Fate of biosolids Cu and Zn in a semi-arid grassland

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A B S T R A C T

Biosolids land application applies varying trace metal amounts to soils. Measuring total soil metals is typically performed to ensure environmental protection, yet this technique does not quantify which soil phases play important metal release or attenuation roles. We assessed the distribution of biosolids-borne Cu and Zn associated with soluble/exchangeable, specifically adsorbed/carbonate-bound, amorphous and crystalline Mn/Fe oxyhydroxide-bound, residual organic, and residual inorganic phases. Biosolids were surface-applied (no incorporation) to experimental plots, at the Meadow Springs Ranch (40 53′46″N, 104 52′28″W) which is owned by the city of Fort Collins, CO, USA, in 1991 at rates of 0, 2.5, 5, 10, 21, and 30 Mg ha^{-1}. Plots were split in half in 2002, with one-half receiving biosolids at rates identical to 1991 rates. In 2003, 0–8, 8–15, and 15–30-cm soil depths were collected and subjected to 4 M HNO₃ digestion and sequential fractionation. The 4 M HNO₃ extraction suggested downward Cu transport, while Zn was immobilized in the soil surface. The sequential extraction procedure, more sensitive to changes in soil metal pools, suggested that repeated biosolids application did not affect vertical Zn movement, but did increase the downward transport potential of organically complexed Cu. In the given time, organically complexed Cu was likely mineralized and subsequently associated with soil mineral oxide phases. Because bioavailability of Cu is associated with dissolved phases, and soluble/exchangeable Cu concentrations were below detection limits in the subsoil, a reduction in environmental quality should be minimal. Still, we advocate that on coarse-textured semi-arid soils, biosolids application rates should match the plant N needs to avoid potential downward trace metal transport.

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1. Introduction

Biosolids land application is a major method of disposal in the US, with approximately 50% land applied (US EPA, 2007a); in US Environmental Protection Agency (US EPA) Region 8, which encompasses Colorado, Montana, North Dakota, South Dakota, Utah, and Wyoming, 85% is land applied (US EPA, 2007b (http://www.epa.gov/unix0008/water/)). This recycling method can greatly benefit municipalities by recycling plant nutrients in an environmentally sound manner when applied at agronomic rates (Barbarick et al., 1992). Our 12-year biosolids study with the city of Fort Collins, Colorado has provided valuable information on the effects of biosolids application to a semi-arid grassland soil. For example, Harris-Pierce (1994) studied surface biosolids application with no incorporation of rates up to 30 Mg ha^{-1} to a city of Fort Collins owned semi-arid grassland. The author noted increasing

concentrations of total Cd, Cu, Mo, and Zn in the soil 0–8-cm depth associated with increasing biosolids application rates. There was some evidence that NO₃–N and salts were leaching to the lowest depth sampled (15–30 cm), with increased leaching associated with increasing biosolids application. Sullivan et al. (2005) revisited the research site, noting that ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extractable Cu and Mo increased with biosolids application rates up to of 30 Mg ha^{-1} in the 0–15-cm depth. Lacking from these studies, however, was a more detailed analysis pertaining to the fate and transport of biosolids-borne metals within this long-term biosolids-amended semi-arid grassland soil.

The limited studies pertaining to biosolids application in semi-arid settings have focused primarily on total and plant-available soil metal concentrations. Fresquez et al. (1990) studied the effects of increasing biosolids surface application with no incorporation (22.5, 45.0, and 90.0 Mg ha^{-1}) on the 0–15-cm soil depth of degraded semi-arid grassland. The authors observed that DTPA-extractable Cu and Zn concentrations increased with increasing biosolids application in each of four years following biosolids

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application; increases were probably the result of biosolids movement and consequently incorporation into the soil, increased decomposition by microorganisms, and subsequently a decrease in soil pH (Fresquez et al., 1990). Walter et al. (2006b) studied HNO_3–HCl (i.e. total) and DTPA-extractable heavy metal soil concentrations one and five years following 0, 40, 80, and 120 Mg ha^{-1} biosolids application to a degraded Mediterranean soil. Total soil metals did not significantly increase over time. However, an increase in DTPA-extractable Cu and Zn concentrations with increasing biosolids rates five years following application was observed, potentially due to organic matter degradation and incorporation of soluble organic matter into the soil. Brenton et al. (2007) studied trace element leaching through intact columns of semi-arid rangeland soils amended with up to 90 Mg ha^{-1} of surface-applied biosolids. Leachate Cu and Zn concentrations were not affected by biosolids application.

Many biosolids trace metal research studies have focused on soils in agricultural settings. Emmerich et al. (1982a) studied biosolids mixed with soil in the top 15 cm of a column study and found little metal movement below the incorporation zone. They attributed the lack of movement, and thus fate, to metals shifting towards more stable 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 agricultural soil as compared to a control soil. McBride et al. (1999) noticed metal transport into groundwater below an orchard soil amended with 244 Mg ha^{-1} biosolids, associating metal movement with dissolved organic matter. Al-Wabel et al. (2002) utilized soils from a long-term biosolids-amended agricultural site, noting that 26.8 Mg ha^{-1} biosolids increased both dissolved organic carbon and Cu in column effluents. A positive correlation between Cu and dissolved organic carbon was also observed.

More detailed biosolids-amended soil analyses have utilized sequential extraction procedures to operationally define soil metal pools. Sloan et al. (1997) determined the fate of biosolids-borne metals using a sequential extraction procedure and noted >75% of Cu and Zn was found in relatively stable soil fractions following applications of up to 224 Mg ha^{-1} on cultivated soils. Sukkariyah et al. (2005) utilized a sequential extraction on soils from an agricultural setting which received up to 210 Mg ha^{-1} biosolids, showing the greatest Cu and Zn concentrations were associated with soil metal oxides. Guerra et al. (2007) used a sequential extraction procedure to study the effects of biosolids-borne metals to a Mollisol under production agriculture. The authors observed increased Zn in labile fractions associated with biosolids application at 30 Mg ha^{-1}. Copper concentration in the organic matter phase increased following biosolids application, with Cu forming relatively strong complexes with biosolids fulvic acid functional groups (Guerra et al., 2007; Dahlgren et al., 1997). Berti and Jacobs (1996) also used a sequential extraction technique and showed that total biosolids applications of up to 690 Mg ha^{-1} on cropland increased Zn, and to a lesser extent Cu, in potentially plant-available (i.e. bioavailable) forms.

Sequential extractions are time consuming, yet research on soil trace metal behavior following years of biosolids application is needed to understand long-term effects on soils (Sukkariyah et al., 2005). Furthermore, such information is necessary for heavy metal environmental impact purposes as well as for enhancement of biosolids land application regulatory guidelines (Vaca-Paulin et al., 2006). This project presents a unique perspective because previous research utilizing sequential trace metal extraction techniques have mostly targeted biosolids land application in agricultural settings. We focused our efforts on Cu and Zn with the goal of identifying the dominant metal pools present in a semi-arid grassland soil receiving biosolids either once (in 1991) or twice (in 1991 and 2002), and to

explain any discrepancies within or between various soil metal phases. Biosolids used in the current study contained Cu and Zn concentrations at an order of magnitude greater than other metals typically present in biosolids (e.g. Cd, Cr, Mo, Ni, and Pb), making it easier to detect and thus describe in the soils utilized. Our hypotheses were that both long-term (once) and short-term (twice) biosolids applications will: (a) cause persistent changes in soil Cu and Zn fractions, which will affect metal mobility, extractability, and fate; and (b) show the largest containing Cu and Zn pools residing in more resistant soil metal phases.

2. Materials and methods

2.1. Site description, biosolids, and soil sampling

The city of Fort Collins, CO, USA owns the 10,500 ha Meadow Springs Ranch (40 53′46″N, 104 52′28″W) and utilizes it for beneficial biosolids land application. In August 1991, 15 m × 15 m test plots were established at the Meadow Springs Ranch with treatments consisting of 0, 2.5, 5, 10, 21, and 30 Mg biosolids ha^{-1} surface-applied with no incorporation. All treatments were replicated four times in a randomized complete block design. In October 2002 the original plots were split in half. One-half received a second surface with no incorporation application using the same rates at the original plots, creating a split-plot in time study design.

Biosolids samples from the city of Fort Collins, Colorado wastewater treatment facility were collected before each application, kept refrigerated at approximately 3 °C, then total Cu, Zn, and additional metal composition was determined by $HClO_4$–HNO_3–HF–HCl digestion using inductively coupled plasma–atomic emission spectroscopy (ICP–AES; Table 1; Soltanpour et al., 1996). Biosolids pH was determined using a saturated paste extract (Rhoades, 1982), total N via LECO-1000 analysis (Nelson and Sommers, 1996; LECO Corp., St. Joseph, MI), NH_4–N and NO_3–N in a 2 M KCl extract (Mulvaney, 1996), and organic N via subtraction of inorganic from total.

The Meadow Springs Ranch is a semi-arid, shortgrass steppe rangeland community dominated by the perennial grasses blue grama (*Bouteloua gracilis* (H.B.K.) Lag. Ex steud) and western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Love). The research site soil was an Altvan loam, fine-loamy over sandy or sandy-skeletal, mixed, mesic Aridic Argiustoll, 0–3% slopes. The Altvan series consists of deep, well-drained soils that formed in mixed alluvial deposits (NRCS, 1980). Three soil cores from each plot were collected in July 2003 from the 0–8, 8–15, and 15–30-cm depths, composited, placed in Ziploc® bags, then into coolers and returned to Colorado State University. Sampling depth was similar to that described by Harris-Pierce (1994). All soils were immediately air-

Table 1
Characteristics of the 1991 and 2002 Fort Collins, CO, USA biosolids applied to the Meadow Springs Ranch semi-arid rangeland experimental plots.

Parameter[a]	Soil	Biosolids application year	
		1991	2002
Zn (mg kg^{-1})	35	772	652
Cu (mg kg^{-1})	6.1	547	475
Pb (mg kg^{-1})	7.9	120	39
Cr (mg kg^{-1})	9.3	40	21
Ni (mg kg^{-1})	6.6	19	17
Mo (mg kg^{-1})	<0.1	16	19
Cd (mg kg^{-1})	0.3	5.0	2.6
pH	6.3	7.3	6.0
Organic N (%)	0.2	4.2	4.2
NH_4–N (mg kg^{-1})	5.4	3960	5440
NO_3–N (mg kg^{-1})	0.9	102	2.9

[a] All values are presented on a dry weight basis.

Table 2
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 M $Ca(NO_3)_2$; 25 °C; 18 h[b]	Soluble/exchangeable
2	15 mL of 1 M NaOAc (pH 5); 25 °C; 5 h	Specifically sorbed/carbonate-bound
3a[a]	20 mL of 0.1 M $NH_2OH(HCl)$ in 0.01 M HNO_3; 25 °C; 0.5 h	Amorphous Mn/Fe oxyhydroxide-bound
3b	20 mL of 1 M $NH_2OH(HCl)$ in 25% HOAc (pH 2); 95 °C; 6 h	
4	3 mL of 0.02 M HNO_3 + 5 mL of 30% H_2O_2; 85 °C, 4 h, followed by the addition of 5 mL of 3.2 M NH_4OAc + 5 mL of 20% HNO_3; 25 °C; 0.5 h	Organically complexed
5	10 mL of 4 M HNO_3; 95 °C, 16 h	Residual inorganic

[a] Negligible quantities of Cu and Zn were extracted with step 3a, metals associated with the amorphous Mn oxyhydroxide-bound phase (similar to findings of Sloan et al. (1997)). Therefore, data from step 3a were combined with data from step 3b, the amorphous Fe oxyhydroxide-bound metals, resulting in the metal fractionation designation of amorphous Mn/Fe oxyhydroxide-bound.

[b] Solutions from all steps analyzed using inductively coupled plasma–atomic emission spectroscopy.

dried, ground to pass a 2-mm sieve, and subjected to aforementioned analyses (Table 1). In addition, total C content of soils was also determined via LECO-1000 analysis (Nelson and Sommers, 1996). The total number of samples collected was 144, the result of four replications over six treatments by three depths by two application times.

2.2. 4 M HNO_3 soil metal extraction

All soils were extracted with 4 M HNO_3 (Bradford et al., 1975), with the amount of Cu and Zn extracted a close approximation to total soil elements. Barbarick et al. (1997) showed that the 4 M HNO_3 digest extracted an average of 115% Cd, 98% Cu, 138% Pb, 87% Ni, and 104% Zn as compared to a standard nitric-perchloric acid digest. McBride et al. (1997) showed the 4 M 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 M HNO_3-extraction procedure consisted of 1 g of soil placed in a 50 mL digestion tube, predigested in 10 mL of 4 M 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, filtered through Whatman #5 filter paper, and solution Cu and Zn concentrations determined using ICP–AES. The detection limits of ICP–AES were 0.01 mg L^{-1} for both Cu and Zn. Ten percent duplicates and 5% blanks were utilized, and no more than 5% error was accepted during ICP–AES analysis.

2.3. Soil metal fractionation

All soils were sequentially extracted to fractionate soil Cu and Zn chemical pools based on their relative binding strength (Sloan et al., 1997). The fractionation scheme utilized had been shown by Sloan et al. (1997) to differentiate between operationally defined metal pools in biosolids-amended soils. However, the names of several operationally defined pools presented by Sloan et al. (1997) were modified based on a recent review of sequential extraction methodologies by Rao et al. (2008). One gram of soil was placed in a 50-mL centrifuge tube and sequentially extracted for: (1) soluble/exchangeable; (2) specifically adsorbed/carbonate-bound; (3) amorphous Mn/Fe oxyhydroxide-bound; (4) crystalline Mn/Fe oxyhydroxide-bound; (5) organically complexed; and (6) residual inorganic using various extractants. The solution amounts and experimental/analytical conditions utilized are shown in Table 2. The extraction steps at room temperature were performed on a reciprocating shaker at 120 oscillations per min; heated extractions were completed on an orbital shaker in a thermostat-controlled hot water bath. Following each extraction step, samples were centrifuged at $7000 \times g$ for 10 min, the solution decanted into glass vials, then filtered through a 0.2 μm nylon membrane filter and analyzed for Cu and Zn concentrations using ICP–AES.

2.4. Statistical analysis

All data were log_{10} transformed to improve normality and reduce heteroscedascity before analysis (Steel and Torrie, 1980). Then, statistical analysis was performed on all data (all replicates; $n = 4$) per individual depth using a split-plot in time design in the Proc GLM model, SAS software version 9.1 (SAS Institute, 2002) to evaluate the effect of biosolids application rate on 4 M HNO_3 extractable and the fractionation of Cu and Zn concentrations; however, data presented are non-log transformed. We tested our hypotheses at $P = 0.05$, calculated a Fisher's Protected Least Significant Difference (LSD; Steel and Torrie, 1980) when

Table 3
Effect of biosolids rate on the 1991 (single; S) and 2002 (repeated; R) Meadow Springs Ranch semi-arid rangeland 4 M HNO_3 extractable mean ($n = 4$). Cu and Zn concentrations in the 0–8, 8–15, and 15–30-cm soil depths.

Biosolids rate (Mg ha^{-1})	Cu (mg kg^{-1})		Zn (mg kg^{-1})	
	S	R	S	R
0–8-cm depth				
0	1.9 (0.1)[a]	1.5 (0.6)	28 (3)	26 (3)
2.5	2.9 (0.5)	3.2 (0.4)	28 (1)	28 (3)
5	5.7 (0.9)	6.5 (0.7)	33 (3)	36 (1)
10	8.1 (2.1)	14 (3.3)	37 (4)	45 (5)
21	11 (2.2)	26 (4.7)	39 (4)	59 (3)
30	24 (3.0)	39 (8.8)	53 (7)	72 (10)
Trt effect LSD				
Time effect LSD				
Trt × time		*		*
8–15-cm depth				
0	2.5 (0.3)	2.8 (0.8)	31 (3)	30 (5)
2.5	2.4 (0.3)	3.1 (0.9)	29 (1)	29 (4)
5	3.6 (0.4)	3.9 (0.2)	33 (3)	34 (2)
10	3.9 (0.6)	5.4 (1.6)	33 (3)	36 (3)
21	4.2 (1.7)	6.3 (2.0)	31 (7)	36 (8)
30	6.4 (1.2)	6.3 (1.3)	34 (2)	35 (3)
Trt effect LSD		0.8*		NS
Time effect LSD		NS		NS
Trt × time		NS		NS
15–30-cm depth				
0	3.1 (0.3)	3.0 (0.5)	34 (3)	31 (3)
2.5	2.9 (0.3)	3.0 (0.6)	30 (4)	28 (5)
5	3.7 (0.4)	3.8 (0.6)	32 (4)	32 (4)
10	3.7 (0.5)	4.3 (0.6)	33 (1)	32 (2)
21	4.0 (1.7)	7.2 (2.6)	30 (5)	38 (6)
30	5.2 (1.0)	10 (4.9)	34 (1)	40 (8)
Trt effect LSD		2.3*		NS
Time effect LSD		1.0*		NS
Trt × time		NS		NS

NS = Not significant. Statistical analyses were based on log transformed data to improve normality and reduce heteroscedascity.

[a] Values inside parentheses represent 1 standard error of the mean.

* Significance at 5% probability level.

significance was observed within treatments or between timing of application. If a significant interaction existed between treatment and time, significance will only be presented.

3. Results and discussion

3.1. 4 M HNO₃ soil metal extraction

The 4 M HNO₃ extractable Cu and Zn concentrations in the 0–8, 8–15, and 15–30-cm soil depths are presented in Table 3. In the 0–8-cm depth, a treatment by time interaction was observed for Cu and Zn, and thus treatment and time could not be separated. Zinc content was unaffected by increasing single or repeated biosolids application in both the 8–15 and 15–30-cm depths. However, a significant treatment effect was observed for Cu in the 8–15-cm depth, with Cu concentration increasing with increasing single and repeated biosolids applications. And within the 15–30-cm depth, Cu concentration was greater with repeated versus single application. We observed increases in the soil surface because biosolids are a source of these elements, and since biosolids were surface-applied, the 0–8-cm depth was most affected. Others have noted similar increases in total surface soil metal concentrations with biosolids application (Guerra et al., 2007; Sukkariyah et al., 2005; Qiao et al., 2003; McBride et al., 1997; Berti and Jacobs, 1996).

The 4 M HNO₃ extractable metal results suggested both short- and long-term transformations in metal pools associated with single and repeated biosolids applications. Copper results suggested downward transport, particularly with regards to the repeated biosolids application. The Zn results suggested that immobile phases were controlling Zn availability in this system because downward movement was not observed.

3.2. Soil heavy metal fractionation

Comparing total soil metal concentrations over depths can suggest, but do not furnish sufficient evidence regarding the potential transformations and element availability (Srikanth and Reddy, 1991). Trace metals can be associated with various soil fractions which affect their mobility and fate (Sukkariyah et al., 2005), with certain fractions more or less available for extraction with 4 M HNO₃. It is important to keep in mind that sequential extraction methods "operationally define" trace element fractionation into chemical pools and that the extraction is not perfectly selected (Basta et al., 2005). However, sequential extractions can be useful for determining potential bioavailability of metal contaminants (Basta et al., 2005), and therefore gathering such information is important for predicting environmental impact of metals from anthropogenic sources such as biosolids (Vaca-Paulin et al., 2006).

The sequential extraction procedure used targeted easily reducible (i.e. Mn) and more resistant (i.e. Fe) oxyhydroxide fractions. However, as with the findings of Sloan et al. (1997), we

Table 4
Effect of biosolids rate on the 1991 (single; S) and 2002 (repeated; R) Meadow Springs Ranch semi-arid rangeland sequentially extracted mean ($n = 4$). Cu concentrations in the 0–8, 8–15, and 15–30-cm soil depths.

Biosolids rate (Mg ha⁻¹)	Soluble/exchangeable (mg Cu kg⁻¹ soil)		Specifically adsorbed/carbonate-bound (mg Cu kg⁻¹ soil)		Amorphous Mn/Fe oxyhydroxide-bound (mg Cu kg⁻¹ soil)		Organically complexed (mg Cu kg⁻¹ soil)		Residual inorganic (mg Cu kg⁻¹ soil)	
	S	R	S	R	S	R	S	R	S	R
0–8-cm depth										
0	ND[a]	ND	ND	0.2 (0.1)	1.5 (1.0)	3.0 (0.6)	1.1 (0.2)	1.5 (0.4)	2.5 (0.3)	1.8 (0.8)
2.5	ND	ND	ND	0.2 (0.1)	2.4 (0.8)	3.9 (0.5)	1.1 (0.2)	2.1 (0.4)	2.1 (0.2)	2.7 (0.3)
5	ND	ND	0.2 (0.1)	0.5 (0.3)	3.6 (0.7)	5.6 (1.1)	1.4 (0.1)	2.9 (0.9)	2.6 (0.2)	3.1 (1.0)
10	ND	0.4 (0.1)	0.3 (0.1)	0.8 (0.2)	4.7 (0.7)	7.4 (0.7)	1.5 (0.3)	3.1 (0.4)	3.0 (0.1)	3.2 (0.5)
21	0.3 (0.3)[b]	0.9 (0.3)	0.6 (0.2)	1.6 (0.4)	6.8 (1.2)	13 (3.3)	2.2 (0.6)	4.3 (1.1)	2.8 (0.5)	3.0 (0.2)
30	0.6 (0.2)	1.4 (0.3)	1.2 (0.5)	2.3 (1.0)	11 (3.2)	17 (4.8)	2.7 (0.8)	5.6 (2.1)	2.9 (0.6)	3.8 (0.7)
Trt effect LSD					1.5*		1.2*		1.3*	
Time effect LSD					2.0*		1.5*		NS	
Trt × time interaction			*				NS		NS	
8–15-cm depth										
0	ND	ND	ND	0.2 (0.1)	1.4 (1.1)	2.3 (0.7)	1.0 (0.2)	1.5 (0.4)	3.7 (0.9)	2.8 (0.7)
2.5	ND	ND	ND	0.2 (0.1)	1.5 (1.1)	2.8 (0.6)	0.9 (0.1)	1.8 (0.5)	3.0 (0.2)	2.6 (0.8)
5	ND	ND	ND	0.2 (0.1)	1.2 (1.1)	3.1 (0.2)	1.0 (0.2)	2.0 (0.6)	3.4 (0.2)	4.1 (1.6)
10	ND	ND	ND	0.3 (0.1)	1.9 (1.4)	3.7 (1.1)	1.1 (0.2)	2.1 (0.3)	3.4 (0.2)	3.3 (0.4)
21	ND	ND	ND	0.3 (0.2)	2.5 (0.4)	4.4 (0.6)	1.1 (0.1)	2.0 (0.3)	2.8 (0.8)	2.8 (0.3)
30	ND	ND	ND	0.3 (0.1)	3.1 (1.4)	4.5 (0.5)	1.2 (0.1)	2.1 (0.8)	3.0 (0.2)	2.8 (0.9)
Trt effect LSD					1.3*		NS		NS	
Time effect LSD					NS		0.13*		NS	
Trt × time interaction					NS		NS		NS	
15–30-cm depth										
0	ND	ND	ND	ND	1.5 (1.0)	2.6 (0.6)	0.8 (0.1)	1.7 (0.5)	3.3 (0.2)	2.6 (0.5)
2.5	ND	ND	ND	ND	1.3 (1.1)	2.4 (0.4)	0.8 (0.2)	1.4 (0.3)	3.2 (0.3)	3.1 (0.4)
5	ND	ND	ND	0.2 (0.1)	1.8 (0.9)	3.1 (0.1)	0.9 (0.2)	1.8 (0.2)	3.3 (0.2)	2.9 (0.5)
10	ND	ND	ND	ND	2.4 (0.7)	3.2 (0.8)	0.8 (0.1)	1.6 (0.4)	3.1 (0.2)	2.7 (0.5)
21	ND	ND	ND	0.3 (0.1)	2.1 (0.3)	4.2 (0.9)	1.0 (0.1)	2.0 (0.2)	3.0 (0.6)	3.2 (0.6)
30	ND	ND	ND	0.5 (0.3)	2.7 (1.7)	6.2 (1.6)	1.0 (0.2)	2.4 (0.7)	3.1 (0.1)	2.9 (0.6)
Trt effect LSD					1.9*		1.4*		NS	
Time effect LSD					NS		1.3*		NS	
Trt × time interaction					NS		NS		NS	

NS = Not significant. Statistical analyses were based on log transformed data to improve normality and reduce heteroscedascity.

[a] ND = non-detectable.
[b] Values inside parentheses represent 1 standard error of the mean.
* Significance at 5% probability level.

observed negligible amounts of heavy metals associated with the easily reducible Mn oxyhydroxide phase. Therefore, data from the reducible Mn oxyhydroxide pool (designated 3a in Table 2) were combined with that from the Fe oxyhydroxide-associated metal phase (designated 3b in Table 2) with this combined fraction now named amorphous and crystalline Mn/Fe oxyhydroxide-bound.

3.2.1. Copper

In the 0–8-cm depth, soil Cu concentrations were greatest in the amorphous and crystalline Mn/Fe oxyhydroxide-bound fraction for both single (51%) and repeated (53%) biosolids applications (Table 4); lesser amounts of Cu were present in the organically complexed (17–20%) and residual inorganic phases (18–26%). Within the 8–15 and 15–30-cm depths the amorphous and crystalline Mn/Fe oxyhydroxide-bound and residual inorganic fractions dominated, with lesser amounts of Cu in the organically complexed phase. However, the greatest Cu concentrations overall were extracted from the soil surface. We expected to observe greater Cu concentrations in the 0–8-cm depth because this depth was most affected by biosolids applications and Cu concentration was greater than most biosolids metals.

In the soil surface, a significant treatment by time interaction was observed for the specifically adsorbed/carbonate-bound phase, increasing biosolids application rate increased Cu associated with the amorphous and crystalline Mn/Fe oxyhydroxide-bound, organically complexed, and residual inorganic phases, and as compared to the single biosolids application the repeated biosolids application increased amorphous and crystalline Mn/Fe oxyhydroxide-bound and organically complexed Cu phases (Table 4). The majority of these Cu forms would be considered less bioavailable, with findings supported by Torri and Lavado (2008) who applied 150 Mg biosolids ha^{-1} to soil in a greenhouse setting. The authors noted that biosolids application increased the less available forms of Cu. Sloan et al. (1997) showed significant increases in all Cu fractions, but biosolids application increased Cu to the greatest extent in the specifically adsorbed/carbonate-bound and amorphous and crystalline Mn/Fe oxyhydroxide-bound fractions. Hickey and Kittrick (1984) observed nearly equal soil Cu concentrations in the Fe/Mn oxide and organically complexed phases from soil amended with 307 Mg ha^{-1} biosolids. Guerra et al. (2007) incubated soils treated with 30 Mg ha^{-1} biosolids for 60 days, 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.

Based on the significant interaction and the time effect observations in the soil surface, biosolids-borne Cu was presumed added predominantly as amorphous Mn/Fe oxyhydroxide and organically bound phases. Garcia-Delgado et al. (2007) sequentially extracted seven biosolids and observed up to 25% of biosolids-borne Cu to be associated with organic phases while the major of Cu was associated with oxide phases. However, biosolids-borne organically bound Cu has been shown to be between ~50% and 75% of the total Cu present (Alloway, 1995; Wong et al., 2001). Walter et al. (2006a) sequentially extracted anaerobically digested biosolids, pelletized biosolids, and composted biosolids, and found in all instances Cu associated to the greatest extent with the organic phase. Relatively strong complexes with biosolids fulvic acid functional groups are assumed to occur (Dahlgren et al., 1997). Copper forms stable complexes with humic and fulvic acids (Stevenson and Fitch, 1981), with the complexing ability due to high oxygen-containing functional groups such as carboxyl, phenolic, hydroxyl, and carbonyls (Stevenson, 1982).

Within the 8–15 and 15–30-cm depths, significant time effects indicated greater organically complexed Cu with repeated versus single biosolids application. Thus, the organically complexed fraction showed the greatest potential for downward Cu transport shortly following biosolids application, and this fraction was likely responsible for observed differences in 4 M HNO_3 extractable Cu content with depth (Table 4). McBride et al. (1999) noticed metal transport into groundwater from an orchard soil amended with 244 Mg ha^{-1} biosolids. The authors associated metal movement with dissolved organic matter. Al-Wabel et al. (2002) utilized soils from a long-term biosolids-amended agricultural site, noting that 26.8 Mg ha^{-1} biosolids increased both dissolved organic carbon and Cu in column effluents. A positive correlation between Cu and dissolved organic carbon was also observed.

Increases in pH from the soil surface to subsurface (Table 5) could have promoted organically bound Cu downward transport and enhanced organic colloid precipitation in this system. In support, significant increases in total C in the 15–30-cm depth (Table 5) were observed. Although not measured in our study, Bayley (2006) found that soils at the Meadow Springs Ranch contained less than 0.002% inorganic C, and thus increases in subsoil C content were due to organic C downward transport and deposition.

The precipitation of organic C in the subsoil could have enhanced mineralization and released Cu from an organically bound state, subsequently increased Cu associated with soil mineral oxide phases. Supporting this contention, significant treatment effects, not time effects, in the 8–15 and 15–30-cm depths indicated increased amorphous and crystalline Mn/Fe oxyhydroxide-bound Cu content with increased biosolids application. No significant differences were

Table 5
Effect of biosolids rate on the 1991 (single; S) and 2002 (repeated; R) Meadow Springs Ranch semi-arid rangeland mean ($n = 4$). Soil pH and total carbon concentrations in the 0–8, 8–15, and 15–30-cm soil depths.

Biosolids rate (Mg ha^{-1})	pH (%)		Total C (%)	
	S	R	S	R
0–8-cm depth				
0	5.9 (0.1)[a]	6.3 (0.3)	2.02 (0.24)	1.64 (0.25)
2.5	5.8 (0.2)	6.3 (0.4)	2.11 (0.31)	1.93 (0.28)
5	5.7 (0.2)	6.1 (0.1)	2.20 (0.20)	2.14 (0.12)
10	5.5 (0.1)	5.9 (0.1)	2.09 (0.55)	2.43 (0.16)
21	5.9 (0.6)	6.1 (0.3)	2.04 (0.21)	2.56 (0.19)
30	5.5 (0.6)	6.3 (0.3)	2.90 (0.69)	3.71 (0.41)
Trt effect LSD	NS		0.53[*]	
Time effect LSD	0.3[*]		NS	
Trt × time	NS		NS	
8–15-cm depth				
0	6.5 (0.2)	6.7 (0.3)	1.36 (0.13)	1.21 (0.14)
2.5	6.4 (0.0)	6.8 (0.4)	1.16 (0.11)	1.35 (0.31)
5	6.4 (0.1)	6.6 (0.2)	1.52 (0.18)	1.42 (0.14)
10	6.2 (0.0)	6.6 (0.1)	1.26 (0.23)	1.48 (0.17)
21	6.5 (0.6)	6.3 (0.1)	1.26 (0.20)	1.49 (0.25)
30	6.0 (0.6)	6.2 (0.6)	1.58 (0.22)	1.62 (0.24)
Trt effect LSD	NS		NS	
Time effect LSD	NS		NS	
Trt × time	NS		NS	
15–30-cm depth				
0	6.6 (0.1)	6.8 (0.3)	1.05 (0.16)	1.00 (0.12)
2.5	6.6 (0.2)	6.8 (0.4)	1.02 (0.10)	1.34 (0.56)
5	6.7 (0.3)	6.5 (0.3)	1.36 (0.47)	1.14 (0.24)
10	6.4 (0.2)	6.7 (0.3)	0.92 (0.07)	0.99 (0.06)
21	6.7 (0.3)	6.4 (0.3)	1.06 (0.20)	1.27 (0.18)
30	6.1 (0.3)	6.3 (0.6)	1.35 (0.37)	1.81 (0.41)
Trt effect LSD	0.4[*]		0.40[*]	
Time effect LSD	NS		NS	
Trt × time	NS		NS	

NS = Not significant. Statistical analyses were based on log transformed data to improve normality and reduce heteroscedascity.
[a] Values inside parentheses represent 1 standard error of the mean.
[*] Significance at 5% probability level.

observed between increasing biosolids application rates, or repeated versus single biosolids additions, and increasing amorphous and crystalline Mn/Fe oxyhydroxide-bound extractable Mn or Fe content (data not shown). Biosolids-associated Mn or Fe was not transported downward, supporting the contention that the organically complexed phase mineralized and released Cu to the subsoil oxyhydroxide pool.

The potential for environmental degradation associated with biosolids-borne Cu addition, the subsequent increase in soil Cu concentration, and downward organically bound Cu transport should be minimal at this location. According to the US EPA (2008a), the cumulative pollutant loading rate limit for biosolids-borne Cu is 1500 kg ha^{-1}. At the greatest application rate, 30 Mg ha^{-1}, only 31 kg Cu ha^{-1} has been applied with two biosolids applications. However, the city of Fort Collins, CO typically applies biosolids at a rate of $\sim$2.5 Mg ha^{-1} to a parcel of land approximately every 8–10 years (Ron Russell, city of Fort Collins, CO; personal communication). This application rate would supply $\sim$2.5 kg Cu ha^{-1} and thus would take approximately 4800–6000 years to reach cumulative loading limits.

The greatest application rate, 30 Mg ha^{-1}, contained $\sim$2.3 mg kg^{-1} of organically complexed Cu in the 15–30-cm depth. This concentration, if leached and transformed entirely to a soluble species, could reach the 1.3 mg L^{-1} drinking water action level set forth by the US EPA (2008b). However, since colloidal metal species are not as readily bioavailable as dissolved species (Aldrich et al.,

2002) and that the site receives 330–380 mm of mean annual precipitation (NRCS, 1980), the potential for deep organically bound Cu transport and transformation to dissolved species should be minimal. In fact, the bioavailability of metals such as Cu is mainly dependent on the free metal species (Morel, 1983), and free metal species would be associated with the soluble/exchangeable phase which was non-detectable in the 8–15 and 15–30-cm depths, supporting the previous contention. Furthermore, if the 1991 biosolids application acted like the 2002 repeated application, the single 30 Mg ha^{-1} application most likely contained elevated organically bound Cu in the 15–30-cm depth several years following application. Twelve years following application the organically bound Cu has been transformed and is now likely associated with amorphous and crystalline Mn/Fe oxyhydroxides. Thus, the treatment effect and lack of time effect associated with this oxyhydroxide phase. The amorphous and crystalline Mn/Fe oxyhydroxide pool can be translocated below the sampling depth (30 cm) through pedogenesis, yet metals associated with this phase are considered relatively non-bioavailable due to the low-solubility and strong adsorptive behavior of oxyhydroxides.

3.2.2. Zinc

Zinc was found in all fractions to some extent, and concentrations tended to be greatest in the both the Fe/Mn oxides and acid replaceable (organically complexed; 31–42%) and residual inorganic (24–44%) fractions for all depths from single or repeated

Table 6
Effect of biosolids rate on the 1991 (single; S) and 2002 (repeated; R) Meadow Springs Ranch semi-arid rangeland sequentially extracted mean ($n = 4$). Zn concentrations in the 0–8, 8–15, and 15–30-cm soil depths.

Biosolids rate (Mg ha^{-1})	Soluble/exchangeable (mg Zn kg^{-1} soil)		Specifically adsorbed/carbonate-bound (mg Zn kg^{-1} soil)		Amorphous Mn/Fe oxyhydroxide-bound (mg Zn kg^{-1} soil)		Organically complexed (mg Zn kg^{-1} soil)		Residual inorganic (mg Zn kg^{-1} soil)	
	S	R	S	R	S	R	S	R	S	R
0–8-cm depth										
0	0.6 (0.6)[a]	ND	1.0 (0.4)	1.0 (0.8)	21 (4)	12 (2)	6.2 (0.6)	6.9 (1.4)	16 (2.6)	14 (2.0)
2.5	ND[b]	0.4 (0.4)	1.6 (1.5)	3.2 (1.5)	17 (4)	15 (2)	7.1 (2.5)	8.0 (1.3)	18 (3.6)	17 (0.7)
5	0.3 (0.3)	3.1 (1.7)	2.6 (2.1)	2.9 (1.6)	22 (4)	24 (4)	6.2 (0.9)	10 (3.3)	18 (4.8)	18 (3.7)
10	0.7 (0.9)	5.7 (0.8)	3.4 (0.7)	4.6 (1.6)	22 (4)	28 (1)	7.0 (1.5)	9.9 (2.2)	15 (3.0)	17 (3.6)
21	0.3 (0.3)	12 (2.2)	3.5 (2.0)	12 (4.0)	27 (4)	38 (4)	6.8 (1.8)	9.2 (1.8)	14 (4.3)	12 (4.0)
30	1.4 (1.4)	15 (10)	3.1 (1.1)	15 (2.5)	33 (7)	48 (10)	6.7 (1.4)	9.8 (2.7)	13 (3.5)	15 (6.0)
Trt effect LSD	NS		1.7[*]				NS		NS	
Time effect LSD	NS		1.8[*]				1.3[*]		NS	
Trt × time interaction	NS		NS		[*]		NS		NS	
8–15-cm depth										
0	2.7 (4.7)	0.3 (0.5)	0.3 (0.3)	2.4 (4.2)	13 (1)	16 (3)	6.4 (1.2)	8.6 (1.5)	27 (3.4)	19 (5.3)
2.5	ND	ND	1.1 (0.8)	ND	16 (2)	13 (2)	5.8 (0.8)	10 (2.2)	24 (0.5)	22 (2.6)
5	ND	ND	0.8 (0.4)	0.5 (0.5)	16 (2)	17 (3)	7.0 (0.8)	11 (2.4)	24 (5.5)	24 (3.6)
10	4.4 (7.6)	0.4 (0.7)	1.3 (0.6)	2.1 (1.3)	18 (2)	18 (4)	7.0 (1.2)	10 (1.5)	24 (3.2)	20 (1.0)
21	7.9 (7.9)	0.6 (0.9)	0.8 (0.6)	1.3 (0.4)	19 (4)	20 (4)	6.3 (1.1)	9.4 (3.2)	21 (2.2)	19 (5.9)
30	4.8 (5.5)	1.0 (0.8)	1.2 (0.6)	1.4 (0.5)	21 (2)	19 (3)	7.6 (2.7)	8.6 (1.9)	20 (3.0)	22 (2.8)
Trt effect LSD			NS		NS		NS		NS	
Time effect LSD			NS		NS		NS		NS	
Trt × time interaction			NS		NS		NS		NS	
15–30-cm depth										
0	5.6 (8.0)	ND	0.9 (1.0)	ND	20 (3)	13 (1)	7.7 (1.5)	9.9 (2.5)	25 (2.9)	20 (2.7)
2.5	ND	ND	2.7 (3.9)	ND	16 (3)	12 (3)	6.0 (0.6)	11 (4.7)	23 (2.3)	19 (2.3)
5	5.3 (7.4)	1.4 (2.3)	1.0 (0.8)	0.9 (1.5)	17 (3)	15 (2)	7.4 (0.9)	11 (3.1)	24 (1.7)	19 (5.1)
10	4.2 (7.2)	ND	1.0 (0.6)	2.0 (2.0)	17 (2)	16 (2)	6.9 (1.4)	8.3 (2.0)	20 (1.4)	16 (3.0)
21	3.0 (5.1)	4.9 (7.4)	2.8 (3.0)	1.4 (1.6)	18 (4)	20 (3)	6.9 (1.4)	13 (5.8)	18 (5.6)	20 (4.0)
30	1.3 (1.7)	1.3 (1.3	1.0 (0.4)	2.8 (1.5)	20 (6)	25 (6)	6.5 (1.5)	10 (1.9)	18 (3.0)	15 (3.2)
Trt effect LSD			NS		NS		NS		NS	
Time effect LSD			NS		NS		NS		NS	
Trt × time interaction			NS		NS		NS		NS	

NS = Not significant. Statistical analyses were based on log transformed data to improve normality and reduce heteroscedascity.
[a] Values inside parentheses represent 1 standard error of the mean.
[b] ND = non-detectable.
[*] Significance at 5% probability level.

biosolids plots (Table 6). Sloan et al. (1997) found the greatest Zn concentrations in the Fe/Mn oxides and acid replaceable (organically complexed) with lesser amounts in the residual inorganic fraction. Rieuwerts et al. (1998) pointed out the importance of hydrous Mn and Fe oxides in adsorbing, among other divalent metal cations, Zn.

In the 0–8-cm depth, increasing biosolids application rate increased specifically adsorbed/carbonate-bound Zn, repeated biosolids application increased Zn associated with the specifically adsorbed/carbonate-bound and organically complexed phases, and a significant treatment by time interaction was observed for Zn associated with the amorphous and crystalline Mn/Fe oxyhydroxide-bound phase (Table 6). Sloan et al. (1997) observed an increase in Zn associated with all fractions as biosolids application rate increased from 0 to 180 Mg ha^{-1}. Hickey and Kittrick (1984) noted Zn content in several soil fractions associated with biosolids application of 307 Mg ha^{-1}.

Biosolids added to soils in the present study most likely contained Zn associated with residual (i.e. organically complexed and amorphous Mn/Fe oxyhydroxide phases) and relatively bioavailable or mobile (i.e. specifically adsorbed/carbonate-bound) phases. Ahumada et al. (2004) noted that 23% of biosolids-borne Zn was present in mobile forms, yet the residual fraction was the predominant form in biosolids-amended soil. Regardless, given time the specifically adsorbed/carbonate-bound phase should transformed to more resistant phases within the soil surface. This phase was not leached, as no significant differences were observed in the 8–15 and 15–30-cm depths.

4. Conclusions

Biosolids land application is a major method of disposal in the US. Land application has been proven to recycle plant nutrients, but biosolids still add metals to soils. Techniques for measuring total soil metals are typically performed to ensure environmental protection, yet these do not quantify which soil phases play important roles in dictating metal release or attenuation. We utilized both 4 M HNO$_3$ and a sequential extraction technique to understand Cu and Zn transformations and transport in long-term single (1991) or short-term repeated (2002) plots receiving 0, 2.5, 5, 10, 21, and 30 Mg biosolids ha^{-1}.

The 4 M HNO$_3$ extractable Cu results suggested downward transport influenced by both increasing biosolids application rates and by repeated biosolids land application. The Zn results suggested that immobile phases were controlling Zn availability in this system because downward movement was not observed. Sequential extraction procedure results showed that the dominant metal forms in our semi-arid system were comparable to that found in other agricultural systems. Copper and Zn concentrations tended to be greatest in the amorphous and crystalline Mn/Fe oxyhydroxide phase. Thus, we accepted our hypothesis that long-term (single) and short-term (repeated) biosolids applications would show the largest containing Cu and Zn pools residing in more resistant phases.

Biosolids applications as single, repeated, or increasing in rate, affected several soil Cu and Zn fractions, and thus we accepted our hypothesis that metal changes due to biosolids land application would be persistent and affect mobility, extractability, and fate. Within the 0–8-cm depth, biosolids applications affected Zn associated with the specifically adsorbed/carbonate-bound, amorphous and crystalline Mn/Fe oxyhydroxide-bound, and organically complexed phases; downward transport of Zn was not observed. In the 0–8-cm depth, biosolids applications affected Cu associated with the specifically adsorbed/carbonate-bound, amorphous and crystalline Mn/Fe oxyhydroxide-bound, organically complexed, and residual inorganic phases. Opposite of the Zn findings, the

potential for downward Cu transport associated with the organically complexed phase was enhanced by the repeated biosolids application, and with time Cu was likely transformed to the less bioavailable amorphous and crystalline Mn/Fe oxyhydroxide-bound phase.

Biosolids contain elevated Zn and Cu concentrations as compared to other elements. Thus, the environmental impact of Zn and Cu from anthropogenic sources such as biosolids needs to be quantified. In semi-arid grassland settings similar to that used in this study, downward transport of organically complexed Cu can be enhanced by biosolids application. However, Cu bioavailability is dictated by dissolved species which were all below detection limits in all subsoil samples. This finding, in addition to Cu transformations from organically complexed to oxyhydroxide-bound phases, should reduce any threat to degradation of environmental quality.

Acknowledgements

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