomic practices, and histories.

BIOSOLIDS land application is a major method of disposal in the USA, with approximately 50% being land applied (USEPA, 2007a); in U.S. Environmental Protection Agency (USEPA) Region 8, which encompasses Colorado, Wyoming, Utah, Montana, North Dakota, and South Dakota, 85% of biosolids are land applied (USEPA, 2007b). This recycling method can greatly benefit municipalities by recycling plant nutrients in an environmentally sound manner if proper agronomic practices are followed. Our 20-yr biosolids project with the cities of Littleton and Englewood, Colorado (L/E) has provided valuable information on the effects of biosolids-borne metals application to dryland winter wheat agroecosystem soils. For example, Barbarick et al. (1997) suggested the use of ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) instead of 4 mol L^{-1} HNO$_3$ for monitoring soil trace metal concentrations because the AB-DTPA extraction is a more sensitive technique for elucidating changes in soil metal concentrations. In a follow-up study, Barbarick et al. (1998) observed significant AB-DTPA–extractable trace element accumulations in the plow layer as affected by L/E biosolids. Lacking from these studies was a more detailed analysis pertaining to the fate of biosolids-borne metals within this long-term, biosolids-amended agricultural soil.

In a column study, Emmerich et al. (1982a) studied a soil heavily amended with biosolids and found little metal movement below the incorporation zone. They attributed the lack of movement to metals shifting toward more stable, residual forms after soil incorporation (Emmerich et al., 1982b). Dowdy et al. (1991) and Sloan et al. (1998), however, observed increased trace metal concentrations with depth in biosolids-treated soil as compared with a control soil. McBride et al. (1999) noticed metal transport into ground water below a soil amended heavily with biosolids and associated the movement with dissolved organic matter. Al-Wabel et al. (2002) used soils from the Barbarick et al. (1998) study site, noting that biosolids application increased dissolved organic carbon (DOC) and Cu in column effluents. A positive correlation between Cu and DOC was also observed.

More complete, detailed biosolids-amended soil analyses have used sequential extraction procedures to operationally define

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Abbreviations: AB-DTPA, ammonium bicarbonate-diethylenetriaminepentaacetic acid; DOC, dissolved organic carbon; ICP-AES, inductively coupled plasma–atomic emission spectrometry; L/E, Littleton and Englewood, Colorado; USEPA, U.S. Environmental Protection Agency.

soil metal pools. Sloan et al. (1997) determined the fate of biosolids-borne metals using a sequential extraction procedure and noted >75% of Cr, Cu, Ni, Pb, and Zn to be found in relatively stable soil fractions after biosolids applications of up to 224 Mg ha^{-1}. The authors also noted that the majority of Cd was located in more bioavailable (and thus potentially more mobile) forms. Sukkariyah et al. (2005) used a sequential extraction on soils receiving up to 210 Mg ha^{-1} biosolids, showing that the greatest concentrations of Zn, Cu, and Ni were associated with soil metal oxides; Cd was only detected in the exchangeable and specifically adsorbed phases, similar to the findings of Sloan et al. (1997).

Exchangeable and specifically adsorbed phases can be considered readily bioavailable. Guerra et al. (2007) used a sequential extraction procedure to study the effects of biosolids-borne metal additions to a Mollisol. Biosolids application at 30 Mg ha^{-1} increased Zn in labile fractions, a finding similar to previous reports (Thompson et al., 2001; Hseu, 2006). Copper concentration in the organic matter phase increased after biosolids application, with Cu forming relatively strong complexes with biosolids fulvic acid functional groups (Guerra et al., 2007; Dahlgren et al., 1997). Al-Wabel et al. (2002) observed a correlation between DOC and Cu content in biosolids-amended soil leachates. Berti and Jacobs (1996) showed that total biosolids land applications of up to 690 Mg ha^{-1} increased Cd, Ni, and Zn in bioavailable fractions, increased Cu and Cr to a lesser extent, and did not increase bioavailable Pb.

Sequential extractions are time consuming, but research on soil-trace metal behavior after years of biosolids application is needed to understand long-term effects on agricultural soils (Sukkariyah et al., 2005). Furthermore, such information is necessary for heavy metal environmental impact purposes and for improvement of biosolids land application regulatory guidelines (Vaca-Paulín et al., 2006). Our project presents a more complete assessment of metal mobility, extractability, and fate, providing further evaluation of recycling biosolids on agricultural lands. Our goal was to identify the dominant metal pools in a soil under a dryland wheat agroecosystem after 20 yr of biennial biosolids application and to explain any discrepancies within or between various soil metal phases. Our hypotheses were that repeated, long-term biosolids applications (i) cause permanent changes in soil Cd, Cr, Cu, Mo, Ni, Pb, and Zn fractions, which in turn affect metal mobility, extractability, and fate and (ii) show the largest pools of Cd, Cr, Cu, Mo, Ni, Pb, and Zn reside in more resistant phases.

Materials and Methods

Site Description

This study is part of a larger experiment that was described by Barbarick et al. (1995). The dryland winter wheat (*Triticum aestivum* L.) agroecosystem study was started in the summer of 1982 on plots approximately 30 km east of Brighton, Colorado, USA. Briefly, mean annual maximum and minimum temperatures, mean annual precipitation, and the annual growing season are 19°C, 2°C, 35 cm, and about 150 d, respectively (USDA-NRCS,

1974). A dryland summer fallow rotation system was used in which one crop is produced every other year. The soil was a Platner loam (fine, smectitic, mesic Aridic Paleustoll).

Biosolids: Generation, Application, Experimental Design, and Metals Content

Littleton/Englewood, Colorado wastewater treatment facility biosolids were generated by anaerobic digestion of domestic sewage water, followed by approximately 2 mo of sand-bed drying. Every 2 yr from 1982 to 2002 (except in 1998), we applied L/E biosolids at rates equivalent to 0, 6.7, 13.4, 26.8, and 40.3 dry Mg biosolids ha^{-1} to 3.6- by 17.1-m plots sown with winter wheat. We did not apply biosolids in 1998 because we had been informed that the land where the plots were located may be sold. The 6.7 Mg ha^{-1} application rate was considered "agronomic" for dryland winter wheat, supplying the N needs of the crop throughout the growing season. The 40.3 Mg ha^{-1} rate was discontinued in 1992 due to excessive accumulation of soil NO$_3$–N. Since 1992, the study of the 40.3 Mg ha^{-1} rate has focused on system recovery. We used four replications of all biosolids application rates in a randomized, complete block arrangement. We weighed the biosolids (solids content of 530–880 g kg^{-1}) and corrected for moisture content, evenly spread them over the plots using a front-end loader, hand raked them to improve the uniformity of distribution, and incorporated the biosolids to a depth of 10 to 15 cm with a rototiller.

Biosolids samples were collected before each application and kept refrigerated at approximately 3°C, and Cd, Cr, Cu, Mo, Ni, Pb, and Zn composition was determined by HClO$_4$–HNO$_3$–HF-HCl digestion using inductively coupled plasma–atomic emission spectroscopy (ICP-AES) (Soltanpour et al., 1996). Table 1 provides the biosolids-borne metal concentrations applied from 1982 through 2002, and Table 2 provides the cumulative biosolids-borne metals loadings as a function of application rate.

Soil Sampling and Basic Soil Analysis

Immediately after the July 2003 winter wheat harvest, we collected composite soil samples (two to three cores per plot) from the 0- to 20-cm (plow layer) and 20- to 60-cm depth from all treatments and replicates. This led to a total of 40 samples collected, the result of four replications over five treatments by two depths. We obtained samples near the center of each plot to avoid the biosolids redistribution problem associated with tillage operations (Yingming and Corey, 1993). We immediately air-dried the soil samples and then crushed them to pass a 2-mm sieve.

Soil from the 0- to 20-cm depth was analyzed for pH (Thomas, 1996) and electrical conductivity (Rhoades, 1996) using a saturated paste extract. Organic C content was determined as the difference between total C (Nelson and Sommers, 1996) and inorganic C (Sherrod et al., 2002), and AB-DTPA–extractable (Barbarick and Workman, 1987) Fe, Mn, and Ca were analyzed using ICP-AES. The data are presented in Table 3.

Table 1. Characteristics of Littleton/Englewood, Colorado, biosolids used at the dryland winter wheat site near Bennett, Colorado, 1982–2002. Biosolids were not applied in 1998 due to a potential land sale.

Parameter	1982	1984	1986	1988	1990	1992	1994	1996	2000	2002	Mean
					mg kg^{-1} (dry weight basis)						
Cd	<30†	8	10	12	9	7	7	6	2	1	7 (3)‡
Cr	256	175	131	113	165	98	80	59	17	10	110 (72)
Cu	1040	462	359	917	865	862	493	657	352	326	634 (256)
Mo	59	12	7	20	32	22	22	24	6	16	22 (14)
Ni	73	56	47	66	93	177	65	52	1	8	64 (46)
Pb	271	531	206	228	134	30	81	27	23	10	154 (154)
Zn	1980	751	618	1050	1300	1300	816	652	370	351	920 (476)

† We did not include this concentration in the cumulative metal loading calculation (Table 2) because we could not accurately determine Cd with the instrumentation available at the time.

‡ Values within parentheses represent 1 SEM.

4 mol L^{-1} HNO$_3$ Soil Metal Extraction

All soils were extracted with 4 mol L^{-1} HNO$_3$ (Bradford et al., 1975), with the amount of metal extracted a close approximation to total soil elements. Barbarick et al. (1997), using soils from this research site, showed that the 4 mol L^{-1} HNO$_3$ digest extracted an average of 115% Cd, 98% Cu, 138% Pb, 87% Ni, and 104% Zn as compared with a standard nitric-perchloric acid digest. McBride et al. (1997) showed the 4 mol L^{-1} HNO$_3$ digestion to be as effective as a standard nitric-perchloric acid digest for solubilizing Cd, Cr, Cu, Ni, Pb, and Zn. The 4 mol L^{-1} HNO$_3$–extraction procedure consisted of 1 g of soil placed in a 50-mL digestion tube, predigested in 10 mL of 4 mol L^{-1} HNO$_3$ overnight, then digested at 80°C for 6 h with vortexing every 2 h. The solution was brought to a final volume of 12.5 mL and filtered through Whatman #5 filter paper, and solution metal concentrations were determined using ICP-AES.

Soil Heavy Metal Fractionation

All soils were sequentially extracted to fractionate soil heavy metal chemical pools based on their relative binding strength (Sloan et al., 1997). One gram of soil was placed in a 50-mL centrifuge tube and sequentially extracted for (i) soluble/exchangeable, (ii) specifically adsorbed/carbonate bound, (iii) amorphous Mn hydroxyoxide bound, (iv) amorphous Fe hydroxyoxide bound, (v) organically complexed, and (vi) residual inorganic using various extractants. Designation of metal phases followed suggestions by Rao et al. (2008), with the exception of amorphous Fe hydroxyoxide–bound met-

als. Rao et al. (2008) suggested the use of "amorphous Fe/Mn hydroxyoxide–bound," but the extractant used in step 4 released approximately 55 times more Fe than Mn into solution. Thus, the majority of metals were associated with Fe phases and supported the naming of this phase as "amorphous Fe hydroxyoxide–bound." The solution amounts and experimental/analytical conditions used are shown in Table 4. The extraction steps at room temperature were performed on a reciprocating shaker at 120 oscillations min^{-1}; heated extractions were completed on an orbital shaker in a thermostat-controlled hot water bath. After each extraction step, samples were centrifuged at $7000 \times g$ for 10 min. The solution was decanted into glass vials and filtered through a 0.2-μm nylon membrane filter and analyzed for metal concentrations using ICP-AES. Percent recovery of total metals recovered from the sequential extraction procedure was compared with the 4 mol L^{-1} HNO$_3$ soil extraction.

Statistical Analysis

The experimental design consisted of five treatments (0, 6.7, 13.4, 26.8, and 40.3 dry Mg biosolids ha^{-1}) by two depths (0–20 and 20–60 cm) by four replicates, for a total of 40 soil samples collected. All data was tested for normality; then analyses were performed on all data (all replicates; $n = 4$) using the Proc GLM model in SAS software version 9.1 (SAS

Table 2. Biosolids application rates and cumulative biosolids and metal loadings at the dryland winter wheat site near Bennett, Colorado, from 1982–2002.

Biosolids application rate yr^{-1}	Cumulative biosolids loading rate	Cd	Cr	Cu	Mo	Ni	Pb	Zn
	Mg ha^{-1}			kg ha^{-1} (dry weight basis)				
0	0	0	0	0	0	0	0	0
6.7	67	0.41	7.40	42.4	1.48	4.27	10.3	61.6
13.4	134	0.83	14.8	84.9	2.97	8.54	20.7	123
26.8	268	1.66	29.6	170	5.93	17.1	41.3	246
40.3†	202	1.59	33.8	147	5.25	13.5	55.2	230

† The 40.3 Mg ha^{-1} rate was discontinued in 1992 due to excessive accumulation of soil NO$_3$–N. Between 1982 and 1990, five applications of 40.3 Mg ha^{-1} were applied.

Table 3. Effect of biosolids rate on the 2003 harvest soil pH, electrical conductivity (EC), organic C content, and ammonium bicarbonate-diethylenetriaminepentaacetic acid–extractable Fe, Mn, and Ca in the 0- to 20-cm depth at the dryland winter wheat site near Bennett, Colorado.

Constituent	Biosolids application rate (Mg ha^{-1})					Significance	LSD
	0	6.7	13.4	26.8	40.3†		
pH	6.8	6.4	6.2	6.0	6.2	NS‡	
EC, dS m^{-1}	0.63	1.64	1.78	2.64	0.91	*	0.94
Total C, %	0.84	1.25	1.28	2.05	1.36	*	0.47
Inorganic C, %	0.06	0.05	0.03	0.04	0.01	NS	
Organic C, %	0.78	1.20	1.25	2.01	1.35	*	0.45
Fe, mg kg^{-1}	6.5	15.6	17.2	23.6	23.9	*	9.7
Mn, mg kg^{-1}	11.0	23.0	26.2	34.7	21.4	*	10.2
Ca, mg kg^{-1}	417	387	409	395	435	NS	

* Significant at the 0.05 probability level.

† The 40.3 Mg ha^{-1} rate was discontinued in 1992 due to excessive accumulation of soil NO$_3$–N. Between 1982 and 1990, five applications of 40.3 Mg ha^{-1} were applied.

‡ NS, not significant.

Table 4. Sequential extraction steps and conditions used (based on Sloan et al., 1997; modified from Gibson and Farmer, 1986).

Step	Extractant and conditions	Metal fraction removed
1	25 mL of 0.5 mol L^{-1} Ca(NO_3)$_2$; 25°C; 18 h	soluble/exchangeable
2	15 mL of 1 mol L^{-1} NaOAc (pH 5); 25°C; 5 h	specifically sorbed/carbonate bound
3	20 mL 0.1 mol L^{-1} NH_2OH(HCl) in 0.01 mol L^{-1} HNO_3; 25°C; 0.5 h	amorphous Mn oxyhydroxide bound
4	20 mL 1 mol L^{-1} NH_2OH(HCl) in 25% HOAc (pH 2); 95°C; 6 h	amorphous Fe oxyhydroxide bound
5	3 mL 0.02 mol L^{-1} HNO_3 + 5 mL 30% H_2O_2; 85°C, 4 h, followed by the addition of 5 mL 3.2 mol L^{-1} NH_4OAc + 5 mL 20% HNO_3; 25°C; 0.5 h	organically complexed
6	10 mL 4 mol L^{-1} HNO_3; 95°C; 16 h	residual inorganic

Institute, 2002) to evaluate the effect of biosolids application rate on 4 mol L^{-1} HNO_3 and soil heavy metal fractionation. We tested our hypotheses at P = 0.05 and calculated a Fisher's protected LSD when significance was observed.

Results and Discussion

4 mol L^{-1} HNO_3 Soil Metal Extraction

Increasing biosolids application rate significantly increased total soil Cr, Cu, Pb, and Zn in the 0- to 20-cm depth (Table 5). Differences did not exist between increasing biosolids application rate and metal concentrations in the 20- to 60-cm depth (Table 5), but a statistically nonsignificant trend was observed for detectable metals. We expected to observe increases in the soil surface because biosolids are a source of these elements and were incorporated into this depth. Others have noted similar increases in total soil metal concentrations (Guerra et al., 2007; Sukkariyah et al., 2005; Qiao et al., 2003; McBride et al., 1997; Berti and Jacobs, 1996).

Total Cr, Cu, Pb, and Zn concentrations extracted from the 40.3 Mg ha^{-1} biosolids-amended plots, 13 yr since being discontinued, were still influenced by biosolids application (Table 5). The 40.3 Mg ha^{-1} plots received a total of 202 Mg biosolids ha^{-1}, as compared with 134 and 268 total Mg biosolids ha^{-1} for the 13.4 and 26.8 Mg ha^{-1} applied plots, respectively (Table 2), and thus it might be expected that total metal concentrations would fall between those present in the 13.4 and 26.8 Mg ha^{-1} plots. However, total metal applied should provide a stronger relationship between the discontinued and other application rates than the total biosolids applied.

Total Cr applied was slightly greater in the 40.3 Mg ha^{-1} versus 26.8 Mg ha^{-1} plots (Table 2), and thus we could expect concentrations to be similar between the two rates, as was observed (Table 5). Total Cu applied with the 40.3 Mg ha^{-1} rate was more similar to the 26.8 Mg ha^{-1} rate, yet the 4 mol L^{-1} HNO_3 soil Cu concentration in the discontinued 40.3 Mg ha^{-1} plots was similar to that at the 6.7 and 13.4 Mg ha^{-1} rates. Copper, over time, may have been transformed to more resistant phases not easily extracted with 4 mol L^{-1} HNO_3. Total Pb applied with the 40.3 Mg ha^{-1} rate was much greater than the 26.8 Mg ha^{-1} rate, yet the 4 mol L^{-1} HNO_3 Pb soil concentration in the 40.3 Mg ha^{-1} plots was slightly less than the 26.8 Mg ha^{-1} rate, suggesting that transformations to forms more resistant to 4 mol L^{-1} HNO_3 had occurred. Total Zn applied with the 40.3 Mg ha^{-1} rate was more similar to the 26.8 Mg ha^{-1} rate, yet the 4 mol L^{-1} HNO_3 soil Zn concentration in the

40.3 Mg ha^{-1} plots was similar to the 6.7 and 13.4 Mg ha^{-1} rates. This also suggests that Zn in labile phases was, over time, fixed as a more resistant form not easily extractable by 4 mol L^{-1} HNO_3.

The decrease in the 40.3 Mg ha^{-1} rate Cu, Pb, and Zn soil surface concentrations could not be fully explained by metal movement into the subsoil or dilution due to the subsoil depth increment sampled. Over the entire study period, total Cu, Pb, and Zn applied to the soil with the 40.3 Mg ha^{-1} rate were approximately 66, 25, and 103 mg kg^{-1}, respectively. The 40.3 Mg ha^{-1} soil surface Cu content was approximately 31 mg kg^{-1}, so if Cu leaching were solely occurring, then 35 mg kg^{-1} of Cu would have been transported into the 20- to 60-cm depth. However, subsoil Cu content in the 40.3 Mg biosolids ha^{-1} treatment was only 9.4 mg kg^{-1}. Assuming the control (0 Mg ha^{-1}) subsoil was unaffected by biosolids application, it contained 7.8 mg kg^{-1} of Cu, and thus an increase of 1.6 mg Cu kg^{-1} associated with the 40.3 Mg ha^{-1} would have occurred. Based on soil dilution (i.e., sampling the 20- to 60-cm depth, or 40 cm total), 35 mg kg^{-1} of Cu would have only moved into the top 1.8-cm of subsoil to produce an observed increase of 1.6 mg Cu kg^{-1} over the background subsoil Cu content. Performing a similar calculation for Pb and Zn suggests movement only into the top 1 and 6 cm of subsoil, respectively. These results further support the contention that soil surface metal concentrations were transformed to more resistant forms.

Buekers et al. (2007) noted that soil fixation of Zn, Cu, Ni, and Cd salts increased over time and suggested that Cd, Zn, and Ni fixation was related to pH-dependent diffusion into oxides and that Cd and Zn were also related to carbonate co-precipitation. Ma et al. (2006) studied Cu transformations for various soils over a 2-yr incubation period and showed that after water-soluble Cu was added to soil, concentrations decreased rapidly as determined by isotopic analysis, total and free Cu in soil pore water, and diffusive gradient in thin film. Ma et al. (2006) suggested that soil pH, Cu precipitation/nucleation, and Cu^{2+} hydrolysis followed by diffusion controlled Cu availability with time. Tye et al. (2003) added 300 and 3.0 mg kg^{-1} Zn and Cd to soil and measured metal availability over 818 d. Soils low in pH tended to show little decrease in lability, whereas higher-pH soils showed greater decreases in availability. The authors suggested that changes in Cd and Zn availability resulted from time-dependent metal fixation followed by a readjustment of soil pH. Rajaie et al. (2008) added $NiSO_4$ to soil and incubated for up to 16 wk. Near the beginning of the experiment, Ni seemed to be more

Table 5. Effect of biosolids rate on the 2003 wheat harvest soil 4 mol L⁻¹ HNO₃ extractable metal concentrations, distribution of and total metals sequentially extracted, and percent recovery of total metals extracted sequentially as compared with 4 mol L⁻¹ HNO₃ extractable metals, 0- to 20-cm soil depth. ($n = 4$).

Biosolids rate	4 mol L⁻¹ HNO₃	A†	B	C	D	E	F	Sequential extraction total metal	Recovery
Mg ha⁻¹					mg kg⁻¹				%
					Cd				
0	ND‡ (ND)§	ND	1.151	ND	ND	ND	ND	1.151	
6.7	ND (ND)	ND	0.830	0.028	ND	ND	ND	0.857	
13.4	ND (ND)	ND	1.092	0.028	ND	ND	ND	1.120	
26.8	ND (ND)	ND	0.714	0.032	ND	ND	ND	0.745	
40.3¶	ND (ND)	ND	1.005	0.024	ND	ND	ND	1.029	
Significance			NS#	NS					
LSD									
					Cr				
0	4.8 (6.5)	ND	ND	ND	3.78	0.65	10.8	15.3	317
6.7	7.5 (7.0)	ND	ND	ND	5.04	1.00	11.5	17.5	232
13.4	7.3 (7.2)	ND	ND	ND	5.30	1.12	11.3	17.7	242
26.8	10.6 (7.5)	ND	ND	ND	6.18	1.58	9.85	17.6	166
40.3	9.8 (7.3)	ND	ND	ND	6.32	1.45	11.2	18.9	194
Significance	* (NS)				NS	NS	NS		
LSD	2.1								
					Cu				
0	6.2 (7.8)	0.06	0.05	ND	4.07	0.96	2.97	8.11	131
6.7	21.4 (8.9)	ND	0.71	ND	9.94	1.47	3.80	15.9	74
13.4	22.8 (10.0)	ND	1.14	ND	13.0	1.88	4.07	20.1	88
26.8	53.5 (9.6)	0.09	1.98	ND	20.1	2.39	4.47	29.0	54
40.3	30.9 (9.4)	0.10	1.49	ND	19.9	2.10	4.60	28.2	91
Significance	* (NS)	NS	*		*	*	*		
LSD	12.0		0.86		10.61	0.71	1.18		
					Mo				
0	ND (ND)	0.282	ND	ND	0.507	ND	0.044	0.833	
6.7	ND (ND)	0.037	ND	ND	0.535	ND	0.108	0.680	
13.4	ND (ND)	0.056	ND	ND	0.547	ND	0.106	0.708	
26.8	ND (ND)	0.075	ND	ND	0.771	ND	0.144	0.990	
40.3	ND (ND)	0.186	ND	ND	0.619	ND	0.152	0.957	
Significance		NS			NS		*		
LSD							0.062		
					Ni				
0	5.97 (7.86)	0.49	0.53	0.16	2.50	0.52	2.53	6.72	113
6.7	7.64 (8.22)	1.49	0.66	0.15	2.83	0.54	2.54	8.22	108
13.4	7.25 (8.56)	1.27	0.95	0.14	3.01	0.56	2.53	8.46	117
26.8	8.87 (8.71)	2.56	0.68	0.05	2.69	0.57	2.29	8.84	100
40.3	7.61 (8.83)	1.67	0.93	0.21	4.05	0.58	2.49	9.92	130
Significance	NS (NS)	*	*	NS	NS	NS	NS		
LSD		1.04	0.31						
					Pb				
0	0.95 (2.15)	0.046	0.650	0.059	5.455	ND	ND	6.210	656
6.7	2.76 (2.54)	0.038	1.199	0.033	8.106	0.062	ND	9.438	342
13.4	2.85 (2.58)	ND	1.185	0.019	9.702	0.136	0.015	11.06	389
26.8	6.70 (2.63)	ND	1.320	0.026	12.39	0.180	0.100	14.02	209
40.3	6.01 (2.59)	ND	1.252	0.024	12.28	0.118	0.020	13.70	228
Significance	* (NS)	NS	*	NS	NS	NS	NS		
LSD	3.1		0.299						
					Zn				
0	41 (42)	2.41	3.98	ND	25.8	5.09	26.9	64.2	155
6.7	65 (44)	5.64	4.10	ND	46.2	5.02	28.4	89.4	137
13.4	66 (46)	0.92	5.34	0.12	51.2	5.49	27.9	91.0	137
26.8	97 (45)	4.33	6.90	0.34	64.5	3.39	25.2	105	108
40.3	78 (46)	3.41	7.53	0.08	62.5	5.41	27.0	106	135
Significance	* (NS)	NS	NS	NS	*	NS	NS		
LSD	14				18.4				

* Significant at the 0.05 probability level.

† Sequential extraction designations: A, soluble/exchangeable; B, specifically adsorbed/carbonate–bound; C, amorphous Mn hydroxyoxide–bound; D, amorphous Fe hydroxyoxide–bound; E, organically complexed; F, residual inorganic.

‡ ND, nondetectable.

§ Values in parentheses for 4 mol L⁻¹ HNO₃ represent 20- to 60-cm soil depth metal concentrations.

¶ The 40.3 Mg ha⁻¹ rate was discontinued in 1992 due to excessive accumulation of soil NO3–N. Between 1982 and 1990, five applications of 40.3 Mg ha⁻¹ were applied.

NS, not significant.

bioavailable; given time, Ni became less available, associating with oxides to a greater extent.

Soil Heavy Metal Fractionation

Total soil metal concentrations do not furnish sufficient evidence regarding the potential transformations and element availability (Srikanth and Reddy, 1991) because trace metals can be associated with various soil fractions that affect their mobility and fate (Sukkariyah et al., 2005). Therefore, gathering such information is necessary for predicting the environmental impact of metals from anthropogenic sources such as biosolids (Vaca-Paulin et al., 2006). Sequential extraction methods "operationally define" trace element fractionation into chemical pools, and the extraction is not perfectly selected (Basta et al., 2005). However, sequential extractions can be useful in determining potential bioavailability of metal contaminants (Basta et al., 2005).

To differentiate metals associated with various soil fractions, we used a heavy-metal sequential extraction (Sloan et al., 1997; Gibson and Farmer, 1986), analyzing for Cd, Cr, Cu, Mo, Ni, Pb, and Zn associated with (i) soluble/exchangeable, (ii) specifically adsorbed/carbonate–bound, (iii) amorphous Mn hydroxyoxide-bound, (iv) amorphous Fe hydroxyoxide–bound, (v) organically complexed, and (vi) residual inorganic soil phases within the 0- to 20-cm and 20- to 60-cm soil depths. The names of several operationally defined pools (Sloan et al., 1997) were modified based on a recent review of sequential extraction methodologies (Rao et al., 2008). The 0- to 20-cm soil depth metal fractionation results are presented in Table 5. As with the 4 mol L^{-1} HNO_3 extraction, no significant differences were observed in the 20- to 60-cm sequential trace metal chemistry, and therefore the data are not shown.

Cadmium

Within the 0- to 20-cm depth, nearly all measurable soil Cd was found in the specifically adsorbed/carbonate phase (97–100%), with minor amounts bound to amorphous Mn hydroxyoxides. Sloan et al. (1997) found similar fractionation results, noting a significant amount of Cd additionally associated with the exchangeable fraction. The Sloan et al. (1997) findings, however, were based on observations from a biosolids-amended site that received a cumulative Cd content ranging from 9 to 25 kg ha^{-1}. Our site received a maximum cumulative Cd load of 1.66 kg ha^{-1} (Table 2). Biosolids application did not significantly affect soil Cd content within either depth for all soil phases, likely due to the relatively small cumulative Cd quantities added with increasing biosolids application.

Biosolids-borne Cd has been shown to be relatively available (Walter et al., 2006), with extractable Cd concentrations measured in biosolids amended soils (Berti and Jacobs, 1996). Li et al. (2001) noted an increase in Cd sorption with increasing biosolids application, and on removal of the soil organic phase, Cd sorption persisted. The authors attributed adsorption to the inorganic fraction provided by biosolids. Hettiarachchi et al. (2003) showed reduced soil Cd adsorption by removing the organic C and Fe/Mn fractions. An increase in the AB-DTPA–extractable Mn concentration was observed with increasing biosolids application (Table 3), potentially influencing Cd adsorption to the amorphous Mn hydroxyoxide phase.

Chromium

Within the 0- to 20-cm depth, soil Cr concentration was dominated by the residual inorganic fraction (56–71%), with lesser amounts located in the amorphous Fe hydroxyoxide–bound fraction (25–35%); minor quantities were present in the organically complexed phase (4–9%). Chromium tends to form stable, residual mineral phases because at pH and redox conditions prevailing in most soils, Cr mainly occurs as the slightly soluble $Cr(OH)_3$ (de Haan and Zwerman, 1978). Biosolids-borne Cr is most likely immobile and thus unavailable in the soluble form (Wong et al., 2001). Walter et al. (2006) used a sequential extraction procedure, noting that >95% of biosolids-borne Cr resided as insoluble forms. Biosolids application rate in the current study did not affect Cr fractionation.

Others have noted changes in soil Cr fractionation with biosolids application. Sloan et al. (1997) applied up to 224 Mg biosolids ha^{-1} and found most Cr to reside in the amorphous Fe hydroxyoxide fraction, with lesser amounts found in the organically complexed and inorganic fractions. McGrath and Cegarra (1992) noted that Cr in the less labile (i.e., resistant) fraction dominated all fractions even after several decades after approximately 400 Mg ha^{-1} of total biosolids application. Sims and Kline (1991) studied co-composted biosolids application at rates up to 44 Mg ha^{-1} and showed that Cr concentrations in soil increased primarily in more resistant phases. All of these phases are considered relatively immobile.

Copper

Soil Cu concentrations were greatest in the amorphous Fe hydroxyoxide fraction (50–70%) in the 0- to 20-cm depth, with lesser amounts found in the residual inorganic (15–37%), organically complexed (7–12%), and specifically adsorbed/carbonate–bound (1–7%) fractions. Biosolids application caused an increase in the 0- to 20-cm depth specifically adsorbed/carbonate–bound, amorphous Fe hydroxyoxide fraction, organically complexed, and residual inorganic phases. Increases in the amorphous Fe hydroxyoxide and organically complexed phases were most likely due to increasing biosolids application rate increasing AB-DTPA–extractable Fe content and organic C percentage (Table 3). Sloan et al. (1997) showed significant increases in all Cu fractions, but biosolids application increased Cu to the greatest extent in the adsorbed and amorphous Fe hydroxyoxide fraction. Guerra et al. (2007) incubated soils treated with 30 Mg ha^{-1} biosolids for 60 d, observing an increase in Cu associated with the organic matter fraction. Ahumada et al. (2004) treated several soils with 30 Mg biosolids ha^{-1} and showed that biosolids increased Cu in the stable residual phases.

We expected to observe an increase in the organically complexed Cu phases because Cu tends to make strong complexes with organic soil compounds. Moreover, the biosolids-borne,

organically bound Cu has been shown to be between approximately 50 and 80% of the total Cu present (Alloway, 1995; Walter et al., 2006; Wong et al., 2001). Relatively strong complexes with biosolids fulvic acid functional groups are assumed to occur (Dahlgren et al., 1997). In our system, biosolids-borne Cu was probably added as an organically complexed phase and given time was mineralized and released and created associations with the specifically adsorbed/carbonate, organically complexed, and residual inorganic phases. Of these phases, the specifically adsorbed/carbonate phase has a greater potential to contribute to downward Cu transport. However, biosolids did not affect Cu fractionation in the 20- to 60-cm depth (data not shown). A nonsignificant trend was present in the 20- to 60-cm depth of increasing amorphous Fe hydroxyoxide–bound Cu with increasing biosolids application rate (data not shown). Al-Wabel et al. (2002) used the 26.8 Mg ha^{-1} soils from the current study site, noting that biosolids application increased DOC and Cu in column effluents, with Cu concentrations approaching 0.30 mg L^{-1}. Thus, leachate Cu concentrations from all biosolids rates would be low enough to be less of a health concern based on the USEPA drinking water standard of 1.3 mg Cu L^{-1} (USEPA, 2007c).

Molybdenum

Soil Mo concentration was greatest in the amorphous Fe hydroxyoxide fraction (61–79%) in the 0- to 20-cm depth. This phase most likely dominated because Mo reacts strongly with iron hydroxyoxides (Bohn et al., 1985; Sparks, 2003). Biosolids with a high Fe oxide content also adsorb molybdate and decrease its phytoavailability (Basta et al., 2005). Brinton and O'Connor (2000) observed an increase in total soil Mo but a decrease in Mo solubility with biosolids addition to an uncontaminated soil. The decrease in Mo solubility was attributed to amorphous Fe and Al phases present in biosolids.

Ippolito et al. (2007) noted that biosolids applied to the current research site contained appreciable quantities of total Fe (14.2 ± 5.4 g Fe kg^{-1}). This was most likely due to Fe$_2$(SO$_4$)$_3$ addition at the wastewater treatment facility inflow to reduce digester-gas H$_2$S production. Increasing biosolids application rate increased extractable soil Fe content (Table 3), and the biosolids Fe present was in part responsible for Mo sorption in the amorphous Fe hydroxyoxide fraction. Although biosolids application did not significantly affect amorphous Fe hydroxyoxide–bound Mo, the trend was toward increasing biosolids application increasing Mo in this phase.

An increase in the residual inorganic Mo phase with increasing biosolids application was observed in the 0- to 20-cm residual depth. Molybdenum may have formed occluded phases within stable mineral species. This phase may be considered residual inorganic if adsorption chemistry followed that of surface adsorption, followed by diffusion into the lattice of mineral phases. Lang and Kaupenjohann (1999) observed continued extractability of soil Mo even after Fe mobilization had ceased, suggesting interlattice Mo adsorption by mineral phases such as Fe-oxides. Smith and Leeper (1969) added Mo to soil and incubated for up to 10 mo and showed up to 40% of the added

Mo was bound in recalcitrant forms. The authors suggested that Mo was slowly transformed from more bioavailable forms to more resistant forms a few months after Mo application.

Nickel

Soil Ni concentration was greatest in the amorphous Fe hydroxyoxide fraction (34–41%), with slightly less amounts in the residual inorganic fraction (25–38%), in the 0- to 20-cm depth. The amorphous Fe hydroxyoxide fraction dominated most likely because biosolids increased the extractable soil Fe content (Table 3) and thus increased the number of reactive surfaces for Ni adsorption. Rajaie et al. (2008) added Ni to calcareous soils but over time found that iron oxides had the greatest Ni retention capacity. Fischer et al. (2007) showed that Ni adsorption to goethite increased with time and suggested that early adsorption was related to particle surface interactions, whereas continued adsorption was affected by diffusion in to goethite micropores.

Biosolids application did increase Ni in the soluble/exchangeable and specifically adsorbed/carbonate–bound phases in the 0- to 20-cm depth. Barbarick et al. (1998) observed increased extractable Ni in the 0- to 20-cm soil depth with repeated biosolids applications up to 26.8 Mg ha^{-1}. Others (Sloan et al., 1997; Sims and Kline, 1991; McGrath and Cegarra, 1992; Guerra et al., 2007) have shown that biosolids-applied Ni increased the more easily extractable soil Ni forms.

The biosolids used in our study most likely contained enough Ni in relatively mobile phases to cause a significant change in the exchangeable soil fractions. Fuentes et al. (2004) showed that 23% of total Ni in anaerobically digested biosolids was available as measured using a diethylenetri-aminepentaacetic acid extraction. Garcia-Delgado et al. (2007) and Guerra et al. (2007) found up to 20% of Ni present in biosolids as an exchangeable form. Partitioning of biosolids-borne Ni in relatively mobile fractions should increase the potential for plant uptake (Sloan et al., 1997) and thus accumulation in the food chain. However, Ni phytotoxicities would limit plant growth before negative effects on other organisms would occur (Pierzynski et al., 2000). In our study, available Ni concentrations are low enough to pose less of a risk in terms of degradation of environmental quality.

Lead

Within the 0- to 20-cm depth, Pb concentration was greatest in the amorphous Fe hydroxyoxide fraction (86–90%), with lesser amounts found in the specifically adsorbed/carbonate–bound fraction (9–13%). Sloan et al. (1997) found similar results with biosolids applications up to 180 Mg ha^{-1}. Others have shown Pb to be adsorbed to this phase within unamended soils (Ramos et al., 1994; Zhu and Alva, 1993), with Pb forming strong associations with hydroxyoxides (McKenzie, 1980; Bruemmer, 1986). Although biosolids did not significantly affect Pb in the amorphous Fe hydroxyoxide fraction, a trend of increasing Pb content with increasing biosolids application was present, most likely due to the increase in extractable Fe associated with increasing biosolids rate (Table 3). Berti and Jacobs

(1996) observed an increase in Fe and Mn oxide Pb-associated fractions, with biosolids application rates up to 690 Mg ha⁻¹. Guerra et al. (2007) showed that approximately 80% of biosolids-borne Pb is bound to Fe oxides.

Increasing biosolids application rate did increase the specifically adsorbed/carbonate–bound Pb in the 0- to 20-cm depth. Sims and Kline (1991) noted a significant increase in the amount of soil-extractable Pb after application of 44 Mg ha⁻¹ co-composted biosolids. Increases may have been due to the biosolids form of Pb. Walter et al. (2006) showed that approximately 27% of anaerobically digested biosolids-borne total Pb was present in an easily extractable form. Wong et al. (2001) showed that although the majority of biosolids-borne Pb was found in the residual phase, between 2.2 and 32.2% was plant available. Barbarick et al. (1998) observed increased extractable Pb in the 0- to 20-cm soil depth with repeated biosolids applications up to 26.8 Mg ha⁻¹ with no observable downward Pb transport. These findings suggest that Pb mobility is of low concern in soils receiving repeated biosolids applications at rates several times greater (26.8 Mg ha⁻¹) than those considered agronomic (6.7 Mg ha⁻¹).

Zinc

The Zn concentration was greatest in the the amorphous Fe hydroxyoxide (40–61%) and residual inorganic (24–42%) fractions in the 0- to 20-cm depth. Sloan et al. (1997) found the greatest Zn concentrations in the amorphous Fe hydroxyoxide phase, with lesser amounts in the residual inorganic fraction. Rieuwerts et al. (1998) pointed out the importance of hydrous Mn and Fe oxides in adsorbing Zn and other divalent metal cations.

Increasing biosolids application rate increased Zn in the 0- to 20-cm amorphous Fe hydroxyoxide fraction, likely due to the increase in extractable Fe content (Table 3). Biosolids with a high Fe oxide content may adsorb Zn, as with molybdate, and decrease its phytoavailability. Therefore, the increase in this Zn phase may be a signature of biosolids application. Sloan et al. (1997) observed increases in Zn associated with the Fe oxide phase with increasing biosolids application. Sukkariyah et al. (2005) also noted a significant increase in oxide-associated Zn with increasing biosolids application rates, with the metal-oxide fraction containing the greatest Zn concentration out of all fractions measured. Ahumada et al. (2004) noted that 23% of biosolids-borne Zn was present in mobile forms, but the residual fraction was the predominant form in biosolids-amended soil.

Discontinued 40.3 Mg ha⁻¹ Biosolids-Amended Plots

The 40.3 Mg ha⁻¹ biosolids-amended plots, 13 yr since being discontinued, contained metal concentrations greater than the control (i.e., background) but similar to that with all other biosolids application rates, when considering only those fractions significantly affected by biosolids application rate (Table 5). Shifts to more resistant Cu, Pb, and Zn forms, as suggested by the 4 mol L⁻¹ HNO₃ extraction, were not observed most likely because the sequential extraction technique was more sensitive to minute changes in metal form. McGrath and Cegarra (1992) concluded that after biosolids application ceased, soil chemical forms remained the same for long periods of time, implying that bioavailability remains unaltered. This conclusion seems to hold true for our study.

Percent Recovery of Total Sequential versus 4 mol L⁻¹ HNO₃ Extractable Metals

Given all biosolids application rates, the sequential extraction procedure performed relatively well in terms of percent recovery of Cu (54–131%) and Ni (100–113%) (Table 5). Cadmium and Mo recovery were not determined because the 4 mol L⁻¹ HNO₃ extraction did not detect Cd or Mo above the detection limit. However, the total sequential extraction procedure recovered greater concentrations of Cr (166–317%), Pb (209–656%), and Zn (108–155%). For each metal analyzed, Sloan et al. (1997) found that the sum of the chemical fractions was usually within 20% of the 4 mol L⁻¹ HNO₃ extractable metals.

The overestimation in Cr and Zn content may have been due to, as suggested by Sposito et al. (1982), the result of the 4 mol L⁻¹ HNO₃ extraction in the sequential extraction procedure being more effective than the 4 mol L⁻¹ HNO₃ extraction because of soil preconditioning by previous extractions in the sequential procedure. In addition, some overestimation may be due to differences in extraction time. The 4 mol L⁻¹ HNO₃ extraction required a 6-h extraction time; the residual inorganic fractionation step, which used 4 mol L⁻¹ HNO₃, required a 16-h extraction period. Dowdy et al. (1991) noted the inability of 4 mol L⁻¹ HNO₃ to extract most of the added biosolids-borne Zn, suggesting slow diffusion into the interior of soil minerals and/or adsorption on to specific sorption sites that are essentially unavailable to 4 mol L⁻¹ HNO₃ (Bruemmer, 1986).

Overestimation of Pb content appeared to be due to Pb extraction frmo the amorphous Fe hydroxyoxide phase. The sequential extraction improved Pb desorption from this phase, and as suggested by Bruemmer (1986), the Pb in this phase was likely unavailable for extraction by 4 mol L⁻¹ HNO₃.

Conclusions

Soils were sampled after 20 yr of biennial biosolids application at rates of 0, 6.7, 13.4, 26.8, and 40.3 dry Mg biosolids ha⁻¹. The 40.3 Mg ha⁻¹ rate was discontinued in 1992 due to excessive accumulation of soil NO₃–N, and since then this portion of the study has focused on system recovery. Increasing biosolids application rate significantly increased 4 mol L⁻¹ HNO₃ extractable soil Cr, Cu, Pb, and Zn in the 0- to 20-cm depth but did not affect metals in the 20- to 60-cm depth, suggesting negligible downward metal mobility. We expected to observe increases in the soil surface because biosolids are a source of these elements and were incorporated into this depth. The discontinued 40.3 Mg ha⁻¹ 4 mol L⁻¹ HNO₃ results suggested equilibrium shifts to more stable Cu, Pb, and Zn phases.

To further assess shifts in metal equilibrium with repeated biosolids application or time since cessation, we used a heavy-metal sequential extraction. We found significant changes within and between metal pools within the 0- to 20-cm depth but no

significant change in the 20- to 60-cm depth, reconfirming negligible downward trace metal movement. Within the 0- to 20-cm depth, nearly all measurable soil Cd was present in the specifically adsorbed/carbonate–bound phase, a phase that could contribute to Cd mobility. Therefore, we rejected the hypothesis that repeated, long-term biosolids applications contain Cd in more resistant phases. Soil Cr was present in highly immobile phases, most likely present in the low solubility form, $Cr(OH)_3$. Soil Cu dominated the amorphous Fe hydroxoxide fraction and to a lesser extent organically complexed and residual inorganic phases. We expected to observe an increase in the organically complexed Cu phase because Cu tends to make strong complexes with organic soil compounds and organic complexes present in biosolids. Copper in all three forms is considered relatively immobile. Soil Mo, Ni, Pb, and Zn concentrations were greatest in the amorphous Fe hydroxoxide fraction in the 0- to 20-cm depth. This phase is relatively immobile and dominated the system because these metals react strongly with iron hydroxyoxides. Biosolids that contain a high Fe oxide content, as in this study, can also adsorb Mo and Zn and reduce their availability. The increase in Fe hydroxoxide–bound Mo and Zn reflects the biosolids $Fe_2(SO_4)_3$ signature and a subsequent increase in AB-DTPA–extractable soil Fe content. We accepted the hypothesis that repeated, long-term biosolids applications contain Cr, Cu, Mo, Ni, Pb, and Zn in more resistant phases.

Increasing biosolids application caused increases in relatively immobile Cu, Mo, and Zn, and mobile Cu, Ni, and Pb phases. Therefore, we accepted the hypothesis that repeated, long-term biosolids applications cause persistent changes in soil Cu, Mo, Ni, Pb, and Zn mobility, extractability, and fate; we rejected the hypothesis that repeated, long-term biosolids applications cause persistent changes in soil Cd and Cr. The 40.3 Mg ha^{-1} application rate, 13 yr since being discontinued, was similar to all other biosolids application rates when considering only those fractions significantly affected by biosolids application rate. Apparently, soil chemical forms of these metals have remained the same years after biosolids application ceased, implying that bioavailability remains unaltered. Mobile phases affected by biosolids application have not contributed to significant downward metal movement, as observed in a lack of a biosolids rate effect in the 20- to 60-cm depth. Application of biosolids, even at repeated rates several times greater than those considered agronomic, has not significantly contributed to trace metal mobility through the soil profile. Consequently, there is little risk of downward metal transport and contamination of water supplies for comparable soils under similar agronomic practices and histories.

Acknowledgments

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