

Copper Impacts on Corn, Soil Extractability, and the Soil Bacterial Community

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Abstract: Dairies use CuSO₄ footbaths to control hoof infections, with Cu accumulation in agricultural soils realized when spent footbaths are disposed of in waste lagoons and subsequently used for irrigation. We investigated the effect of Cu applications (up to 1000 mg kg⁻¹) to a Xeric Haplocalcid (Declo series) and a Typic Calciaquoll (Logan series) on corn (*Zea mays* L.) growth and Cu concentration, soil total and diethylenetriaminepentaacetic acid (DTPA)-extractable Cu, and the soil bacterial community diversity using ribosomal intergenic spacer analysis followed by cluster analysis. Copper application up to 250 mg kg⁻¹ did not affect corn growth; at 500 mg Cu kg⁻¹, corn growth was reduced by up to 80%. The 250 and 500 mg kg⁻¹ Cu application rates increased corn Cu content grown in Declo soil, whereas the 500 mg kg⁻¹ Cu application rate increased Logan soil corn Cu. Regardless of initial application rate, 60% to 75% of the added Cu was still plant available. Comparing DTPA-extractable Cu with corn Cu concentrations for the Declo and Logan soils, 130 or 220, and 215 or 300 mg kg⁻¹ of DTPA-extractable soil Cu would be detrimental in terms of sheep and cattle dietary Cu intake, respectively. As Cu concentrations increased, bacterial diversity decreased, and species evenness remained high, suggesting that few phylotypes predominated within the ribosomal intergenic spacer analysis profiles. To prevent excessive corn Cu accumulation and negative impacts on the soil bacterial community, it is recommended that available soil Cu not exceed 130 mg kg⁻¹ in agroecosystems associated with these soil series.

Key words: Copper, corn, soil bacterial community, DTPA-extractable copper.

(*Soil Sci* 2010;175: 586–592)

Copper accumulation in agricultural soils can occur through anthropogenic inputs such as application of Cu-containing fungicides, Cu-related mining and manufacturing, and application of liquid or solid waste residuals (Yu et al., 2002). Agricultural soil Cu accumulation is also a concern because of routine dairy operations. In south-central Idaho, soil Cu concern is increasing because the state is currently the third largest dairy-producing state in the United States, with approximately 550,000 head of dairy cows as of 2008 (USDA NASS, 2008). Dairies use between 5% and 10% (12,500 to 25,000 mg L⁻¹) CuSO₄ concentrations in footbath to control hoof infections, with spent footbaths typically washed out of dairy barns and into liquid

waste lagoons. Once CuSO₄ enters the waste lagoon, soluble Cu concentration decreases because of dilution and Cu binding to organic phases (Stehouwer and Roth, 2009). Approximately 90% to 95% of the Cu is held by organic phases in the waste lagoon (Stehouwer and Roth, 2009); however, 625 to 2500 mg Cu L⁻¹, depending on initial footbath Cu content, remains soluble and thus available. Because excessive plant Cu uptake can be toxic to plants, Cu-enriched liquid dairy waste applied as irrigation water to agricultural crops raises concerns regarding how plants and soils are impacted.

When added to soils, Cu can occur in several forms, such as (i) in the soil solution, (ii) on exchange sites, (iii) specifically sorbed, (iv) occluded in soil oxides, (v) in the lattice structure of primary and secondary minerals, and (vi) in organic residues and living organisms (Adriano, 1986). In soils with pH values greater than 7.0, soluble Cu can react to form CuO, CuCO₃, or mixed hydroxycarbonate mineral species (McBride and Bouldin, 1984; Ponizovsky et al., 2007), although most of the Cu is strongly adsorbed to soil organic matter (OM) in basic soils because of greater OM solubility and increasing pH-dependent charge associated with increasing soil pH (McBride and Blasiak, 1979). Cavallaro and McBride (1978) showed that more than 80% of Cu added to neutral pH soil was in complexed form, whereas McBride and Blasiak (1979) showed that 99.9% of total soluble Cu was complexed with organic phases at pH 8. McBride and Bouldin (1984) found evidence of malachite precipitation in a Cu-contaminated calcareous soil (~3%–4% free carbonate), yet the soil contained 5.3% OM, and greater than 95.5% of the Cu in soil solution was complexed with organic species. When present in calcareous soils, it has been shown that increasing OM content decreases the quantity of plant-available Cu (Ghasemi-Fasaei et al., 2006) because of Cu complexation with organic species.

Overapplication of soluble Cu to soils, regardless of potential soil precipitation or complexation reactions, may detrimentally impact plants. Phytotoxicity symptoms are often associated with Cu and include stunted growth, chlorosis, necrosis, and death of the crop (Pierzynski et al., 2000). However, Cu mainly impairs root growth through inhibition of lateral root development and new seedling root growth initiation (Pahlsson, 1989). Brun et al. (2001) studied corn (*Zea mays* cv. Gaucho) root and shoot growth when grown in Cu-contaminated vineyard soils (up to 251 mg total Cu kg⁻¹). Root Cu concentrations (23–584 mg kg⁻¹) were greater than shoot concentrations (7–17 mg kg⁻¹), with total Cu and cation exchange capacity, and total Cu, soil pH, and OM explaining 81% and 85% of the variability in roots and shoots, respectively. Unfortunately, the authors did not measure root or shoot weight that could have accounted for decreases in accumulated biomass with greater soil Cu concentrations. Sonmez et al. (2006) applied increasing concentrations of either Cu oxychloride or CuSO₄·5H₂O to tomato (*Lycopersicon esculentum* [L.] Mill. Cv. F144), noting in both cases a decrease in plant height, total yield, number of fruit, and dry root weight with increasing Cu addition. Strandberg et al. (2006) studied Cu-spiked soil effects on growth of Black Bindweed (*Fallopia convolvulus*),

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Received April 29, 2010.

Accepted for publication September 21, 2010.

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ISSN: 0038-075X

DOI: 10.1097/SS.0b013e3181fe2960

showing that shoot growth was affected at a Cu dose greater than 200 mg kg⁻¹, reduced to 50% at 280 mg kg⁻¹, and was absent at more than 400 mg kg⁻¹.

Not only are excessive Cu concentrations detrimental to plants, they may also be toxic to microorganisms. Microorganisms are generally more sensitive to Cu and other heavy metal stresses than other organisms in the soil ecosystem (Giller et al., 1998). Ranjard et al. (2006) investigated the effects of Cu, applied at 16 and 48 kg ha⁻¹, on indigenous soil microorganisms in a calcareous silty clay. Using principal component analysis, the authors showed that 2 months after Cu application, control soil bacterial and fungal populations were significantly separated from Cu-treated soils regardless of application rate. However, at 4 and 12 mth after application, no differences existed between treatments and the control. Ranjard et al. (2006) suggested that a transitory effect of the Cu stress might be partly caused by the progressive reduction of soil Cu bioavailability over time. Yet time does not always reduce bioavailability, as shown by Sauve (2006), who noted that soil OM degradation by microorganisms was inhibited by 10%, 20%, and 50% with 154, 193, and 285 mg Cu kg⁻¹ soil, respectively at a site exposed to decades of Cu contamination.

The objectives of this study were to quantify the effects of Cu addition on (i) corn growth, (ii) soil total and subsequently extractable Cu characteristics, and (iii) soil bacterial community structure and diversity. Soil chemical characteristics were also used to determine an estimate of extractable soil Cu associated with corn copper content and the maximum tolerable Cu level in cattle and sheep based on National Research Council (2005) guidelines. Cattle were chosen because of their consumption of corn silage and the large dairy population in Idaho (~550,000 head; USDA NASS, 2008), whereas sheep were chosen because they are more sensitive to dietary Cu as compared with cattle.

MATERIALS AND METHODS

Soils

The 0- to 30-cm depth of the Declo (coarse-loamy, mixed, superactive, mesic Xeric Haplocalcid) and Logan (fine-silty, mixed, superactive, mesic Typic Calciaquoll) soil series were used for this study as they are extensive in south-central Idaho (63,900 and 6700 ha, respectively; USDA NRCS, 2009). Both soils were also chosen because of similar chemical characteristics (Table 1), except for inorganic and organic C percentage, which could potentially influence Cu adsorption and thus our estimate of extractable soil Cu associated with corn copper content and animal dietary Cu intake. Soil pH (Thomas, 1996) and electrical conductivity ([EC] Rhoades, 1996) were determined on a saturated paste extract, and cation exchange capacity *via* the method outlined by Sumner and Miller (1996) for soils containing carbonates. Total C was determined using the dry combustion method outlined by Nelson and Sommers (1996), whereas CaCO₃ and inorganic C content were determined by a modified pressure-calimeter method (Sherrod et al., 2002); organic C was determined via the difference between total C and inorganic C, and OM was calculated by multiplying organic C content by 1.724. Nitrate-N and NH₄-N were determined following methods outlined by Mulvaney (1996), and Ca, Mg, Na, K, Fe, P, Mn, Zn, Mo, and Cu using a 4-M HNO₃ digest (Bradford et al., 1975).

Experimental Setup

Soils were air-dried and passed through a 0.64-cm sieve before use. Then 1.5 kg of soil was placed into individual 23-cm-tall × 10-cm-diameter pots with no drain holes. All

TABLE 1. Chemical Characteristics of the Declo and Logan Soil Series

Property	Units	Declo Soil Series	Logan Soil Series
pH		7.9	7.9
EC	dS m ⁻¹	1.02	0.73
CEC	cmol _c kg ⁻¹	14	14
Total C	%	2.0	7.3
CaCO ₃	%	11	49
Inorganic C	%	1.3	5.9
Organic C	%	0.7	1.4
Organic matter	%	1.2	2.5
NO ₃ -N	mg kg ⁻¹	11.6	9.82
NH ₄ -N	mg kg ⁻¹	5.20	4.75
Ca [†]	mg kg ⁻¹	30,560	85,600
Mg	mg kg ⁻¹	6180	29,800
Na	mg kg ⁻¹	398	414
K	mg kg ⁻¹	1430	866
Fe	mg kg ⁻¹	3290	1150
P	mg kg ⁻¹	706	405
Mn	mg kg ⁻¹	252	291
Zn	mg kg ⁻¹	27.2	27.0
Mo	mg kg ⁻¹	ND	ND
Cu	mg kg ⁻¹	6.52	5.04

[†]Ca, Mg, Na, K, Fe, P, Mn, Zn, Mo, and Cu determined using a 4-M HNO₃ digest (Bradford et al., 1975).

pots received N (KNO₃ in liquid form) at a rate equivalent to 213 kg N ha⁻¹ based on University of Idaho fertilizer recommendations for corn silage with a yield goal of 45 Mg ha⁻¹ (Brown and Westermann, 1988). Pots were brought to approximately 70% of field capacity using tap water three times per week during the study period. Five corn plants were seeded into each pot and allowed to establish over a 2-week period, after which pots were thinned to three plants per pot. Pots then received Cu solutions, as CuSO₄, at concentrations of 0 (control), 50, 100, 250, 500, or 1000 mg Cu kg⁻¹, and plants were allowed to grow for an additional 30 days. The experimental design was completely randomized with four replicates.

Plant and Soil Chemical Analyses

From each pot, live plants were harvested approximately 2.54 cm above the soil surface 30 days after Cu was applied, placed in paper bags, oven dried at 60°C for 72 h, biomass was determined, and then ground to pass a 20-mesh sieve. A 0.50-g subsample was placed in a 100-mL beaker and ashed at 500°C for 5 h. The samples were allowed to cool, weighed, and then 10 mL of 1 M HNO₃ was added. The samples were then heated on a hot plate until condensation no longer occurred on the inside of the beaker. Then, all samples were brought to a 50-mL final volume by weight with deionized H₂O, stirred, filtered through Whatman no. 50 filter paper, and analyzed for total Cu using inductively coupled plasma–optical emission spectroscopy.

After harvest, soils were removed from pots, air-dried, and ground to pass a 2-mm sieve. Soils were extracted for plant-available Cu using diethylenetriaminepentaacetic acid ([DTPA] Lindsay and Norvell, 1978) and with 4 M HNO₃ (Bradford et al., 1975), a close approximation to total soil Cu. McBride et al. (1997) showed the 4-M HNO₃ digestion to be as effective as a

standard nitric–perchloric acid digest for solubilizing, among other elements, soil Cu.

All soil and plant statistical tests were performed using the Proc GLM model in SAS software version 9.1 (SAS institute, 2002). Differences within each P fraction were examined using analysis of variance at a significance level of $\alpha = 0.05$, with mean separation determined using Fisher protected least significant difference (LSD) procedure. The mean separation test aided in the determination of a recommended available soil Cu threshold value at which Cu became an issue with regard to corn Cu content and animal dietary Cu consumption.

Soil Bacterial Community Analysis

A total of 100 g each of prestudy and treatment soil subsamples were collected immediately after plant harvest and stored moist at -80°C in anticipation of bacterial community analysis. The prestudy soils consisted of soil devoid of plant and copper additions, and all soil subsamples for bacterial analysis were collected from the corn root zone. One replicate of each subsample was selected at random, and DNA was extracted using a SoilMaster DNA Extraction Kit (Epicentre, Madison, WI). The DNA was further purified using an UltraClean GelSpin DNA Extraction Kit (MO BIO Laboratories, Inc., Carlsbad, CA). Final DNA quantity and quality were determined via Biophotometer (Eppendorf AG, Hamburg, Germany), and electrophoresis on a 1% agarose gel stained with SYBR Safe (Invitrogen, Carlsbad, CA). Only DNA with a 260:280 ratio between 1.7 and 1.9, with most of the fragments greater than 5 kb in size, was used in this study.

Ribosomal intergenic spacer analysis (RISA) was performed on extracted DNA using primers ITSF (5'-GTCGTAACAAGG TAGCCGTA-3') and ITSReub (5'-GCCAAGGCATCCACC-3') to amplify the variable length region between the 16S and 23S rDNA genes of the soil bacterial populations (Cardinale et al., 2004). Final reaction concentration of reagents were 10 ng of DNA, $1 \times$ polymerase chain reaction buffer, 1.5 U of *Taq* DNA polymerase (New England Biolabs, Ipswich, MA), 0.2 mmol/L of each deoxynucleoside triphosphate, and 0.25 $\mu\text{mol/L}$ of each primer in a final volume of 25 μL . Amplifications were performed at 94°C for 3 min, followed by 30 cycles of 94°C for 45 sec, 55°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 7 min. A total of 1 μL of each amplification reaction was loaded, and RISA fragments were resolved on 3.7% KB^{plus} polyacrylamide gels (LI-COR Inc., Lincoln, NE) of 66 cm length and 0.2 mm thickness. Gels were preconditioned at 3000 V/60 A and 40°C for 30 min. Profiles were run under denaturing con-

ditions for 15 h at 3000 V/60 A on a LiCor 4300 DNA Analyzer (LI-COR Inc., Lincoln, NE). Gel images were analyzed using TotalLab TL120 v2006f software (Nonlinear Dynamics, Newcastle upon Tyne, United Kingdom). Bands (hereafter referred to as phylotypes) were determined, and matched between soil RISA profiles based on their electrophoretic mobility (Rf).

Bacterial diversity was calculated using both the Shannon-Wiener Index ([*H*] Margalef, 1958) and Reciprocal Simpson Diversity Index ([1/*D*] Simpson, 1949). For both indices, p_i was calculated as $P_i = n_i/N$, with n_i representing the peak intensity of individual phylotypes to the *i*th phylotype in a profile, and N representing the total peak intensity of the profile. Evenness ([*J*] Pielou, 1966) of the soil RISA profiles was calculated using sample richness (*S*), which was calculated as the total number of phylotypes for a given profile.

Cluster analysis of soil RISA profiles was performed by the unweighted pair-group method with arithmetic averages (UPGMA) using a Pearson correlation coefficient, in TotalLab TL120DM v2006b (Nonlinear Dynamics, Newcastle upon Tyne, United Kingdom).

RESULTS AND DISCUSSION

Corn Growth and Corn Cu Concentration

Corn growth was unaffected by Cu application up to 250 mg kg^{-1} for both soils (Table 2), suggesting that corn is relatively tolerant of Cu. However, at a Cu application rate of 500 mg kg^{-1} , corn growth was reduced by 80% and 70% for the Declo and Logan soils, respectively. No corn plants survived the 1000 mg kg^{-1} Cu application rate. Sonmez et al. (2006) applied up to 2000 $\text{mg Cu kg soil}^{-1}$ to tomato (*Lycopersicon esculentum* [L.] Mill. Cv. F144), noting that total yield, fruit number, dry root weight, and plant height decreased with increasing application rate. Ginocchio et al. (2006) showed that greater than approximately 300 mg Cu kg^{-1} , lettuce (*Lactuca sativa* var. capitata) yield began to decrease.

Corn Cu concentrations increased with increasing Cu application rate (Table 2). The 250- and 500- mg kg^{-1} Cu application rates caused a significant increase in corn Cu content grown in Declo soil; only the 500 mg kg^{-1} Cu application rate caused an increase in the Logan soil. Because the pH of the two soils were similar (pH 7.9), differences within and between the two soils were likely caused by quantities of CaCO_3 and OM present, which helped form insoluble Cu precipitates and organic Cu complexes; the Declo and Logan soils contained 11% and 49% CaCO_3 , and 1.2% and 2.5% OM, respectively (Table 1).

TABLE 2. Influence of Copper Application on Corn (*Zea Mays* L.) Growth and Copper Concentration in the Declo and Logan Soil Series

Cu Application, mg kg^{-1}	Declo Soil Series		Logan Soil Series	
	Corn Growth, g Pot^{-1}	Corn Cu Concentration, mg kg^{-1}	Corn Growth, g Pot^{-1}	Corn Cu Concentration, mg kg^{-1}
0	8.48 (1.80) a	2.45 (0.31) a	22.8 (1.30) a	0.67 (0.22) a
50	8.15 (1.27) a	3.70 (0.95) a	22.9 (0.96) a	0.92 (0.21) a
100	8.12 (2.85) a	6.10 (1.99) a	24.0 (1.32) a	2.17 (0.79) a
250	8.07 (1.20) a	23.1 (9.96) b	23.8 (1.82) a	4.88 (2.67) a
500	1.75 (0.49) b	81.9 (1.54) c	6.75 (2.33) b	48.2 (28.6) b

The 1000 mg kg^{-1} Cu application was not included in the statistical analysis because no plants survived. Values within parentheses represent one SEM. Different lowercase letters within a column indicate a significant difference between copper application rates (Fisher LSD, $\alpha = 0.05$, $n = 4$).

TABLE 3. Influence of Copper Application on Total (4 M HNO₃) and DTPA-Extractable Copper in the Declo and Logan Soil Series

Cu Application (mg kg ⁻¹)	Declo Soil Series		Logan Soil Series	
	Total Cu	DTPA Cu	Total Cu	DTPA Cu
0	6.52 (0.28) a	0.99 (0.23) a	5.03 (0.55) a	0.72 (0.02) a
50	54.3 (8.64) ab	30.0 (3.02) ab	43.6 (8.77) a	33.1 (6.26) ab
100	107 (9.41) b	64.8 (5.21) b	87.1 (15.0) ab	66.4 (5.61) b
250	272 (18.3) c	163 (20.7) c	212 (49.7) b	154 (31.6) c
500	455 (55.5) d	288 (25.5) d	515 (135) c	323 (39.7) d
1000	924 (95.2) e	615 (55.1) e	1040 (200) e	704 (36.0) e

Values within parentheses represent one SEM. Different lower case letters within a column indicate a significant difference between copper rates (Fisher LSD, $\alpha = 0.05$, $n = 4$).

In soils with pH values greater than 7.0, soluble Cu has been shown to react and form CuO, CuCO₃, Cu₂(OH)₂CO₃ (malachite), and Cu(OH)₂ (McBride and Bouldin, 1984; Ponizovsky et al., 2007; Ma et al., 2006). Rodriguez-Rubio et al. (2003) showed that Cu sorption on calcareous soils was enhanced by addition of calcite and OM, but Cu sorption decreased with the removal of the soil carbonate fraction. The authors suggested that CuO, Cu(OH)₂, and malachite were controlling Cu availability. McBride and Bouldin (1984) found evidence of malachite precipitation in a Cu-contaminated calcareous soil, yet the soil contained 5.3% OM and greater than 95.5% of the Cu in soil solution was complexed with organic species. Maftoun et al. (2002) concluded that Cu retention in calcareous soils was related to CaCO₃ and OM, yet McBride and Blasiak (1979) suggested that most of the Cu is strongly adsorbed to OM in calcareous soils because of both greater OM solubility and greater pH-dependent charge present. When present in calcareous soils, it has been shown that increasing OM content decreases the quantity of plant-available Cu (Ghasemi-Fasaei et al., 2006) because of Cu complexation with organic species. Between 80% and 100% of Cu added to neutral or calcareous soils has been shown to be organically complexed (Cavallaro and McBride, 1978; McBride and Blasiak, 1979).

Soil Total and DTPA-Extractable Cu and Associated Corn Cu Content

Soil total and DTPA-extractable Cu content increased with increasing Cu application rate (Table 3) as was expected. Between 60% and 66% of the total Cu measured in the Declo soil was plant available, as determined by the DTPA extraction, whereas 63% to 75% was plant available in the Logan soil. The remainder of Cu was likely sorbed to OM or precipitated as inorganic phases, as previously outlined. Other research has shown lower DTPA-extractable Cu concentrations over time; Ghasemi-Fasaei et al. (2006) demonstrated that after 20 days of incubation, DTPA extracted only 20% of the total soluble Cu (5 mg kg⁻¹) added to calcareous soils.

Soil DTPA-extractable Cu was used to determine an estimate of extractable soil Cu associated with corn copper content and the maximum dietary tolerable Cu level in cattle and sheep (40 and 15 mg kg⁻¹, respectively) that will not impair health or performance based on National Research Council (2005) guidelines (Fig. 1). Third-order polynomial equations fit the observed Declo and Logan data best (both $R^2 = 0.999$; dashed lines, Fig. 1). Based on the predicted fit for the Declo soil, 130 and 215 mg kg⁻¹ of DTPA-extractable soil Cu would result in

the maximum dietary level in corn and thus would be detrimental to sheep and cattle health in terms of dietary Cu intake, respectively. Following the same approach for the Logan soil, 220 and 300 mg kg⁻¹ of DTPA-extractable soil Cu would be detrimental to the health of both animal species, respectively. These plant-available soil Cu concentrations are much greater than the typical background soil total Cu content, which ranges from 2 to 100 mg Cu kg⁻¹, with an average estimated at 30 mg Cu kg⁻¹ (Lindsay, 1979). It should be kept in mind that domestic animals would likely receive feedstock from a variety of sources and locations. Thus, the elevated Cu content from feedstock produced in areas receiving Cu-containing dairy lagoon liquid waste would probably be reduced below tolerable levels.

Stehouwer and Roth (2009) suggested that if copper was added gradually to soil (<11 kg ha⁻¹ year⁻¹, which is not uncommon [Rankin, 2008]), then approximately 170 kg ha⁻¹ could be added to light-textured, low-OM soils ([<1%] Stehouwer, personal communication) without causing crop toxicity. Heavier textured soils with moderate to high OM contents could receive

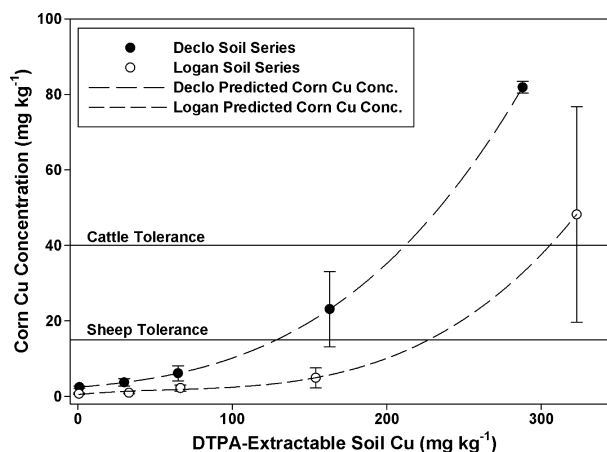


FIG. 1. Corn copper concentration versus diethylenetriaminepentaacetic acid (DTPA)-extractable soil copper concentration. Dashed lines represent the predicted corn copper concentration as compared with either Declo or Logan DTPA-extractable Cu content. Solid horizontal lines represent sheep (15 mg kg⁻¹) or cattle (40 mg kg⁻¹) maximum tolerable dietary Cu content based on National Research Council (2005) recommendations.

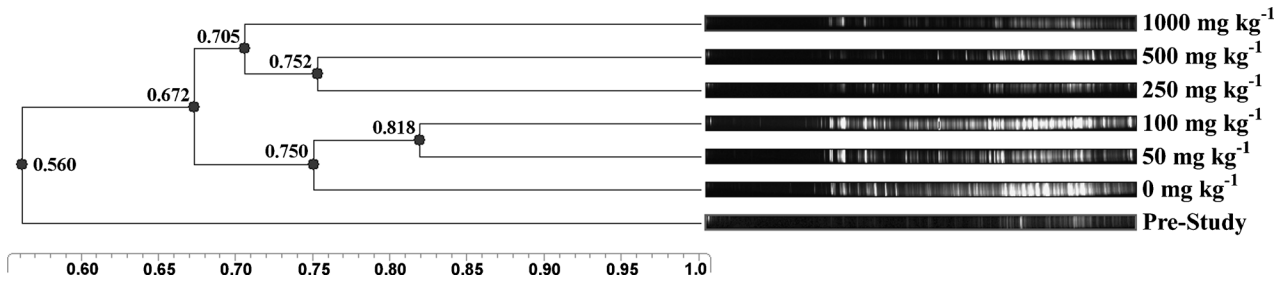


FIG. 2. The UPGMA cluster analysis of the bacterial RISA profiles from Declo soil with increasing Cu application rates (0, 50, 100, 250, 500, 1000 mg Cu kg⁻¹). The percentages of similarity among the RISA profiles were calculated using the Pearson coefficient. Each profile represents one subsample of each treatment chosen at random.

three to five times as much copper without experiencing crop toxicity symptoms (Stehouwer and Roth, 2009). However, Klingberg (2009) recommended that continued use of high-copper content manures on the same field for many years should be avoided because of Cu toxicity and immobility. Nevertheless, Cu extractability and bioavailability have been repeatedly shown to decrease over time (Ghasemi-Fasaei et al., 2006; Novak et al., 2004; Brennan et al., 1986; McLaren and Ritchie, 1993).

Bacterial Community Analysis

The RISA cluster analyses demonstrated that both Cu-fortified Declo and Logan soil series planted with corn exhibited a shift in bacterial communities as they were treated with increasing levels of Cu. In both soil series, as the Cu concentrations increased, the levels of similarity among the RISA profiles decreased. For Declo soils (Fig. 2), clustering was observed between bacterial RISA profiles in the control soil (no copper treatment), as well as the profiles of soils treated with low levels of copper (i.e., 50–100 mg Cu kg⁻¹). A second cluster was observed between bacterial RISA profiles of soils treated with greater Cu concentrations (250–1000 mg Cu kg⁻¹). Logan soils demonstrated a similar clustering pattern (Fig. 3), with profiles of control and low level Cu treatment soils forming one cluster, and profiles of two high level (250–500 mg Cu kg⁻¹) Cu treatments forming a second cluster. In the Logan soil series, the 1000 mg kg⁻¹ Cu-treated soil profile demonstrated markedly less similarity (0.1449) than the remainder of the profiles. Overall, these clustering patterns were indicative of changes within the overall bacterial community and coincide with a study performed by Frostegård et al. (1993), which demonstrated significant

changes in phospholipid fatty acid profiles when Cu concentrations increased more than 130 mg kg⁻¹. Differences between the bacterial community profiles of the pre-study and control soils were most likely the result of the introduction of corn (Garbeva et al., 2006) and were much more pronounced in the Declo soils (0.190) than the Logan soils (0.040). It should be noted that the separation of the 1000 mg kg⁻¹ Cu treatment profile in the Logan soil series could be the result of the loss of plant growth in this treatment. This could also describe the separation of the 1000 mg kg⁻¹ Cu treatment in the Aberdeen soil series from the 250 and 500 mg kg⁻¹ Cu treatments, although the change in similarity between the profiles in these treatments occurred at a much lower extent (0.047).

In addition to direct examination of the RISA profiles, diversity indices of the bacterial communities within both the Declo and Logan soil series were compiled. In both studies, diversity trended upward ($[H$ and $1/D]$ Table 4) at lower Cu concentrations (i.e., 50–100 mg kg⁻¹). As Cu concentrations continued to increase, however (i.e., 500–1000 mg kg⁻¹), diversity levels dropped. Throughout the study, species evenness ($[J]$ Table 4) remained high, indicating that there were few phylotypes predominant within the RISA profiles. Initial increases in diversity may be attributed to an increase in Cu bioavailability. The Logan and Declo control soils contained between 0.7 and 1.0 mg kg⁻¹ of DTPA-extractable Cu (Table 3), which may have been low enough to initially limit species diversity. Another explanation is that Cu, at lower concentrations, produced a stress response within the bacterial community (Huysman et al., 1994); the result of this stress response would have been exhibited as an increase in species diversity. Yachi and Loreau (1999) have

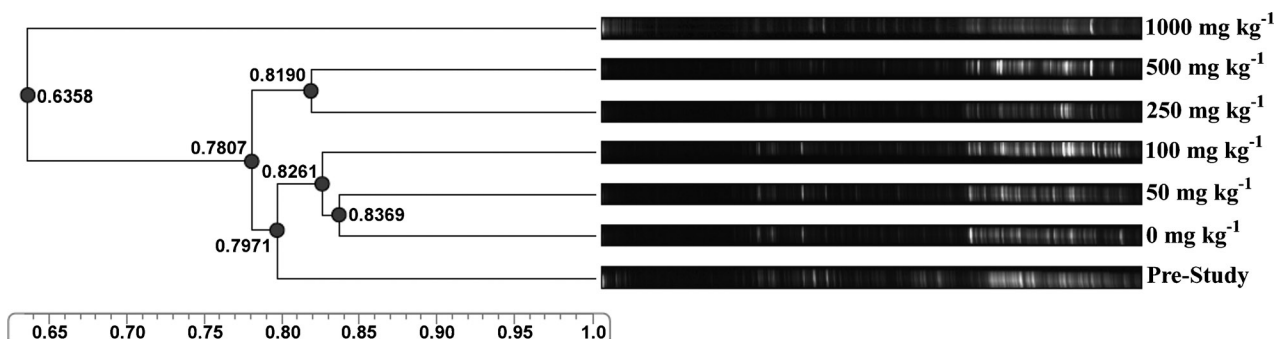


FIG. 3. The UPGMA cluster analysis of the bacterial RISA profiles from Logan soil with increasing Cu application rates (0, 50, 100, 250, 500, 1000 mg Cu kg⁻¹). The percentages of similarity among the RISA profiles were calculated using the Pearson coefficient. Each profile represents one subsample of each treatment chosen at random.

TABLE 4. Microbial Diversity Indices as Affected by Corn (*Zea Mays* L.) and Copper Application to the Declo and Logan Soil Series

Soil series	Plant	Cu Application (mg kg ⁻¹)	H	J	1/D	Richness
Declo	None	0	3.74	0.8763	28.37	74
Declo	<i>Z. mays</i>	0	3.90	0.8480	35.38	81
Declo	<i>Z. mays</i>	50	3.99	0.8954	37.19	86
Declo	<i>Z. mays</i>	100	4.04	0.8915	41.96	87
Declo	<i>Z. mays</i>	250	3.75	0.9056	28.24	63
Declo	<i>Z. mays</i>	500	3.59	0.8738	24.89	60
Declo	<i>Z. mays</i>	1000	3.63	0.8803	25.89	59
Logan	None	0	4.04	0.9275	44.36	78
Logan	<i>Z. mays</i>	0	4.03	0.9219	42.09	79
Logan	<i>Z. mays</i>	50	4.14	0.9480	52.33	79
Logan	<i>Z. mays</i>	100	4.09	0.9192	46.24	86
Logan	<i>Z. mays</i>	250	4.04	0.9485	46.07	71
Logan	<i>Z. mays</i>	500	3.85	0.9030	34.51	71
Logan	<i>Z. mays</i>	1000	3.88	0.9526	40.29	59

H: microbial diversity calculated using the Shannon-Wiener Index; J: evenness of the soil RISA profiles; 1/D: microbial diversity calculated using the Reciprocal Simpson Diversity Index. Richness was calculated as the total number of phylotypes for a given profile.

hypothesized that such increases in species diversity, in response to environmental stressors, are an attempt to protect the overall bacterial community against declines in function. It is known, however, that Cu does have bactericidal properties (Borkow and Gabbay, 2004), which could explain the eventual decreases in bacterial diversity at the greater Cu application rates.

CONCLUSIONS

The objectives of this investigation were to identify Cu application effects on corn growth and Cu concentration, total and DTPA-extractable soil Cu content, and the soil bacterial community. In addition, soil DTPA-extractable Cu was used to determine an estimate of extractable soil Cu associated with corn copper content and the maximum tolerable Cu level in cattle and sheep based on National Research Council (2005) guidelines. Findings showed that corn growth was unaffected by Cu applications up to 250 mg kg⁻¹; Cu rates greater than 250 mg kg⁻¹ increased corn Cu concentrations and reduced growth. In soil, DTPA-extractable Cu content increased with increasing Cu application, and after 30 days since Cu application, 60% to 75% of the added Cu was still plant available. The predicted DTPA-extractable soil Cu content associated with corn Cu concentrations and detrimental sheep or cattle dietary intake was determined. Higher than 130 and 215 mg kg⁻¹ of DTPA-extractable soil, Cu was associated with the maximum dietary Cu limit for sheep and cattle in Declo soil, respectively; 220 and 300 mg kg⁻¹ of DTPA-extractable soil Cu would be detrimental for sheep and cattle in Logan soil, respectively. Bacterial community analysis showed that community structure was unaltered up to 100 mg Cu kg⁻¹. However, as Cu concentrations increased, the similarity among RISA profiles, as well as bacterial diversity, decreased.

Based on the predicted DTPA-extractable soil Cu to tolerable animal dietary consumption values, it is suggested that plant-available soil Cu concentrations not exceed 130 mg kg⁻¹ in these agroecosystems. Most states do not have environmental regulations in place regarding Cu handling and land application. However, as with any land application of waste program, it is suggested that soil testing is conducted on a routine basis to ensure optimum crop yields along with environmental protection.

ABBREVIATIONS

ANOVA: analysis of variance;
DTPA: diethylenetriaminepentaacetic acid;
OM: organic matter;
RISA: ribosomal intergenic spacer analysis.

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