

# Copper and Zinc Speciation in a Biosolids-Amended, Semiarid Grassland Soil

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## Abstract

Predicting trace-metal solid-phase speciation changes associated with long-term biosolids land application is important for understanding and improving environmental quality. Biosolids were surface-applied (no incorporation; 0, 2.5, 5, 10, 21, and 30 Mg ha<sup>-1</sup>) to a semiarid grassland in 1991 (single application) and 2002 (repeated application). In July 2003, soils were obtained from the 0- to 8-, 8- to 15-, and 15- to 30-cm depths in all plots. Using soil pH, soluble anion and cation concentrations from 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> extractions, dissolved organic C (DOC) content, and an estimate of solid phase humic and fulvic acids present, Cu and Zn associated with minerals, hydrous ferric oxides (HFO), organically complexed, electrostatically bound to organic matter (OM), or DOC phases was modeled using Visual Minteq. Scanning electron microscopy and energy-dispersive X-ray analysis (SEM-EDXRA) was also used to identify solid-phase metal associations present in single and repeated biosolids-amended soils. Based on soil solution chemistry in all depths, as modeled using Visual Minteq, >90% of the Cu and >95% of the Zn from the single or repeated biosolids-applied soils were sorbed electrostatically or as mono- or bidentate solid-phase OM complexes. Up to 10 and 5% of the Cu and Zn, respectively, was associated with HFO, with negligible amounts associated with DOC. The SEM-EDXRA of clay-sized separates from all soil depths led to direct observation of Fe–Cu and Fe–Zn associations. Results implied that after surface-applying biosolids either once or twice with up to 30 Mg ha<sup>-1</sup>, some shifts occurred in phases controlling Cu and Zn solubility, but solution concentrations remained below drinking water standards.

**B**IOSOLIDS (i.e., sewage sludge) land application is a major method of recycling in the United States, with approximately 50% land applied (USEPA, 2012); in USEPA Region 8, which encompasses Colorado, Montana, North Dakota, South Dakota, Utah, and Wyoming, 85% is land applied (USEPA, 2013b). This recycling method can greatly benefit municipalities by recycling plant nutrients in an environmentally sound manner rather than disposing of biosolids. Within USEPA Region 8, a long-term (>12 yr) biosolids project with the City of Fort Collins, CO has provided valuable information on the effects of biosolids application to a semiarid grassland soil, particularly with respect to heavy-metal dynamics. For example, Harris-Pierce (1994) studied surface biosolids application with no incorporation at rates up to 30 Mg ha<sup>-1</sup> to a semiarid grassland, noting increased total Cd, Cu, Mo, Pb, and Zn concentrations in the soil at 0- to 8-cm depth associated with increasing biosolids application rates. A decrease in Cr and Ni concentrations associated with both increasing biosolids application and depth to 30 cm was also observed. Ippolito et al. (2009) revisited the Harris-Pierce research site in 2003 and found increasing total soil Zn and Cu in the 0- to 8-cm soil depth associated with increasing biosolids application rates. The authors also observed evidence of organically complexed downward Cu transport.

Lacking from these studies, however, was an analysis of the bioavailability of soil metals as a function of long-term biosolids land application. Basta et al. (2005) suggested that research is needed in predicting the long-term fate and bioavailability of trace elements in biosolids-amended soils. Furthermore, adequate assessment of the potential hazards imparted to trace-metal soil accumulation must take into account not only the concentration of trace metals but also the solid-phase chemical forms of these trace metals (Essington and Mattigod, 1991).

Essington and Mattigod (1991) analyzed biosolids-amended soil particles using scanning electron microscopy energy-dispersive X-ray analysis (SEM-EDXRA) following size, density, and magnetic fractionation. The authors found evidence of sphalerite ((Zn,Fe)S), chalcopyrite (CuFeS<sub>2</sub>), Pb-silicate, and Cr–Ni–Fe soil associations. Light-density fractions were similar

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**Abbreviations:** DOC, dissolved organic carbon; HFO, hydrous ferric oxides; ICP–AES, inductively coupled plasma–atomic emission spectroscopy; OM, organic matter; SEM–EDXRA, scanning electron microscopy and energy-dispersive X-ray analysis.

to heavy-density particles, yet the light fraction was apparently associated with organic matter (OM) via particle agglomeration. Hettiarachchi et al. (2006) used micro-X-ray fluorescence microprobe analysis and showed a strong association between Fe and Cd, Cr, Pb, and Zn, emphasizing the importance of Fe in adsorption–desorption of metals in both biosolids and biosolids-amended soils. The authors further showed that associations between Fe and Cu were reduced on organic carbon removal, suggesting that organic coatings on Fe were present.

Previous studies have also used wet chemistry techniques to elucidate metal–soil binding mechanisms. Li et al. (2001) noted increased soil Cd adsorption with increased biosolids application, and on removal of the organic phase, Cd adsorption persisted. This finding suggested that biosolids added an inorganic fraction that increased both soil Cd adsorption capacity and Cd stability. The biosolids inorganic fraction added was most likely an Fe- or Mn-(hydr)oxide phase, because Hettiarachchi et al. (2003) showed that removal of both the organic and Fe or Mn fractions from biosolids reduced soil Cd adsorption. Brown et al. (2003) showed that composted biosolids containing poorly ordered (i.e., amorphous) Fe and Mn oxyhydroxides, in combination with biosolids organic phases, resulted in a reduction in soil Pb bioavailability. Sukkariyah et al. (2005) used a sequential extraction on soils from an agricultural setting receiving up to 210 Mg ha<sup>-1</sup> biosolids showing the greatest concentrations of Cu, Zn, and Ni associated with soil metal oxides. Jaynes and Zartman (2005) studied inorganic phases present in New York, NY biosolids, observing >20% poorly crystalline Fe phosphates containing metals such as Cr, Cu, and Zn. Donner et al. (2013) noted that Cu and Zn formed associations with sulfide species in fresh biosolids; Zn was also associated with Fe-oxide phases. Upon aging, Cu formed associations with organic phases, while Zn-sulfide species decreased and Zn associated with Fe-oxide and Zn-PO<sub>4</sub> phases increased.

When applied to soils, biosolids-borne metals interact with soil- and biosolids-borne constituents, which, in turn, can affect downward transport. Emmerich et al. (1982a) researched a heavily amended (476 Mg ha<sup>-1</sup>) biosolids soil in a column study and found little metal movement below the incorporation zone. They attributed the lack of movement, and thus fate, to metals shifting toward more stable, residual forms after soil incorporation (Emmerich et al., 1982b). However, Sloan et al. (1998) observed increased trace-metal concentrations (including Cu and Zn) with depth in biosolids-treated (180 Mg ha<sup>-1</sup>) agricultural soil as compared with a control soil. Downward metal transport was potentially attributed to bioturbation and cotransport with soil colloids. McBride et al. (1997) noticed Cu transport into groundwater below an orchard soil amended with 244 Mg ha<sup>-1</sup> biosolids, associating Cu with an organically complexed mobile form. The authors also noted that Zn was relatively immobile, remaining in the topsoil in a plant-available form. Ippolito et al. (2009) used a sequential extraction scheme to discern differences in a semiarid grassland soil trace-metal pools associated with a single or repeated, increasing biosolids application of up to 30 Mg ha<sup>-1</sup>. Similar to the findings of McBride et al. (1997), biosolids-borne Cu was added to the system as an organically complexed phase that appeared to be transported downward and then, given time, transformed to a more stable oxyhydroxide phase. Zinc applied with biosolids was immobile, with chemical

changes only observed in the soil surface; the soil surface was dominated by a Zn-Mn/Fe oxide phase.

The objective of this study was to further support findings by Ippolito et al. (2009) by predicting Cu and Zn metal–soil solid-phase associations and identify which phases were controlling Cu and Zn solubility in a biosolids-amended semiarid grassland soil. A 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> solution was the chosen extractant to closely mimic soil ionic strength and could provide a measure of metal bioavailability. Metals extracted with this method account for only a small fraction of the total metals present in soil. The geochemical model Visual Minteq version 3.0 (Gustafsson, 2006) and soluble-metal concentration data were used, and observations were supported with the use of SEM-EDXRA. Based on our previous findings (Ippolito et al., 2009), we hypothesized that Visual Minteq would predict Fe-oxide phases as controlling Cu and Zn availability, and that DOC would enhance downward Cu movement and availability in the repeated biosolids application treatments.

## Materials and Methods

### Site Description, Biosolids, and Soil Sampling

In August 1991, 15- by 15-m test plots were established at the 10,500-ha Meadow Springs Ranch (40°53'46" N, 104°52'28" W; owned and operated by the City of Fort Collins, CO) with treatments consisting of 0-, 2.5-, 5-, 10-, 21-, and 30-Mg biosolids ha<sup>-1</sup>. Biosolids were 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 application with no incorporation using the same rates as the original plots.

Biosolids samples from the city of Fort Collins, CO wastewater treatment facility were collected before each application and kept refrigerated at approximately 3°C until analyzed. Total Cd, Cu, Cr, Mo, Ni, Pb, and Zn composition was determined by HClO<sub>4</sub>–HNO<sub>3</sub>–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 in 1991 and 2002 and the biosolids-borne metals loadings as a function of application rate.

The Meadow Springs Ranch is a semiarid, shortgrass steppe rangeland community dominated by the perennial grasses blue grama [*Bouteloua gracilis* (Willd. ex Kunth) Lag. Ex Griffiths] and western wheatgrass [*Pascopyrum smithii* (Rydb.) Á. Löve]. The research site soil was an Altvan loam (fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Aridic Argiustoll) on 0 to 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- to 8-, 8- to 15-, and 15- to 30-cm depths, composited, placed in Ziploc bags then into coolers, and returned to Colorado State University. All soils were immediately air-dried and ground to pass a 2-mm sieve.

### Soil Analyses

Soil pH was determined using a saturated paste (Thomas, 1996). Soluble PO<sub>4</sub> (Kuo, 1996) and SO<sub>4</sub> (Bettany and Halstead, 1972) were extracted by shaking 4.0 g of soil in 40 mL of 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> at 120 rpm for 30 min at 25°C and

**Table 1.** Trace metal characteristics of the 1991 and 2002 Fort Collins, CO biosolids and biosolids metals applied to the Meadow Springs Ranch semiarid rangeland experimental plots.

Parameter	Biosolids application rate													
	Mg ha <sup>-1</sup>													
	Application year		0		2.5		5		10		21		30	
	1991	2002	1991	2002	1991	2002	1991	2002	1991	2002	1991	2002	1991	2002
	— mg kg <sup>-1</sup> (dry wt. basis) —		Metal added, kg ha <sup>-1</sup> (dry wt. basis)											
Cu	547	475	0	0	1.37	1.19	2.74	2.38	5.47	4.75	11.5	9.98	16.4	14.2
Zn	772	652	0	0	1.93	1.63	3.86	3.26	7.72	6.52	16.2	13.7	23.2	19.6
Cd	5.0	2.6	0	0	0.01	0.01	0.03	0.02	0.05	0.03	0.10	0.05	0.15	0.08
Cr	40	21	0	0	0.10	0.05	0.20	0.10	0.40	0.21	0.84	0.44	1.20	0.63
Mo	16	19	0	0	0.04	0.05	0.08	0.10	0.16	0.19	0.34	0.40	0.48	0.57
Ni	19	17	0	0	0.05	0.04	0.10	0.08	0.19	0.17	0.40	0.36	0.57	0.51
Pb	120	39	0	0	0.30	0.10	0.60	0.20	1.20	0.39	2.52	0.82	3.60	1.17

then filtering through Whatman #42 filter paper. The PO<sub>4</sub> concentration was analyzed colorimetrically using the ascorbic acid method (Murphy and Riley, 1962) at 882 nm, while the SO<sub>4</sub> concentration was determined turbidimetrically using QuikChem Method No. 12-116-10-1-C on a QuikChem QC8500 automated ion analyzer (Hach Company). Dissolved organic C (DOC) was determined by shaking 5 g of soil in 20 mL of deionized H<sub>2</sub>O at 120 rpm for 1 h at 25°C then filtering through a 0.45-µm membrane filter and analyzing for DOC

using a TOC-5050A total organic C analyzer (Shimadzu Corp.). Soluble Cu and Zn concentrations were determined by shaking 4.0 g of soil in 40 mL of 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> at 120 rpm for 2 h at 25°C (Houba et al., 2000) then filtering through Whatman #42 filter paper and then analyzing using ICP-AES. This solution was also analyzed for Cd, Cr, Fe, K, Mg, Mn, Mo, Na, Ni, and Pb content using ICP-AES. Average soil pH, PO<sub>4</sub>, SO<sub>4</sub>, DOC, Cu, and Zn concentrations for the three soil depths, by rate, are presented in Table 2. Average soil Fe,

**Table 2.** Mean soil pH, soluble PO<sub>4</sub>, SO<sub>4</sub>, dissolved organic C (DOC), Cu, and Zn concentrations for the 0- to 8-, 8- to 15-, and 15- to 30-cm depths from the Meadow Springs Ranch semiarid rangeland biosolids-amended soils. Mean values were used as inputs in Visual Minteq, along with mean metal concentrations in Supplemental Table S1.

Biosolids rate	pH†		PO <sub>4</sub> ‡		SO <sub>4</sub> ‡		DOC§		Cu‡		Zn‡	
	S¶	R#	S	R	S	R	S	R	S	R	S	R
Mg ha <sup>-1</sup>	mg kg <sup>-1</sup>											
	0–8 cm											
0	5.9	6.3	0.90	0.95	33.2	8.48	3.59	3.79	0.039	0.005	0.176	0.112
2.5	5.8	6.3	1.80	1.56	25.0	13.5	7.20	6.22	0.014	BD††	0.181	0.165
5	5.7	6.1	3.53	2.20	43.2	34.8	14.1	8.78	0.026	BD	0.283	0.309
10	5.5	5.9	6.00	5.88	29.7	127	24.0	23.5	0.053	0.023	0.742	0.675
21	5.9	6.1	8.01	10.1	53.1	428	32.2	40.3	0.068	0.188	0.735	1.209
30	5.5	6.3	13.9	12.4	46.9	629	55.6	49.4	0.133	0.247	1.805	1.458
	8–15 cm											
0	6.5	6.7	0.37	0.48	13.6	5.85	1.47	1.90	0.012	BD	0.037	0.077
2.5	6.4	6.8	0.39	0.54	15.3	18.6	1.55	2.18	BD	BD	0.037	0.048
5	6.4	6.6	0.51	0.53	20.0	15.5	2.03	2.13	BD	BD	0.028	0.080
10	6.2	6.6	0.76	0.80	31.1	55.3	3.05	3.20	0.015	BD	0.162	0.062
21	6.5	6.3	1.55	1.40	17.0	144	6.19	5.59	0.013	BD	0.040	0.127
30	6.0	6.2	3.38	2.41	27.8	235	13.5	9.65	0.028	BD	0.184	0.239
	15–30 cm											
0	6.6	6.8	0.34	0.42	14.8	10.5	1.34	1.67	BD	BD	0.036	0.099
2.5	6.6	6.8	0.32	0.44	11.3	7.12	1.27	1.76	BD	BD	0.008	0.020
5	6.7	6.5	0.36	0.45	25.8	9.05	1.44	1.81	BD	BD	0.024	0.071
10	6.4	6.7	0.43	0.48	23.1	17.1	1.70	1.92	BD	BD	0.125	0.037
21	6.7	6.4	0.54	0.64	20.1	70.6	2.15	2.55	BD	BD	0.040	0.086
30	6.1	6.3	1.44	2.45	26.5	171	5.75	9.78	0.016	0.012	0.138	0.246

† pH was determined using a saturated paste extract.

‡ PO<sub>4</sub>, SO<sub>4</sub>, Cu, and Zn were determined using a 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> extraction.

§ DOC was determined using a water extraction.

¶ S = single biosolids application (1991).

# R = repeated biosolids application (2002).

†† BD = below inductively coupled plasma-atomic emission spectroscopy detection.

K, Mg, Mn, and Na in the single and repeated biosolids plots, for all depths, ranged from 0.007 to 1.041, 10.2 to 159, 10.7 to 249, 0.149 to 19.8, and 0.43 to 8.27 mg kg<sup>-1</sup>, respectively (Supplemental Table S1). All other metals (Cd, Cr, Mo, Ni, and Pb) were below ICP–AES detection limits. More soils information, including total soil metal content, can be found in Ippolito et al. (2009).

## Geochemical Modeling

Solid and solution phases controlling Cu and Zn activity were predicted using Visual Minteq version 3.0 (Gustafsson, 2006). Model inputs included fixed ionic strength at 0.01, soil pH, PO<sub>4</sub>, SO<sub>4</sub>, soluble Cu, Zn (Table 1), and other detectable 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> extractable metal concentrations (Supplemental Table S1) for each treatment and soil depth within the single or repeated biosolids-applied plots. Quartz, gibbsite, and ferrihydrite (aged) were added as infinite solids, and all potential mineral phases were allowed to precipitate. Metal activities were determined using the Davies equation in Visual Minteq.

Potential solid-phase OM- and DOC-metal sorption was modeled using the Stockholm Humic Model function in Visual Minteq. This model was adapted for modeling systems where humic substances are part of both the solid and solution phase (Gustafsson and Van Schaik, 2003). The DOC (Table 1) was entered into the model for all biosolids-treated soils and depths; the model assumed that 100% of all DOC phases were present as dissolved fulvic acid. The model also required solid-phase humic and fulvic acid concentrations as inputs. These were estimated by first measuring soil C content in all treatments and depths using a LECO-1000 CHN autoanalyzer (Nelson and Sommers, 1996). The plots did not contain inorganic C as determined by the pressure transducer method (Sherrod et al., 2002; data not shown). The contribution of background (i.e., control) soil and biosolids to the soil humic and fulvic acid content (Supplemental Table S2) were estimated as follows: (i) background soil C content was multiplied by 1.74 to convert to OM. Then, assuming soil OM contained 70% humic substances (Brady and Weil, 1999), OM content was converted to humic and fulvic acid content by assuming 75 and 25% of the humic substances were humic and fulvic acids, respectively, as found in grassland soils (Stevenson, 1982); (ii) the contribution of biosolids to soil humic and fulvic acid content was estimated based on the increase in soil C content due to biosolids application as compared with the control. The difference in soil C content was then multiplied by 1.74, and the humic and fulvic acid contents were estimated by assuming 17% of the biosolids-derived OM was equally distributed between the humic and fulvic acid fractions (Riffaldi et al., 1982). The sum of humic and fulvic acids from the background soil and the biosolids were entered separately into the model.

Potential metal sorption onto hydrous ferric oxides (i.e., ferrihydrite; one surface, plane geometry) was also taken into account by using the HFO model (Dzombak and Morel, 1990) in Visual Minteq. The model assumes hydrous ferric oxides (HFO) with strong and weak binding sites as the main adsorbing surface. As suggested by Dzombak and Morel (1990) and present as initial set values in Visual Minteq, we used an HFO surface area estimate of 600 m<sup>2</sup> g<sup>-1</sup>, the mass of adsorbing surface (56.8 g

HFO L<sup>-1</sup>), and concentration of strong (0.003 mol L<sup>-1</sup>) and weak (0.128 mol L<sup>-1</sup>) binding sites.

Both the OM- and DOC-metal sorption and HFO modeling approaches were used based on our previous sequential extraction wet chemistry findings (Ippolito et al., 2009). Those findings showed that downward Cu transport was associated with organically complexed phases shortly after biosolids land application. Given time, Cu began forming associations with poorly ordered (i.e., amorphous) Fe- and Mn-(hydr)oxide phases. Zinc tended to form strong associations with poorly ordered Fe- and Mn-(hydr)oxide phases regardless of short- or long-term biosolids application. However, the Zn content increased in the specifically sorbed and organically complexed phases shortly after biosolids application.

## Scanning Electron Microscopy and Energy-Dispersive X-Ray Analysis

The clay-sized fraction from each treatment and soil depth was separated without the removal of OM following the method outlined by Gee and Or (2002) using 50 g L<sup>-1</sup> sodium hexametaphosphate. Wet clay samples were placed in small aluminum boats, dried in an oven at 60°C for several days, and then placed into 20-mL glass scintillation vials. Carbon paint was applied to a 10- by 10-mm Al stub, clays were gently sprinkled on the surface, and the system was allowed to dry for more than 24 h before analysis. Approximately three particles per sample were examined at an accelerating voltage of 20 kV and a working distance between 9.3 and 10.3 mm using a JEOL-JSM 6500F Thermal Assist Emission Scanning Electron Microscope (JEOL Ltd.) in backscattered electron mode. To qualitatively identify solid-phase elemental associations, energy-dispersive X-ray analysis (EDXRA) was performed with a NORAN System SIX X-ray microanalysis system (Thermo Fisher Scientific, Inc.) equipped with a NanoTrace Si(Li) detector (Thermo Electron Corp.).

## Results and Discussion

### Copper

The phases controlling Cu availability, as determined by Visual Minteq, are presented in Table 3. In all cases, Cu bound to DOC fulvic acid phases was negligible and thus did not control Cu availability. Within the single and repeated biosolids-applied soils, however, Visual Minteq predicted that mono- or bidentate OM solid phases dominated Cu availability (79–93%) as compared with the other phases present. Monodentate and bidentate Cu bonding occur as innersphere complexes to either one or two oxygen atoms, respectively, and are often not reversible (i.e., stable; Sparks, 2003). Innersphere complexes appear to be stable for relatively long periods of time (at least 12 yr) in this system.

Copper, electrostatically bound onto solid phases from OM, accounted for approximately 4 to 12% of the phases controlling Cu availability (Table 3). Electrostatic attraction mechanisms are strongly pH dependent (Sparks, 2003), as illustrated within the single biosolids application with 0- to 8-cm depth. The pH of the 0 and 21 Mg ha<sup>-1</sup> biosolids-applied soils both equaled 5.9, while soil pH values in the other treatments ranged from

5.5 to 5.8 (Table 2). The increase in pH likely led to greater pH-dependent charge on solid OM phases, attracting more Cu to the OM surface and thus increasing the electrostatically bound Cu.

Within the single and repeated biosolids-applied soils and in all cases, Visual Minteq predicted that the soil solution was undersaturated with respect to Cu-bearing mineral phases (data not shown). Karthikeyan et al. (1997) showed that when HFO was present, solution Cu concentrations were undersaturated with respect to certain mineral phases (e.g.,  $\text{Cu}(\text{OH})_2$ ). The authors suggested that Cu surface precipitation and substitution in the HFO lattice occurred. Hydrous ferric oxides accounted for 3 to 9% of the phases controlling Cu availability (Table 3), suggesting that HFO surface precipitation of Cu may have occurred.

Although Cu forms strong complexes with organic phases, Ippolito et al. (2009) suggested that, over time, biosolids-borne organically complexed Cu would be mineralized and transformed to create associations with Fe-oxyhydroxide phases. In fact, the authors showed that in the 0- to 8-, 8- to 15-, and 15- to 30-cm depths of the single and repeated biosolids-treated soils, Cu was found largely associated with Fe- and Mn-oxyhydroxide phases. In this coarse-textured soil, it also may have been possible that particles containing Fe and Mn oxyhydroxides, were transported downward following biosolids application. These phases could have sorbed solution Cu and led to below-detection-limit Cu concentrations, which was especially evident in the repeated biosolids treated plots (Table 3). Copper bound to soil HFO phases may be considered less bioavailable as compared with

dissolved phases (e.g., Torri and Lavado, 2008; Sloan et al., 1997; Hickey and Kittrick, 1984). Although Fe- and Mn-oxyhydroxide phases dominated this system (Ippolito et al., 2009), they do not appear to control Cu availability.

Previous modeling efforts have shown that large quantities (>99%) of Cu were sorbed to dissolved OM (Benedetti et al., 1996; Temminghoff et al., 1997). More specifically, it has been shown that 50 to 75% of the Cu present within biosolids is organically complexed (Alloway, 1995; Wong et al., 2001), and that downward transport of biosolids-borne Cu occurred with dissolved organic phases (Al-Wabel et al., 2002; Ippolito et al., 2009; McBride et al., 1997). The current modeling effort does not support downward Cu transport by DOC phases but does support the contention that Cu forms strong associations with organic phases and that these phases are likely controlling and dominating Cu availability. In this coarse-textured soil, it may have been possible that OM colloids were transported downward and influenced Cu availability, similar to that observed by Sloan et al. (1998). It is important to note that the soluble Cu concentrations within all soil depths (Table 2) are approximately an order to several orders of magnitude lower than the USEPA drinking water maximum contaminant level goal of  $1.3 \text{ mg L}^{-1}$  (USEPA, 2013a) and secondary drinking water standard of  $1.0 \text{ mg L}^{-1}$  (USEPA, 2013c).

## Zinc

The phases controlling Zn availability, as determined by Visual Minteq, are presented in Table 4. In all cases, Zn bound to DOC fulvic-acid phases was negligible and thus did not control

**Table 3. Predicted phases controlling Cu availability, as determined by Visual Minteq (Gustafsson, 2006).**

Biosolids rate	Cu (single application in 1991)				Cu (repeated application in 2002)			
	Electrostatically bound to solid OM†	Mono- or bidentate solid OM complexes	Hydrous ferric oxide bound	Bound to fulvic acid in solution	Electrostatically bound to solid OM	Mono- or bidentate solid OM complexes	Hydrous ferric oxide bound	Bound to fulvic acid in solution
Mg ha <sup>-1</sup>	%				%			
	<b>0–8 cm</b>							
0	7.1	89.3	3.6	0.0	11.3	83.3	5.4	0.0
2.5	6.2	90.3	3.5	0.0	BD‡	BD	BD	BD
5	5.2	91.4	3.5	0.0	BD	BD	BD	BD
10	4.0	92.8	3.1	0.1	6.9	89.0	4.0	0.1
21	7.2	89.1	3.6	0.1	9.2	86.3	4.4	0.1
30	4.3	92.8	2.7	0.2	12.5	83.2	4.2	0.1
	<b>8–15 cm</b>							
0	8.6	81.2	10.2	0.0	BD	BD	BD	BD
2.5	BD	BD	BD	BD	BD	BD	BD	BD
5	BD	BD	BD	BD	BD	BD	BD	BD
10	5.0	86.1	8.9	0.0	BD	BD	BD	BD
21	12.4	79.3	8.3	0.0	BD	BD	BD	BD
30	5.2	87.8	6.9	0.1	BD	BD	BD	BD
	<b>15–30 cm</b>							
0	BD	BD	BD	BD	BD	BD	BD	BD
2.5	BD	BD	BD	BD	BD	BD	BD	BD
5	BD	BD	BD	BD	BD	BD	BD	BD
10	BD	BD	BD	BD	BD	BD	BD	BD
21	BD	BD	BD	BD	BD	BD	BD	BD
30	3.0	87.1	9.9	0.0	9.3	82.8	7.9	0.0

† OM = organic matter.

‡ BD = Cu concentration in the  $0.01 \text{ mol L}^{-1} \text{ CaCl}_2$  extraction was below inductively coupled plasma–atomic emission spectroscopy detection.

Table 4. Predicted phases controlling Zn availability as determined by Visual Minteq (Gustafsson, 2006).

Biosolids rate	Zn (single application in 1991)				Zn (repeated application in 2002)			
	Electrostatically bound to solid OM†	Mono- or bidentate solid OM complexes	Hydrous ferric oxide bound	Bound to fulvic acid in solution	Electrostatically bound to solid OM	Mono- or bidentate solid OM complexes	Hydrous Ferric Oxide Bound	Bound to fulvic acid in solution
Mg ha <sup>-1</sup>	%				%			
	<b>0–8 cm</b>							
0	78.2	21.4	0.4	0.0	87.1	12.4	0.5	0.0
2.5	75.0	24.5	0.5	0.0	87.5	12.1	0.4	0.0
5	71.0	28.5	0.5	0.0	82.5	17.0	0.5	0.0
10	64.0	35.4	0.5	0.1	77.7	21.7	0.5	0.1
21	78.4	21.1	0.4	0.1	83.4	16.1	0.4	0.1
30	65.8	33.6	0.4	0.2	88.4	11.2	0.3	0.1
	<b>8–15 cm</b>							
0	84.5	14.4	1.1	0.0	91.6	7.7	0.7	0.0
2.5	83.5	15.5	1.0	0.0	93.4	6.0	0.6	0.0
5	78.5	20.1	1.4	0.0	92.7	6.7	0.6	0.0
10	73.1	25.5	1.4	0.0	89.4	9.9	0.7	0.0
21	89.1	10.2	0.7	0.0	84.0	15.2	0.8	0.0
30	72.6	26.4	1.0	0.0	83.0	16.3	0.7	0.0
	<b>15–30 cm</b>							
0	63.4	31.8	4.8	0.0	90.9	8.0	1.0	0.0
2.5	75.2	22.1	2.7	0.0	92.1	7.0	0.9	0.0
5	73.4	23.2	3.4	0.0	82.3	16.3	1.4	0.0
10	70.1	27.3	2.6	0.0	90.1	9.0	0.9	0.0
21	89.5	9.5	1.0	0.0	83.1	15.8	1.1	0.0
30	59.9	37.9	2.2	0.0	84.6	14.6	0.8	0.0

† OM = organic matter.

Zn availability. Within the single and repeated biosolids-applied soils, however, Visual Minteq predicted that electrostatically bound Zn dominated Zn availability (60–93%) as compared with the other phases present. As electrostatic interactions are pH dependent, the percentage of Zn available increases (Table 4) with increasing soil pH (Table 2). Based on the modeling results, it also appears that the percentage of electrostatically bound Zn increased following biosolids reapplication. This increase could have accounted for the increase in specifically sorbed and organically complexed Zn phases immediately following biosolids application as observed by Ippolito et al. (2009).

To a lesser extent, mono- and bidentate solid OM complexes contributed to Zn availability (6–38%), while HFO-bound Zn played a relatively negligible role (0.3–4.8%; Table 4). Within both the single and repeated biosolids-applied soils, the model predicted that in all cases the soil solution was undersaturated with respect to Zn-bearing mineral phases (data not shown).

Voegelin et al. (2002) showed that Zn sorption was associated with edge surfaces of phyllosilicates or amorphous Al hydroxides. Ippolito et al. (2009) used a sequential extraction to show that Zn was associated with extractable Fe- and Mn-oxide phases following semiarid grassland biochar application. Others have reported similar findings (Sloan et al., 1997), emphasizing the importance of Fe-oxide metal sorption (Rieuwerts et al., 1998). However, although others have pointed out that biosolids may contain Zn associated with Fe-oxyhydroxide phases (Ippolito et al., 2009; Donner et al., 2013), these phases do not appear responsible for controlling Zn availability in this system. As with Cu, soluble Zn concentrations within all soil depths (Table

2) are approximately five times to several orders of magnitude lower than the USEPA secondary drinking water standard of 5.0 mg L<sup>-1</sup> (USEPA, 2013c).

### Scanning Electron Microscopy and Energy-Dispersive X-ray Analysis

The SEM-EDXRA biosolids-amended clay-sized separates, mainly from the 21 and 30 Mg ha<sup>-1</sup> single and repeated biosolids treated plots, led to direct observation of Cu-, Zn-, and Fe-bearing particles (Fig. 1 and 2), presumably Fe (hydr)oxides. Figure 1C presents the Cu and Fe EDXRA dot map overlay in the 8- to 15-cm depth from the single 30 Mg ha<sup>-1</sup> biosolids treatment. Within the figure, 1% Cu and 9.2% Fe were present at the yellow hatch mark. Similarly, Fig. 2C presents the Fe and Zn EDXRA dot map overlay (0- to 8-cm depth; repeated 21 Mg ha<sup>-1</sup> biosolids treatment) with 3.2% Fe and 0.3% Zn present across the particles. Both figures support the contention that Cu and Zn are at least associated with Fe-bearing phases. Karthikeyan and Elliott (1999) suggested that hydrous oxide adsorption of Cu was due to the formation of some surface precipitates at lower pH values, and surface precipitation was the dominant sorption mechanism above pH 6. Voegelin et al. (2005) suggested the presence of Zn in hydroxides might kinetically control Zn availability. Both surface adsorption and precipitation reactions could have occurred in the current system studied, as shown by the predicted adsorption of Cu and Zn by HFO as well as, occasionally, the predicted precipitation of cupric ferrite (Tables 3 and 4).

The association of Cu with organic phases, however, should not be ruled out based on SEM-EDXRA analysis. This analysis

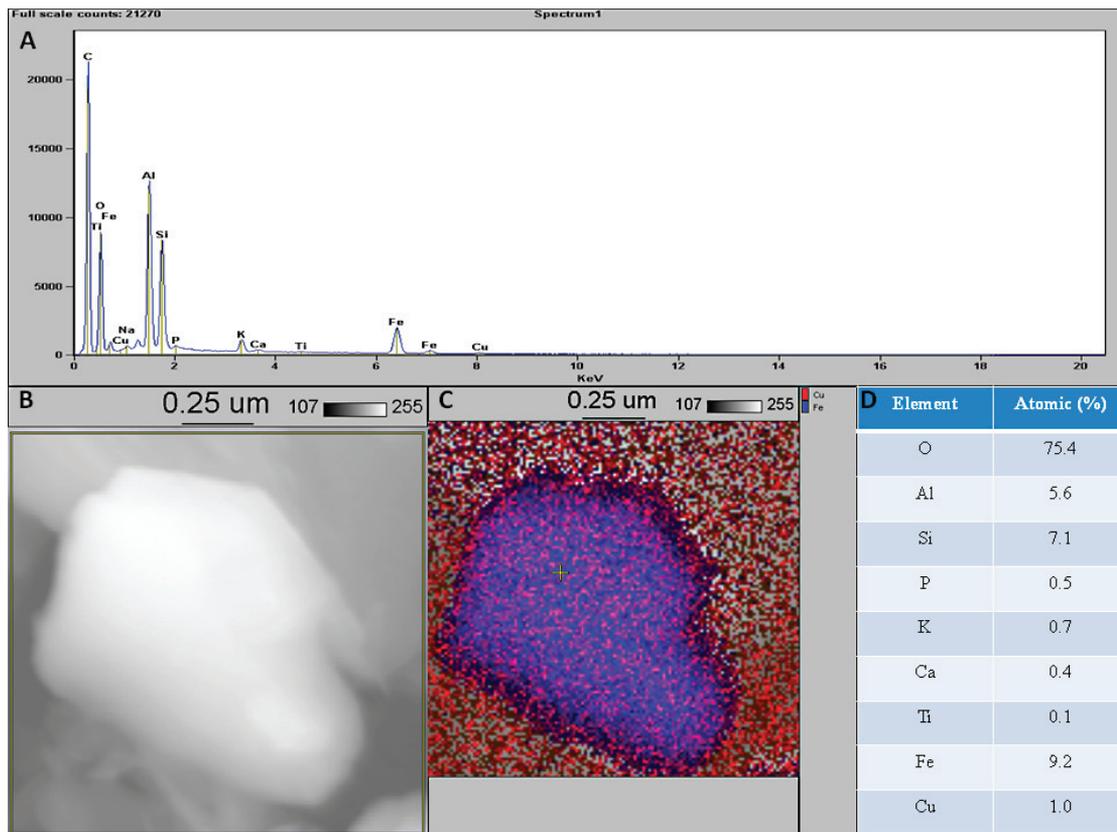


Fig. 1. Scanning electron microscope and energy-dispersive X-ray analysis (EDXRA): (A) spectra; (B) backscattered image; (C) Cu and Fe EDXRA dot map overlay (Cu [red] and Fe [blue] overlap to make pink); and (D) atomic percentage of elements at the yellow hatch mark in (C). Particle was from the 30 Mg ha<sup>-1</sup> single biosolids application (1991), 8- to 15-cm depth, Meadow Springs Ranch semiarid rangeland amended plots.

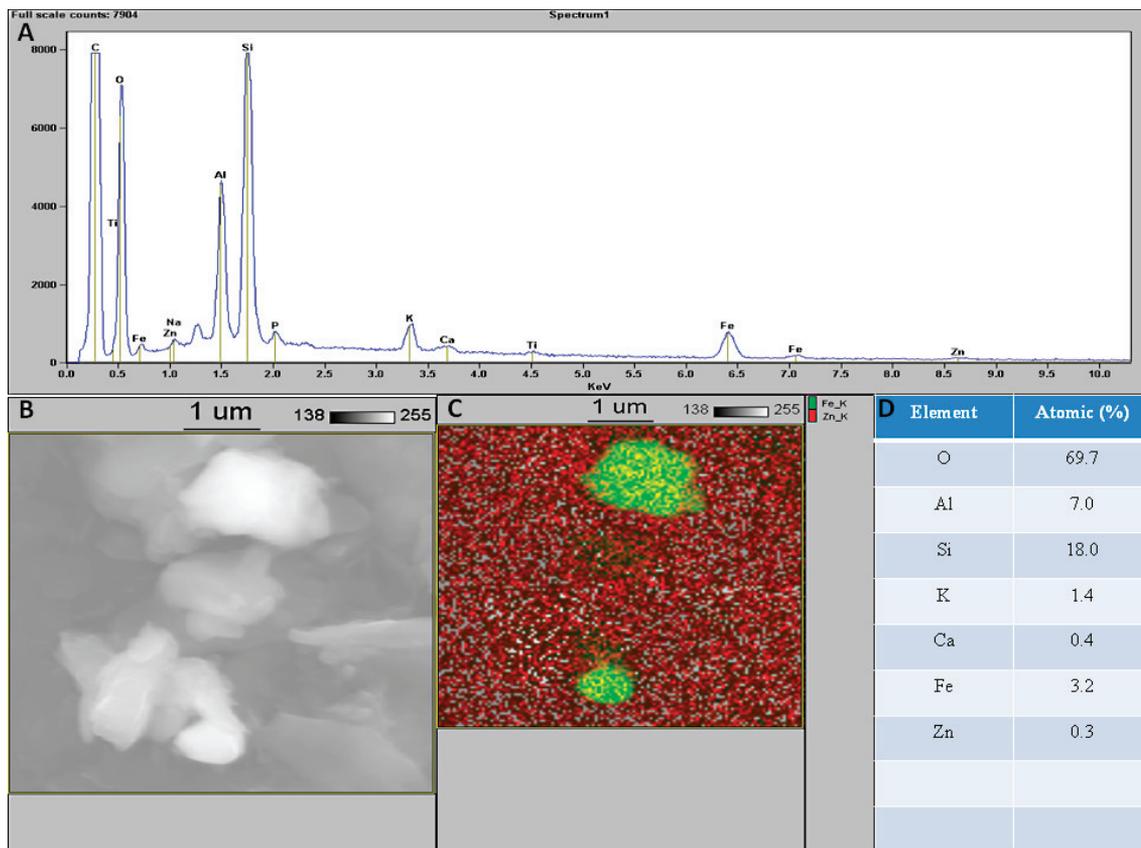


Fig. 2. Scanning electron microscope and energy-dispersive X-ray analysis (EDXRA): (A) spectra; (B) backscattered image; (C) Fe and Zn EDXRA dot map overlay (Fe [green] and Zn [red] overlap to make yellow or orange); and (D) atomic percentage of elements on all particles in (C). Particles were from the 21 Mg ha<sup>-1</sup> repeated biosolids application (2002), 0- to 8-cm depth, Meadow Springs Ranch semiarid rangeland amended plots.

cannot differentiate between C used during sample preparation and sample-borne C. In support of this contention, Hettiarachchi et al. (2006) used micro-X-ray fluorescence microprobe analysis and showed a reduction in soil Fe–Cu associations on organic C removal. The author's findings suggested that organic coatings on Fe surfaces were present, emphasizing the importance of Fe and organic C for Cu sorption.

## Significance of Results

Following a single or repeated biosolids application (surface-applied with no incorporation at rates of up to 30 Mg ha<sup>-1</sup>) soil phases controlling Cu and Zn availability shifted minimally. Our hypotheses regarding the phases controlling Cu and Zn availability were based on observations from a sequential extraction technique and not based solely on metal solubility. Based on the current study findings, we rejected our hypotheses that Fe-oxide phases appeared to control Cu and Zn availability and that DOC enhanced downward Cu movement. Visual Minteq modeling predicted innersphere complexation and electrostatically bound Cu and Zn and, to a lesser extent, HFO-bound Cu and Zn, to control metal availability in this system. Previous research suggested that biosolids metal bioavailability is linked to either biosolids OM chelation (McBride, 1995) or inorganic surface adsorption (Hettiarachchi et al., 2003), as observed in our study. Furthermore, it is possible that bioturbation and colloidal Cu and Zn downward transport has influenced availability with depth, as observed by Sloan et al. (1998). At the application rates used, soluble Cu and Zn concentrations were below USEPA primary and secondary drinking water standards. Identifying and predicting soil phases responsible for controlling metal bioavailability in long-term biosolids land application programs should enhance our understanding and improve environmental quality.

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