Copper and Zinc Accumulation, Profile Distribution, and Crop Removal in Coastal Plain Soils Receiving Long–Term, Intensive Applications of Swine Manure

J. M. Novak, D. W. Watts, K. C. Stone

Abstract. *Trace metals like copper (Cu) and zinc (Zn) are added to animal feed as dietary supplements, and trace metals not assimilated by the animals gut are excreted in manure. The accumulation and movement of Cu and Zn in soils has not been as well documented when compared to nitrogen (N) and phosphorus (P). In cases where plant−available Cu and Zn concentrations accumulate to phytotoxic levels, yield may decline for sensitive crops. We investigated the accumulation of Cu and Zn in the topsoil (0 to 15 cm) and the soil profile (0 to 183 cm deep) and estimated uptake by Coastal Bermuda grass (*Cynodon dactylon *L.) in a Coastal Plain spray field after 4 and 10 years of swine manure effluent application. Effluent was initially applied to a 1 ha portion of this field, which was later enlarged to 5 ha. Top and subsoil samples were collected at nearby control sites that received no swine manure. All soil samples were extracted using Mehlich 3 (M3) and double acid reagent for plant−available and total Cu and Zn concentrations. Comparison of trace metal concentrations in the topsoil after 4 and 10 years of effluent application with control soils showed that total Cu, and M3 and total Zn had accumulated. Mehlich 3 and total Cu and Zn accumulation rates both declined after an increase in spray field area and decrease in effluent application rates. In a 1 ha region of the field, total Cu and Zn concentration both increased between 45 and 90 cm deep after 4 years of effluent applications. Across the 5 ha field, higher subsoil total Cu concentrations relative to controls suggest that Cu leaching occurred; however, leaching of Zn into the total pool was not significant. Estimated times through Coastal Bermuda grass uptake to reduce topsoil M3 Zn concentrations to background levels ranged between 21 to 152 years. Results from this study show that after 10 years of swine manure effluent application, mean topsoil M3 Cu and Zn concentrations were far below concentrations considered phytotoxic to sensitive crops.*

Keywords. *Copper, Plant uptake, Soil accumulation, Swine manure effluent, Zinc.*

Growing swine need trace metals as part of their diet, so metals like Cu and Zn are added to feeds as growth promoters (Smith et al., 1997). Unfortunately, swine do not assimilate 100% of the Cu and Zn contained in the feed; therefore, a portion of these metals is excreted in the manure (Nicholson et al., 1999; Orihara et al., 2002). In confined swine operations in North Carolina, swine manure slurry is collected by housing swine in large barns or buildings where the floor is suspended above a pit. Slurry in the pits is flushed to waste lagoons for treatment and storage (Burkholder et al., 1997; Mallin, 2000). Because of lagoon storage capacity restrictions, the liquid effluent is periodically removed and is surface applied to fields containing row crops or perennial grasses. Trace metals and other macronutrients contained in the effluent can re−enter the soil nutrient cycle via leaching, tillage incorporation, and plant uptake.

Trace metal concentrations in soils are important for healthy crop growth and high yields. Agronomic crops require varying amounts of trace metals depending on such factors as plant species and development stage. Most crops use only a small amount of Cu and Zn to complete their life cycles. Any amount not assimilated through plant uptake can lead to accumulation in the soil.

Agronomic crops can reduce trace metal concentrations through plant uptake and harvesting of aboveground biomass (Baker et al., 2000). Coastal Bermuda grass is a recommended grass species for manure disposal because it has a high N uptake rate (200 kg ha^{-1}; NCSU, 1994), and large amounts of N can be removed when the aboveground biomass is harvested. However, crops like Coastal Bermuda grass remove very little Cu and Zn in the harvested biomass, thereby allowing trace metals to persist in soils at high levels for long periods of time. Zublena (1991) reported that Coastal Bermuda grass removes 0.009 and 0.218 kg of Cu and Zn, respectively, per 7.3 Mg ha^{-1} yield of aboveground biomass.

Time estimates for crops to reduce trace metals concentrations in soils have been modeled by examining relationship between target metal concentrations and the quantity of metal removed in the harvested crop. These projected time estimates can vary from a few years to several hundred years depending on the reduction level required, yield, bioavailability, leaching potential, and plant uptake (McGrath et al., 2000).

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In situations where trace metal loading rates from swine manure effluent or slurry application exceed crop removal rates, accumulations of Cu and Zn have been reported in some areas of the U.S. (King et al., 1985), Asia (Wong, 1985; Eneji et al., 2001; Orihara et al., 2002), and Europe (van Driel and Smilde, 1990; Hansen, 2002). Excessive accumulations of Cu and Zn in soils can produce phytotoxic levels that may reduce yields of sensitive crops (Wong and Bradshaw, 1982; Whitehead, 2000). Although agronomic crops vary in their sensitivity to Cu and Zn (Marschner, 1995; Whitehead, 2000), critical concentrations in North Carolina soils that can be phytotoxic to sensitive plants have been reported as >120 mg kg^{-1} for Zn and >60 mg kg^{-1} for Cu (Mehlich 3 extractable; Tucker et al., 2003). Coastal Bermuda grass is fairly tolerant to high concentrations of Mehlich 3 Cu and Zn in North Carolina soils (David Hardy, North Carolina Department of Agriculture, personal communication, 2003); however, some agronomic crops are sensitive to Zn far below the 120 mg kg^{-1} soil concentration. For instance, peanut (*Arachis hypogaea* L.) sensitivity to soil Zn concentrations has been reported as low as 12 mg kg^{-1} (Keisling et al., 1977). Because peanut is sensitive to soil Zn concentrations, fields used for their production have a Mehlich 3 Zn concentration limit of 20 mg kg^{-1} (Tucker et al., 2003).

In a previous study, Novak et al. (2000) reported soil P accumulation and leaching in a Coastal Bermuda grass spray field located in a North Carolina Coastal Plain watershed. In this study, swine manure was applied at rates much greater than standard practices within the watershed. An opportunity existed to determine if high rates of swine manure caused Cu and Zn accumulation and deep profile movement in the sandy soils. The long projected residence time for these metals could also be measured through examining relationships between trace metal soil concentrations and crop uptake. The objectives of this study were: (1) to determine the effects of long−term (10 years) intensive rates of swine manure effluent application on soil Cu and Zn concentrations in topsoil and subsoil, (2) to determine topsoil Cu and Zn accumulation rates, and (3) to estimate the time period necessary for Coastal Bermuda grass to reduce soil Cu and Zn concentrations to background levels upon cessation of manure application.

Materials and Methods

Site, Soil, and Manure Production Description

The studied field and swine production facility is located in Duplin Co., North Carolina. The county is located in the Middle Coastal Plain physiographic region and within the Cape Fear River basin. Descriptions of the site's landscape features, nearby stream systems, aquifers, and mean rainfall amounts were presented by Novak et al. (1998) and Novak et al. (2003). The studied field is about 5 ha in size and has slopes ranging from 1% to 5%. The dominant soil series is Autryville loamy sand (loamy, siliceous, subactive, thermic Arenic Paleudults) with small inclusions of Lakeland sand (thermic, coated Typic Quartzipsamments).

Swine production commenced at this facility in August 1986. Swine manure was stored in an open−pit lagoon, and effluent was applied in mid− to late−1987 to corn (*Zea mays* L.) grown in a nearby field (Starr Maready, North Carolina Cooperative Extension Service, personal communication, 1998). An unknown quantity of manure was initially applied to a 1 ha portion of the field from 1987 to 1989 (fig. 1). The applications rates were believed to be high because a later study showed that shallow groundwater beneath the field had elevated NO_3−N concentrations (Stone et al., 1998). Because of groundwater quality concerns, the field was expanded to 5 ha and planted with Coastal Bermuda grass in 1990 to facilitate soil nutrient assimilation (Starr Maready, North Carolina Cooperative Extension Service, personal communication, 1998). From 1990 to 1996, manure application rates along with nutrient analyses of the manure are unknown. Stone et al. (1998) modeled a total N loading rate of 2500 kg ha^{-1} $year^{-1}$ for this field through estimates of swine populations and total N contents of typical swine manure effluent. During this period, there was no nutrient analyses on the lagoon effluent applied to this field, so the average total N content of the swine lagoon effluent was assumed to be 0.57 mg L^{-1} (*ASAE Standards*, 1998). The 1 ha field would need to receive approximately 4.33×10^6 L of effluent $year^{-1}$ to supply 2500 kg N ha^{-1} $year^{-1}$ as reported by Stone et al. (1998). This means that between 1987 and 1989, the 1 ha portion of the spray field was estimated to annually receive 4.33×10^6 L of effluent. Increasing the field area to 5 ha between 1990 and 1997 caused a reduction in the estimated effluent application rate to 0.86×10^6 L ha^{-1}. A nutrient management plan for this facility was implemented in January 1997, which recommended a decrease in effluent applications to 1673 kg N $year^{-1}$. Reducing the N load resulted in a decrease in effluent application to approximately 0.58×10^6 L ha^{-1} $year^{-1}$.

Records measuring actual Cu and Zn loading rates for this field between 1987 and 1996 were not available. Estimates for these values, however, were made using two monthly lagoon effluent nutrient analyses reports. From the nutrient analyses report in early 1997 (January and February), the effluent mean Cu and Zn concentrations were 0.82 and 0.74 mg L^{-1}, respectively. Estimates of Cu and Zn loading rates were determined through multiplying effluent application rates by the mean effluent Cu and Zn concentrations. For annual metal loading rates, it was assumed that the effluent mean Cu and Zn concentrations were similar from 1987 to 1997.

Soil Collection and Analyses

Soils were collected using three different schemes, as described in Stone et al. (1995) and Novak et al. (2000). In 1991, during monitoring well installation, soil samples were collected at two locations within the initial 1 ha portion of the spray field in 15 cm increments down to 183 cm (Stone et al., 1995). The 1991 soil samples represent spray field conditions after 4 years of swine manure application. To examine soil conditions after 10 years of manure application, samples were collected in 1997 at three locations approximately 5 m away from the two locations sampled in 1991. These sites were selected in close proximity to the 1991 locations and to minimize possible disturbance to soil drainage patterns around the two wells. One additional soil core was collected because of concern about variations in soil drainage due to a Lakeland sand inclusion encountered during coring. These three soil sampling sites were also located within the initial 1 ha area of the spray field (fig. 1). The cores were collected to 183 cm deep using a bucket auger in 15 cm increments to determine soil profile Cu and Zn concentrations.

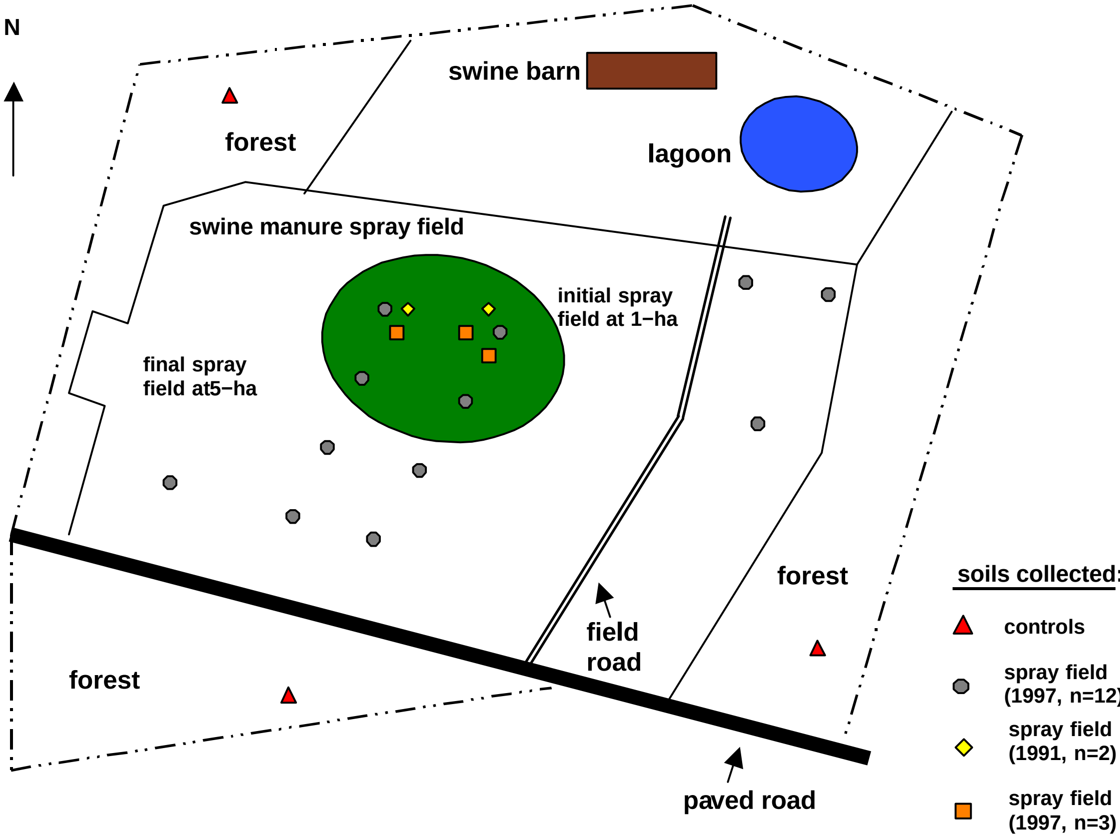

Figure 1. Location of the spray field and sampling sites.

Twelve more sites were sampled in 1997 to account for swine manure applied to other areas in the 5 ha field (fig. 1). Four of these sites were located within the initial 1 ha area, while the remaining eight sites were located in other regions of the spray field. Soil sampling at these 12 locations were limited to depths of 0 to 15 cm and 15 to 45 cm based on an initial hypothesis of minimal Cu and Zn leaching due to the severe effluent application reduction per hectare.

Control soils (*n* = 3) were also collected in 1997, as described by Novak et al. (2000). Sampling depths for the control soils were 0 to 15 cm, 15 to 45 cm, 45 to 90 cm, 90 to 135 cm, and 135 to 183 cm. These soils were collected using coarser depth increments because of minimal P leaching measured in soils receiving no animal manure (Novak et al., 2000). These soils were collected using a bucket auger at three nearby locations (fig. 1). These control sites were mapped as Autryville loamy sand and were in forested locations. Preliminary analysis of soil Mehlich 3 Cu and Zn contents and pH values showed that these sites had no history of swine manure application.

All soil samples were air−dried and ground to pass a 2 mm sieve. Soil pH was measured using a 2:1 (v/v) deionized H_2O to soil ratio. Soil organic carbon (SOC) was measured using a Leco CN2000 analyzer (St. Joseph, Mich.). Soil particle size analysis was preformed using the micro−pipette method of Miller and Miller (1988). Mehlich 3 reagent was used to extract Cu and Zn in each soil sample (Mehlich, 1984). Besides extracting plant−available P, this reagent contains ethylenediaminetetraacetic acid (EDTA), which can chelate and extract labile forms of Cu and Zn (van Raij, 1998). Total Cu and Zn concentrations were determined using the double acid (HNO_3 + HCl) EPA 3050B digestion method (EPA, 2004). Digestion performance was determined using Standard Reference Material 2709 (San Joaquin soil) obtained from the National Institute of Standards and Technology (Gaithersburg, Md.) for QA/QC purposes. The digested Cu and Zn concentrations measured in this study were within the concentration range reported for the San Joaquin soil, indicating acceptable QA/QC performance of the employed acid digestion procedures. Mehlich 3 and total Cu and Zn concentrations were quantified using a Perkin Elmer AAnalyst 300 (Norwalk, Conn.) atomic absorption spectrophotometer.

Estimates of Cu and Zn Accumulation Rates and Downward Movement

The topsoil M3 and total Cu and Zn accumulation rates in the 1 ha area were determined after 4 (1987 to 1991) and 10 (1991 to 1997) years because of variations in effluent application rates and spray field area. It was assumed that the control soil's Cu and Zn concentrations approximate background concentrations before swine manure applications. In the first time period (1987 to 1991), the accumulation rate for the 1 ha portion of the spray field was calculated by subtracting mean control (1987) concentrations from the field mean 1991 concentration and dividing by 4 years. Accumulation rates for the second time period (1991 to 1997) were calculated by subtracting the field mean 1997 concentrations from the field mean 1991 concentrations and then dividing by six years. Downward movement of both M3 and total Cu and Zn (to 183 cm) concentrations from samples collected in the initial 1 ha spray field area and the control soils were plotted as a function of profile depth. It should be noted that except for the topsoil depth, subsoil depth increments were different between control soils (0 years) and soils after 4 and 10 years of effluent application.

Reductions in Trace Metal Concentrations Through Crop Uptake

Projected time estimates were made based on Coastal Bermuda grass uptake data to reduce accumulated M3 trace metal concentrations in spray field topsoil to mean background topsoil concentrations. The projected times needed for target metal abatement were estimated by equations 1, 2 and 3:

$$X_{SF} - X_{BG} = X_{removed} \quad (1)$$

$$X_{removed} \times kg_{soil}\ ha^{-1} = X_{removed}\ kg\ ha^{-1} \quad (2)$$

$$X_{removed}\ kg\ ha^{-1} / X_{plant} = \text{time (years) for } X_{removed} \quad (3)$$

where

X_{SF} = spray field metal concentration (mg kg^{-1})
X_{BG} = background soil metal concentration (mg kg^{-1})
$X_{removed}$ = metal target concentration to deplete (mg kg^{-1})
X_{plant} = Bermuda grass metal uptake for given yield (kg ha^{-1} $year^{-1}$).

This plant nutrient removal equation was applied using the following five assumptions: (1) 100% of the trace metal is bioavailable, (2) the annual M3 extractable concentration is similar, (3) abatement is restricted to plant uptake exclusively, (4) the spray field soil has a bulk density of 1.5 g cm^{-3}, and (5) Coastal Bermuda grass aboveground dry matter yield is 7.3 Mg ha^{-1} $year^{-1}$ (Zublena, 1991). Additionally, reduction estimates were obtained based on a continuous crop of Coastal Bermuda grass and cessation of swine manure application. The removal period was determined using the measured maximum, minimum, and overall spray field topsoil (0 to 15 cm deep) trace metal concentrations.

Statistics

To examine trace metal accumulation after 0, 4, and 10 years of effluent applications to the 1 ha area of the spray field, the mean topsoil Cu and Zn concentrations measured were statistically analyzed using a one−way ANOVA. For this analysis, mean concentrations determined from the controls, 1991, and 1997 sampling locations were used. Potential Cu and Zn downward movement below 15 cm was determined using soils collected in the 1 and 5 ha spray field area. In the 1 ha area, a one−way ANOVA was used to compare mean M3 and total Cu and Zn concentrations in subsoils collected after 0, 4, and 10 years. For this comparison, a weighted mean average was determined for subsoils after 4 and 10 years because of differences in their sampling depth increments compared to the control sampling depths. For the 5 ha field, topsoil and subsoil M3 and total mean Cu and Zn concentrations at 0 and 10 years of effluent application were compared using a t−test. Statistical tests were completed using SigmaStat version 3.0 (SPSS Corp., Chicago, Ill.) with a $P < 0.05$ level of rejection.

Results and Discussion

Soil Properties

Soils from the spray field and control sites are dominated by sands (table 1). Control soils are very strongly acid, with pH values less than 5.0, and are SOC enriched. They have similar properties to other forest−covered, Southeastern Middle Coastal Plain sandy soils (Novak and Bertsch, 1991). Soils in the spray field had lower mean SOC contents and medium acidic pH values (5.8 to 6.0). Thus, after 10 years of swine manure effluent application, pH values of spray field soils increased over 1 pH unit compared to controls. Swine lagoon effluent has an alkaline pH due to the predominance of excreted salts. After application to soil, the effluent acts as a surrogate−liming agent, thereby increasing soil pH.

Table 1. Means of chemical and physical properties for control and spray field soils (SD in parentheses).

Location	Depth (cm)	SOC (g kg^{-1})	pH	Soil Texture (%) Sand	Silt	Clay
Control						
n = 3	0 to 15	16.0 (3.0)	4.5 (0.5)	89	8	3
n = 3	15 to 45	5.0 (2.0)	4.9 (0.2)	89	9	2
Spray field						
n = 12	0 to 15	6.1 (2.0)	6.0 (0.3)	93	5	2
n = 12	15 to 45	2.0 (1.0)	5.8 (0.2)	94	4	2

Estimated Cu and Zn Loadings of Swine Manure Effluent

Because effluent rates applied to this spray field were not recorded prior to 1997, estimates were made by working backwards through estimated total N application rates of 2500 kg ha^{-1} $year^{-1}$ obtained by modeling (Stone et al., 1998) and assuming the effluent contained 0.57 mg total N L^{-1} (*ASAE Standards*, 1998). Based on these assumptions, 4.33×10^6 L $year^{-1}$ of effluent was applied from 1987 to 1989 to a 1 ha portion of the spray field (table 2). Effluent applied per hectare was reduced by 80% to 87% of the initial rate after expansion of the field to 5 ha in 1990 and adoption of a nutrient management plan in 1997.

Cu and Zn loading rates for this spray field were also not recorded; however, estimates were made using effluent application rates and mean Cu and Zn concentrations determined from two monthly nutrient management reports collected in 1997. Associate Cu and Zn loading rates onto the 1 ha portion of the spray field were higher in 1987 to 1989 and, thereafter, were reduced to less than 1 kg ha^{-1} $year^{-1}$. The annual Cu loading rates from swine manure effluent applied to this field are similar to rates reported by Sutton et al. (1983; 0 to 3.18 kg Cu ha^{-1}) and Jondreville et al. (2003; 1.35 to 3.55 kg Cu ha^{-1}). The annual Zn loading rates in this study are similar to swine lagoon effluent applied to soils in North Carolina (Burns et al., 1985).

Trace Metal Concentration, Accumulation, and Profile Distribution

M3 Extractable and Total Trace Metals in the 1 ha Field

Trace metal chemical species is more important than total concentration in determining bioavailability (He et al., 1992; Chaignon et al., 2003) and phytotoxicity (Qian et al., 1996; Gupta and Gupta, 1998). Binding characteristics change between trace metal species; therefore, their interaction with solid and organic phases cause changes in the bioavailable concentration contained in the soil solution (Mattigod et al., 1981; Hesterberg, 1998). If a trace metal's bioavailability exceeds the crop nutrient demand, then it can accumulate. Continued trace metal accumulation will lead to bioavailable

Table 2. Estimated swine manure effluent application rates and Cu and Zn loading rates.

Years	Effluent Application, (L ha^{-1} year^{-1}) × 10^6	Trace Metal Loading Rate (kg ha^{-1} year^{-1})	
		Cu	Zn
1987 to 1989	4.33	3.55	3.21
1990 to 1997	0.86	0.71	0.64
1997+	0.58	0.48	0.43

concentrations that can approach phytotoxic concentrations (Hesterberg, 1998). Trace metal phytotoxicity thresholds levels are commonly determined using reagents containing organic ligands like EDTA or diethylenetriaminepentaacetic acid that chelate cationic trace metals from the total trace metal pool and are classified as plant available (van Raij, 1998; Whitehead, 2000). Usually a very small amount of chelated trace metals are extracted from soils, compared to concentrations in the total trace metal pool, because trace metals are tightly bound through complex and solid formation, and adsorption onto solid surfaces (Bohn et al., 1979; Mattigod et al., 1981; Hesterberg, 1998). If trace metal's binding energies to these phases collectively exceed the binding chelation energy, then the trace metal remains in the unavailable form. It is, however, beneficial to extract and determine trace metals in both the plant−available and total metal pool (unavailable) because dynamic equilibrium processes between these fractions can release unavailable trace metals to replenish the plant−available pool when concentrations decline due to plant uptake or leaching losses.

Background Cu and Zn concentrations were determined in nearby mixed forest−covered control sites that had no history of swine manure application (table 3, 0 years). Control topsoils had low mean M3 (<2 mg kg^{-1}) and mean total Cu and Zn concentrations (<8.1 mg kg^{-1}). Total Zn concentrations in control topsoils were higher than total Cu. After 4 years of effluent application, there were no significant differences between the mean M3 and total Cu and Zn topsoil concentrations. However, 10 years of effluent application significantly increased the M3 mean Zn and the mean total Cu and Zn concentrations relative to concentrations in control soils.

The North Carolina Department of Agriculture uses Mehlich 3 reagent, which contains EDTA, to determine plant−available trace metal concentrations in North Carolina soils (NCDA, 2004) and has established potential phytotoxicity levels (David Hardy, NCDA, personnel communication, 2003). After 10 years of swine manure effluent application to the 1 ha region of the field, the topsoil M3 mean Cu and Zn concentrations (3.37 and 7.42 mg kg^{-1}, respectively) were far below concentrations considered phytotoxic to most agronomic crops grown in North Carolina soils (M3 extractable, >60 and >120 mg kg^{-1} for Cu and Zn, respectively; Tucker et al., 2003).

Table 3. Mean topsoil (0 to 15 cm deep) M3 and total Cu and Zn concentrations in the initial 1 ha portion of the spray field after 0 (control), 4, and 10 years of swine manure effluent application (SD in parentheses).

Metal	Years[a]	M3[b] (mg kg^{-1})	Total (mg kg^{-1})
Cu	0	0.48 (0.30) a	1.25 (0.58) a
	4	2.37 (1.39) a	6.20 (2.22) ac
	10	3.37 (1.88) a	8.52 (3.57) bc
Zn	0	1.69 (1.39) a	8.10 (0.93) a
	4	5.89 (3.13) ac	17.34 (5.39) ac
	10	7.42 (1.68) bc	20.09 (3.22) bc

[a] Soil M3 and total Cu and Zn concentrations measured at 0 year are from nearby control locations without swine manure application.

[b] Mean M3 and total Cu and Zn concentrations tested between years of manure application using a one−way ANOVA; means followed by a different letter are significantly different at the $P < 0.05$ level of rejection.

Trace Metal Accumulation in Topsoil in the 1 ha Field

Sampling soils in a chronosequence allowed for determination of two different M3 and total Cu and Zn concentration accumulation rates within the 1 ha portion of the spray field (table 4). Topsoil M3 Cu and Zn accumulation rates between 1987 and 1991 were almost 3− to 4−fold higher than rates determined from 1991 to 1997. This was due to the initial large effluent volume (4.33 × 10^6 L) that was applied to this area. By expanding the spray field to 5 ha in 1990, effluent applied to the 1 ha portion was reduced to 0.86 × 10^6 L ha^{-1}, which decreased effluent loading by almost 80% of effluent applied between 1987 and 1989. Concomitantly, the M3 Cu and Zn accumulation rates in the 1 ha portion of the field decreased. Both the M3 Cu accumulation rates in this study are much lower than the plant−available (HCl extractable) Cu accumulation rate (4.43 mg Cu kg^{-1} year^{-1}), as reported by Sutton et al. (1983). The difference is due to this study applying effluent with low Cu concentrations, while Sutton et al. (1983) applied swine manure slurry (270 t ha^{-1}) containing high Cu concentrations (between 5.6 and 56.7 mg Cu kg^{-1}). Comparison of the M3 Zn accumulation rates in soils after swine manure effluent application with other literature values was difficult due to a lack of published articles.

Between 1987 and 1991, the total Zn accumulation rate was almost 2−fold higher than the total Cu accumulation rate. This is an interesting finding and is contrary to predictable trends that should be occurring in this field. Predictably, the Cu accumulation rate should be higher than the Zn rate because Coastal Bermuda grass has a lower Cu uptake rate (0.009 kg Cu year^{-1}) compared to Zn (0.218 kg Zn year^{-1}; Zublena, 1991). This dissimilarity may be related to possible changes in the effluent annual mean Cu and Zn concentrations and/or preferential dissolved Cu leaching from the topsoil into subsoil depths. Variations in Cu and Zn concentrations in the effluent are possible; however, the variability in their concentrations cannot be assessed. The alternate explanation may be the binding preference of Cu over Zn to the organic ligands contained within the manures (Adriano, 2001). Mullins et al. (1982) also noted that increased Cu leaching may be related to Cu propensity to be preferentially chelated by ligands contained within the manure.

In the second time period (1991 to 1997), the total Zn and total Cu accumulation rates were similar. The total Cu and Zn

Table 4. Accumulation rates for M3 and total Cu and Zn concentrations in topsoil (0 to 15 cm deep) from the 1 ha portion of the spray field.

Metal	Accumulation Rate (mg kg^{-1} year^{-1})	
	1987 to 1991	1991 to 1997
M3 Cu	0.47	0.17
Total Cu	1.24	0.39
M3 Zn	1.05	0.26
Total Zn	2.31	0.46

accumulation rates in this study are much lower than the values estimated by Jondreville et al. (2003). These researchers estimated total Cu and Zn accumulation rates in French soils treated with swine manure slurry under different diet formulations and crop uptake scenarios. They reported annual soil total accumulation values between 86 and 521 for Cu and between 383 and 1,120 mg kg^{-1} for Zn after applying slurry delivering 0.31 to 1.61 kg Cu ha^{-1} and 1.35 to 3.55 kg Zn ha^{-1}. The variations in total Cu and Zn accumulation rates between this study and Jondreville et al. (2003) are probably related to no correction in accumulation rates for trace metal leaching losses under the proposed scenarios.

A reduction in the M3 Cu and Zn accumulation rates between the two time periods is a substantial finding. Assuming that crop management and the initially high annual effluent applications do not vary, then 128 and 114 years for Cu and Zn, respectively, are required for soils in this spray field to reach phytotoxic M3 concentrations established for crops grown in Coastal Plain soils (60 and 120 mg kg^{-1} for Cu and Zn, respectively; Tucker et al., 2003). By reducing the Cu and Zn accumulation rates through reduced effluent application, the years needed to reach the phytotoxic M3 Cu and Zn concentrations increased to 353 and 261 years, respectively. This is a significant step in environmental protection through altering a manure management practice. By increasing the acreage of the spray field, effluent applications rates were reduced, thereby slowing the accumulation of trace metals to phytotoxic levels for most crops. This positive impact for a manure best management practice used by the livestock producer is worth being stated.

Trace Metal Distribution in Soil Profiles in the 1 ha Field

The soil profile mean M3 and total Cu and Zn concentrations after 0, 4, and 10 years of effluent application to the 1 ha portion of the field show some interesting trends (figs. 2 and 3). There was no influence of manure application on the mean M3 Cu concentrations extracted from subsoil depths after 10 years of effluent application (fig. 2). This shows that results obtained by extracting soils using Mehlich 3 reagent did not show significant Cu leaching for soil profiles within the 1 ha region of the field. Extracting the total Cu fraction, however, showed that Cu had leached into the subsoil

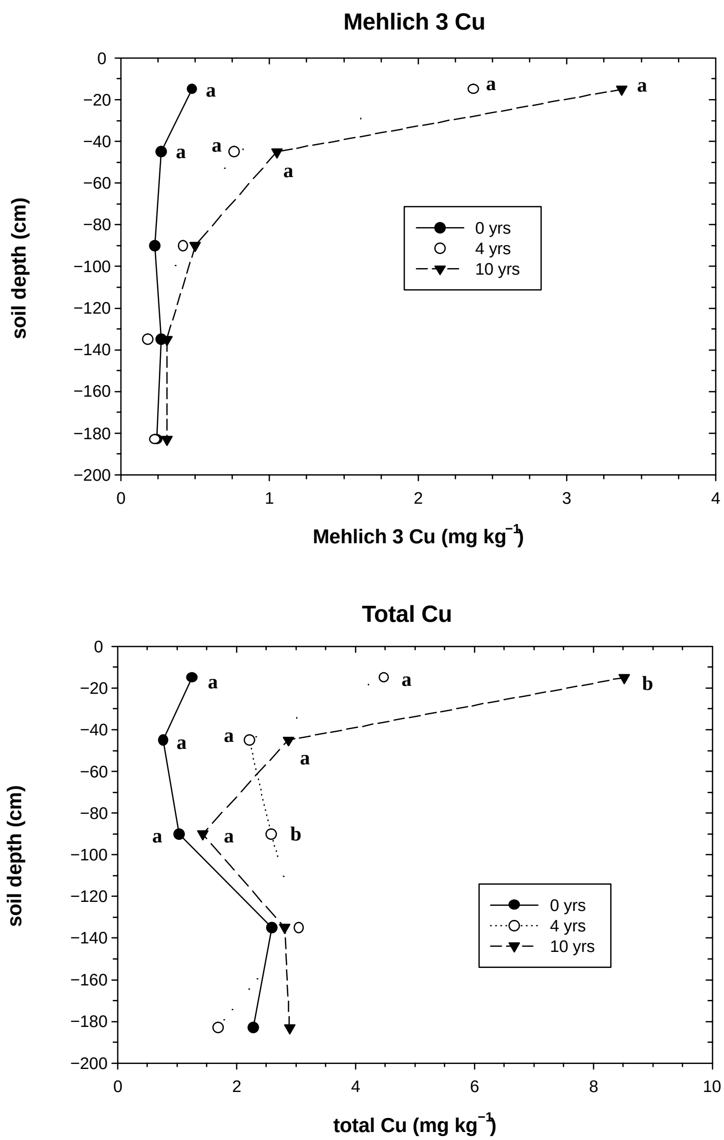

Figure 2. Mean M3 extractable and total Cu concentrations in soils after 0, 4, and 10 years of swine manure effluent application within the initial 1 ha portion of the spray field (mean followed by a different letter are significantly different at the P < 0.05 level of rejection).

horizons at 90 cm because the mean total Cu concentration was different from the control (fig. 2). This implies that Cu leaching into the subsoil horizons was more apparent by extracting the total fraction than the M3 fraction.

Downward movement of Zn (M3 extractable) occurred into subsoils after 4 and 10 years of effluent application (fig. 3). The leaching of dissolved or complexed Zn swelled the M3 extractable pool to a depth of 183 cm. The amount of Zn leaching into the subsoil horizons was sufficient to significantly increase the total Zn fraction at 90 cm; however, below 90 cm, the effect was not significant (fig. 3).

It is interesting that the subsoil mean total Cu and Zn concentrations after 10 years of effluent application declined to values lower than the means in subsoils after 4 years. In fact, both the mean total Cu and Zn concentrations in subsoils after 10 years resemble the controls mean subsoil distribution patterns (figs. 2 and 3). The decline in subsoil total Cu and Zn concentrations is related to a reduction in loading rate (between 80% and 87% relative to the initial rate); therefore, less trace metals would be available to leach into the subsoils and swell the total pool concentration.

Trace Metal Concentrations Across the 5 ha Field

An alternate soil sampling scheme was used in 1997 to examine both M3 and total Cu and Zn concentrations variations across the 5 ha spray field in order to account for field expansion. Twelve sampling locations were selected across the field (fig. 1). Because manure continued to be applied to the initial 1 ha portion of the spray field, 4 of the 12 sites were located within the 1 ha zone (fig. 1). This was done because previous sampling locations were located adjacent to wells, which left a large portion of the 1 ha region not sampled. Only two sampling depths were collected (0 to 15 cm and 15 to 45 cm) because it was theorized that Cu and Zn leaching would be minimal due to the 80% relative reduction in effluent applied per ha.

Similar to M3 Cu concentrations across the 1 ha field, the mean M3 Cu concentrations at both soil depths across the 5 ha field were not significantly different from the control

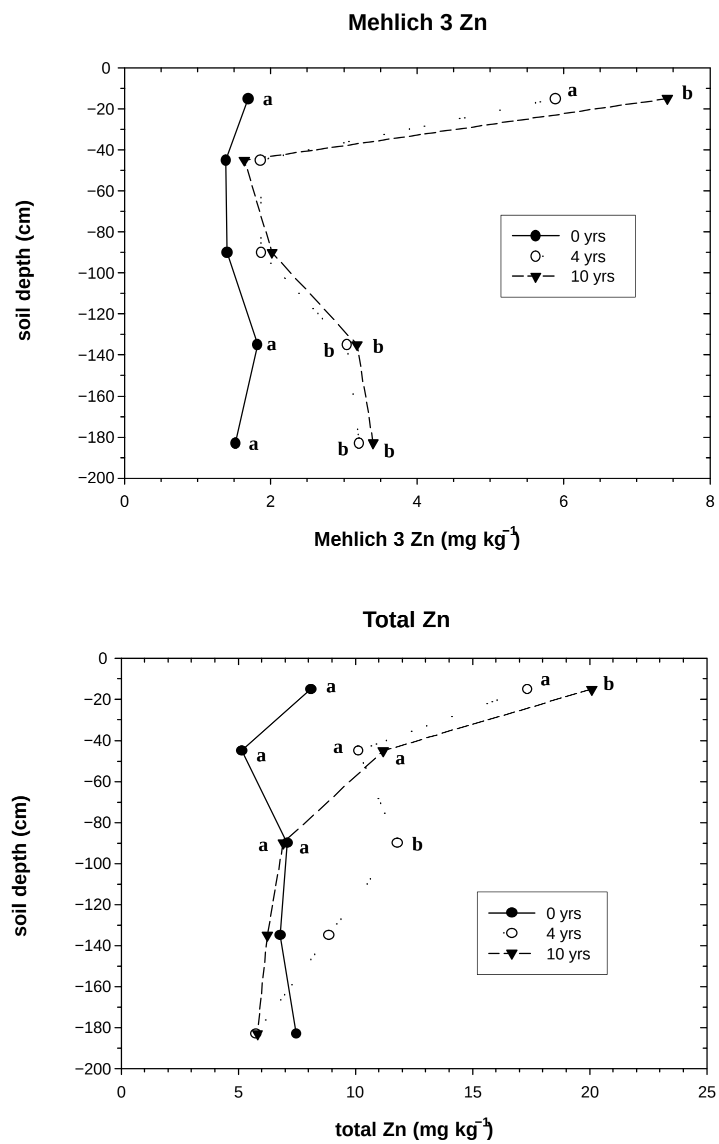

Figure 3. Mean M3 extractable and total Zn concentrations in soils after 0, 4, and 10 years of swine manure effluent application within the initial 1 ha portion of the spray field (mean followed by a different letter are significantly different at the P < 0.05 level of rejection).

Table 5. Mean, minimum, and maximum M3 and total Cu and Zn concentrations in 0 years (control) and after 10 years (1997) of swine manure effluent application across the 5 ha spray field (SD in parentheses).[a]

	M3 ($mg\ kg^{-1}$)		Total ($mg\ kg^{-1}$)	
Metal, depth	0 years	10 years	0 years	10 years
Cu, 0−15 cm				
mean	0.48 (0.30) a	3.59 (2.77) a	1.25 (0.58) a	7.59 (5.38) a
min.	0.17	0.77	0.89	2.70
max.	0.83	10.3	1.93	19.81
Cu, 15−45 cm				
mean	0.27 (0.10) a	0.86 (0.46) a	0.77 (0.02) b	2.33 (1.14) c
min.	0.30	0.39	0.74	0.94
max.	0.37	1.95	0.79	4.47
Zn, 0−15 cm				
mean	1.69 (1.39) b	7.87 (4.67) c	8.10 (0.93) d	23.95 (5.08) e
min.	0.88	3.76	7.53	13.24
max.	3.3	16.43	9.17	29.58
Zn, 15−45 cm				
mean	1.39 (0.78) d	1.33 (0.41) d	5.15 (1.42) f	10.15 (4.21) f
min.	0.62	0.64	3.93	3.3
max.	2.19	1.97	6.71	17.79

[a] Mean M3 and total Cu and Zn concentrations after 0 vs. 10 years of effluent application were tested by depth using a t−test; means followed by a different letter are significantly different at the $P < 0.05$ level of rejection.

(table 5). Except for 2 of the 12 samples, the additional years of swine manure applications did not strongly influence soil M3 Cu concentrations across the field. The two sites with elevated mean M3 Cu concentrations (7.38 and 10.33 mg kg^{-1}) were located in the 1 ha portion of the spray field. Both of these M3 Cu concentrations were still well below the phytotoxic M3 Cu concentration for most crops grown in North Carolina soils (<60 mg kg^{-1}; Tucker et al., 2003).

The topsoil mean M3 Zn concentration across the 5 ha field is significantly different from the control (table 5). Only 4 of the 12 sites had M3 Zn concentrations that were greater than 10 mg kg^{-1}. Three of these sites were located within the initial 1 ha area, while the remaining site was located in the south central part of the spray field. The maximum topsoil M3 Zn concentration (16.43 mg kg^{-1}) was measured at one of these three sites, and this concentration approached the 20 mg kg^{-1} M3 Zn phytotoxic level for peanuts (Tucker et al., 2003). However, the field topsoil mean M3 Zn concentration (7.87 mg kg^{-1}) was well below the M3 Zn concentration considered phytotoxic to other agronomic crops grown in North Carolina soils (120 mg kg^{-1}; Tucker et al., 2003). The remaining 8 sites had topsoil M3 Zn concentration (0 to 15 cm depth) that ranged from 1.19 to 9.15 mg kg^{-1}. The mean M3 Zn concentrations in subsoils (15 to 45 cm deep) from across the 5 ha field were not significantly different from the control (table 5).

Unlike the topsoil mean total Cu concentration after 10 years of effluent application in the 1 ha field, the mean total Cu concentration in the 5 ha field was not significantly different from the control (table 5). This is related to a decrease in Cu loading rates because of field expansion (table 2). In subsoils samples from across the 5 ha field, however, the mean total Cu concentrations after 10 years of effluent application was significantly different from the control. This finding supports the fact that dissolved or complexed Cu leached into subsoil depths and significantly increased the total Cu fraction across the 5 ha field after 10 years of effluent application. In contrast, the mean total Zn concentration at 15 to 45 cm deep in soils from across the 5 ha field was not significantly different from the control. This finding is similar to the total Zn concentration results at 45 cm in soils within the 1 ha area (fig. 3).

Higher M3 Cu and Zn concentrations were extracted from topsoils than from subsoils across the 5 ha field, which is similar to topsoil and subsoil Cu and Zn concentration distribution patterns in two swine manure treated sandy Coastal Plain soils in North Carolina (King et al., 1985) and in 12 New York soils treated with sludge for 15 years (McBride et al., 1997). Previous research showed that larger Cu and Zn concentrations occur in topsoils than in subsoils due to a high association with soil organic matter (Hsu and Lo, 2001; Yin et al., 2002). In this study, however, linear regression analyses between the M3 and total Cu and Zn concentrations in topsoil and subsoil samples collected across the 5 ha spray field showed no significant relationship with the SOC contents ($P > 0.05$, data not shown). Although accumulating in the topsoils, the profile metal concentration distribution patterns strongly suggest that concentrating effluent applications to the smaller area of the spray field (1 ha) created conditions conducive to leaching of dissolved and/or complexed Cu and Zn into subsoil depths, resulting in total metal concentration increases. This finding is similar to reports of Mullins et al. (1982) and Payne et al. (1988), who found Cu leaching to subsoil depths in sandy Virginia soils after application of Cu−enriched swine manure. The finding of significant Zn leaching is contrary to King et al. (1985), who reported that no Zn leaching occurred through a sandy North Carolina soil after swine manure effluent application.

Removal of Accumulated Trace Metals by Coastal Bermuda Grass

The third objective of this investigation was to project the time periods needed for Coastal Bermuda grass to reduce M3 metal concentrations in the spray field topsoil to mean background concentrations. Coastal Bermuda grass was chosen because it is the current grass crop in the spray field and is recommended in North Carolina for fields receiving swine manure (NCSU, 1994).

The projections were determined using only the maximum, minimum, and mean M3 Zn concentrations measured in the spray field topsoil. Projections were not made for M3 Cu concentrations because the field mean concentration was not significantly different from the control (table 5). The years of Coastal Bermuda grass production required to reduce M3 Zn concentrations to background concentrations ranged from 21 to 152 years (table 6). This projection assumes that the field will remain in Coastal Bermuda grass production, effluent application ceases, and the extracted M3 Zn concentration remains similar.

Conclusions

The objectives of this investigation were to determine the effects of long−term intensive rates of swine manure effluent application on the accumulation and movement of Cu and Zn in a Coastal Plain spray field. Cu and Zn concentrations were measured in soils that were collected chronosequentially, and their accumulation rates were determined after 4 and 10 years

Table 6. Years required for Coastal Bermuda grass to reduce accumulated spray field M3 Zn concentrations to mean background concentrations (calculated using maximum, minimum, and mean spray field M3 Zn concentrations).

Metal	Years for Removal of M3 Zn Concentration in the Field		
	Max. Conc.	Min. Conc.	Mean Conc.
Zn	152	21	64

of application. The Cu and Zn fraction in two different pools were extracted (M3 and total) and quantified to examine changes in the plant−available and unavailable pools. The projected time period for Coastal Bermuda grass to reduce M3 Zn concentrations to average background levels was determined using a simple nutrient uptake equation. This investigation found that:

- No significant differences in the mean topsoil M3 Cu concentrations were found between controls and spray field soils after 10 years of swine manure applications; however, the mean M3 Zn concentration was significantly different.
- Accumulation rates in the total Cu and Zn fractions were higher than in the M3 extractable fraction. Reducing swine manure effluent application rates, by increasing the size of the spray field, slowed the rate of both M3 and total Cu and Zn accumulation several fold.
- A few hot spots of high topsoil Cu and Zn concentrations were measured in regions of the spray field. The Zn concentrations in these areas approached the phytotoxic level for peanuts.
- Topsoils collected in the 1 ha area and from across the 5 ha spray field had higher M3 and total Cu and Zn concentrations than subsoils. In the 1 ha area of the spray field, significant total Cu and Zn concentration increases in subsoil depths occurred after 4 years of effluent application, which implies that Cu and Zn leaching occurred and contributed to increases in the total metal pool. Across the 5 ha field, higher total Cu concentrations in subsoil depths relative to controls suggest that Cu leaching into the total Cu pool occurred; however, Zn leaching was not significant. In contrast, extracting subsoils using Mehlich 3 reagent revealed that no significant leaching to 45 cm of Cu and Zn occurred in other regions of the 5 ha spray field.
- It was projected that plant−available Zn would persist for a long time in this spray field under Coastal Bermuda grass due to low uptake and a large M3 extractable Zn pool.

This study showed a positive impact of modifying a manure management practice. By simply increasing the spray field area, a reduction in swine manure effluent application rates was achieved, which resulted in a decline in both topsoil M3 and total Cu and Zn accumulation rates and a reduction in subsoil total concentrations. Simply applying swine manure to more land, however, is not a viable long−term option to decrease trace element accumulation. Land area for manure application in the Coastal Plain region of North Carolina may decline each year because of losses by urbanization, zoning restrictions, and recreational demands.

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References

Adriano, D. C. 2001. *Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals.* 2nd ed. New York, N.Y.: Springer.

ASAE Standards. 1998. D384.1: Manure production and characteristics. St. Joseph, Mich.: ASAE.

Baker, A. J., S. P. McGrath, R. D. Reeves, and J. A. Smith. 2000. Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal−polluted soils. In *Phytoremediation of Contaminated Soil and Water*, 86−107. N. Terry and G. S. Banuelos, eds. Boca Raton, Fla.: CRC Press.

Burkholder, J. M., M. A. Mallin, H. B. Glasgow, L. M. Larsen, M. R. McIver, G. C. Shank, N. Deamer−Melia, D. S. Briley, J. Springer, B. W. Touchette, and E. K. Hannon. 1997. Impacts to a coastal river and estuary from rupture of a large swine waste holding lagoon. *J. Environ. Qual*. 26(6): 1451−1466.

Bohn, H. L., B. L. McNeal, and G. A. O’Connor. 1979. *Soil Chemistry.* 1st ed. New York, N.Y.: John Wiley and Sons.

Burns, J. C., P. W. Westerman, L. D. King, G. A. Cummings, M. R. Overcash, and L. Goode. 1985. Swine lagoon effluent applied to Coastal Bermuda grass: I. Forage yield, quality, and element removal. *J. Environ. Qual*. 14(1): 9−14.

Chaignon, V., I. Sanchez−Neira, P. Herrmann, B. Jaillard, and P. Hinsinger. 2003. Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine−growing area. *Environ. Pollut*. 123(2): 229−238.

Eneji, A. E., T. Honna, and S. Yamamoto. 2001. Manuring effect on rice grain yield and extractable trace elements in soils. *J. Plant Nutr.* 24(7): 967−977.

EPA. 2004. Method 3050B: Acid digestion of sediments, sludges, and soils. Available at: www.epa.gov/epaoswer/hazwaste/test/pdfs/3050b.pdf. Accessed 10 June 2004.

Gupta, U. C., and S. C. Gupta. 1998. Trace element toxicity relationships to crop production and livestock and human health: implications for management. *Commun. Soil Sci. Plant Anal.* 29(11): 1491−1522.

Hansen, M. N. 2002. Risk of heavy metal accumulation in agricultural soil when livestock manure and organic waste is used for fertilization. In *Proc. 4th Int’l Livestock Waste Management Symp.: Global Perspective in Livestock Waste Management*, 269−272.

He, X. T., S. J. Traina, and T. J. Logan. 1992. Chemical properties of municipal solid waste compost. *J. Environ. Qual*. 21(2): 213−329.

Hesterberg, D. 1998. Biogeochemical cycles and processes leading to changes in mobility and chemicals in soils. *Agric. Ecosyst. Environ*. 67(2−3): 121−133.

Hsu, J.−H., and S.−L. Lo. 2001. Effect of composting on characterization and leaching of copper, manganese, and zinc from swine manure. *Environ. Pollut.* 114(1): 119−127.

Jondreville, C., P. S. Revy, and J. Y. Dourmad. 2003. Dietary means to better control the environmental impact of copper and zinc by pigs weaning to slaughter. *Livestock Prod. Sci.* 84(2): 147−156.

Keisling, T. C., D. A. Lauer, M. E. Walker, and R. J. Henning. 1977. Visual, tissue, and soil factors associated with Zn toxicity of peanuts. *Agron. J*. 69(5): 765−769.

King, L. D., P. W. Westerman, G. A. Cummings, M. R. Overcash, and J. C. Burns. 1985. Swine lagoon effluent applied to "Coastal" Bermuda grass: II. Effects on soil. *J. Environ. Qual*. 14(1): 14−21.

McBride, M. B., B. K. Richards, T. Steenhuis, J. J. Russo, and S. Sauve. 1997. Mobility and solubility of toxic metals and nutrients in soil fifteen years after sludge application. *Soil Sci.* 162(7): 487−500.

McGrath, S. P., S. J. Dunham, and R. L. Correll. 2000. Potential for phytoextraction of zinc and cadmium from soils using hyperaccumulator plants. In *Phytoremediation of Contaminated Soil and Water*, 110−128. N. Terry and G. S. Banuelos, eds. Boca Raton, Fla.: CRC Press.

Mallin, M. A. 2000. Impacts of industrial−scale swine and poultry production on rivers and estuaries. *American Scientist* 88(1): 26−37.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants*. 2nd ed. New York, N.Y.: Academic Press.

Mattigod, S. V., G. Sposito, and A. L. Page. 1981. Factors affecting the solubilities of trace metals in soils. In *Chemistry in the Soil Environment*, 203−221. M. E. Stelly, ed. Madison, Wisc.: ASA, CSSA, and SSSA.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Commun. Soil Sci. Plant Anal.* 15: 1409−1416.

Miller, W. P., and D. M. Miller. 1988. A micro−pipette method for soil mechanical analysis. *Commun. Soil Sci. Plant Anal.* 18: 1−15.

Mullins, G. L., D. C. Martens, W. P. Miller, E. T. Kornegay, and D. L. Hallock. 1982. Copper availability, form, and mobility in soils from three annual copper−enriched hog manure applications. *J. Environ. Qual*. 11(2): 316−320.

NCDA. 2004. Soil test methodology. Available at: www.ncagr.com/agronomi/smethod.htm. Accessed 1 June 2004. Raleigh, N.C.: North Carolina Department of Agriculture.

NCSU. 1994. North Carolina agricultural chemicals manual. Raleigh, N.C.: North Carolina State University, College of Agriculture and Life Sciences.

Nicholson, F. A., B. J. Chambers, J. R. Williams, and R. J. Unwin. 1999. Heavy metal contents of livestock feeds and animal manures in England and Wales. *Bioresource Tech.* 70(1): 23−31.

Novak, J. M., and P. M. Bertsch. 1991. The influence of topography on the nature of humic substances in soil organic matter at a site in the Atlantic Coastal Plain of South Carolina. *Biogeochem*. 15: 111−126.

Novak, J. M., D. W. Watts, K. C. Stone, M. H. Johnson, and P. G. Hunt. 1998. Pesticides and metabolites in the shallow groundwater of an eastern Coastal Plain watershed. *Trans. ASAE* 41(5): 1383−1390.

Novak, J. M., D. W. Watts, P. G. Hunt, and K. C. Stone. 2000. Phosphorus movement through a Coastal Plain soil after a decade of intensive swine manure application. *J. Environ. Qual*. 29(4): 1310−1315.

Novak, J. M., K. C. Stone, D. W. Watts, and M. H. Johnson. 2003. Dissolved phosphorus transport during stream storm and base flow conditions from an agriculturally intensive southeastern Coastal Plain watershed. *Trans. ASAE* 46(5): 1355−1363.

Payne, G. G., D. C. Martens, E. T. Kornegay, and M. D. Lindemann. 1988. Availability and form of copper in three soils following eight annual applications of copper−enriched swine manure. *J. Environ. Qual*. 17(4): 740−746.

Qian, J., X. Q. Shan, Z. J. Wang, and Q. Tu. 1996. Distribution and plant availability of heavy metals in different particle−size fractions of soil. *Sci. Total Environ*. 187(2): 131−141.

Orihara, K., K. Kamiyama, and S. Fujiwara. 2002. Characteristics of heavy metal content in animal manure compost. *Japanese J. Soil Sci. Plant Nutr.* 73(4): 403−409.

Smith, J. W., M. D. Tokach, R. D. Goodband, J. L. Nelssen, and B. T. Richert. 1997. Effects of the interrelationship between zinc oxide and copper sulfate on growth performance of early weaned pigs. *J. Animal Sci*. 75(7): 1861−1866.

Stone, K. C., P. G. Hunt, S. W. Coffey, and T. A. Matheny. 1995. Water quality status of a USDA water quality demonstration project in the eastern Coastal Plain. *J. Soil Water Conserv.* 50(5): 567−571.

Stone, K. C., P. G. Hunt, M. H. Johnson, and T. A. Matheny. 1998. Nitrate−N distribution and trends in shallow groundwater on an eastern Coastal Plains watershed. *Trans. ASAE* 41(1): 59−64.

Sutton, A. L., D. W. Nelson, V. B. Mayrose, and T. D. Kelly. 1983. Effects of copper in swine manure on corn and soil. *J. Environ. Qual*. 12(2): 198−203.

Tucker, M. R., D. H. Stokes, and C. E. Stokes. 2003. Heavy metals in North Carolina soils: Occurrence and significance. Raleigh, N.C.: North Carolina Department of Agriculture and Consumer Services. Available at: www.ncagr.com. Accessed 11 June 2004.

van Driel, W., and K. W. Smilde. 1990. Micronutrients and heavy metals in Dutch agriculture. 1990. *Fert. Res*. 25: 115−126.

van Raij, B. 1998. Bioavailable tests: Alternatives to standard soil extractions. *Comm. Soil Sci. Plant Anal.* 29(11−14): 1553−1570.

Whitehead, D. C. 2000. *Nutrient Elements in Grassland.* New York, N.Y.: CABI.

Wong, M. H., and A. D. Bradshaw. 1982. A comparison of the toxicity of heavy metals, using root elongation of rye grass (*Lolium perenne*). *New Phytol*. 91: 255−261.

Wong, M. H. 1985. Heavy metal characteristics of soils and crops from auto traffic, sewage sludge, pig manure, and chemical fertilizer. *Agric. Ecosyst. Environ*. 13(2): 139−185.

Yin, Y., C. A. Impellitteri, S.−J. You, and H. E. Allen. 2002. The importance of organic matter distribution and extract soil: Solution ratio on the desorption of heavy metals from soils. *Sci. Total Environ*. 287(1−2): 107−119.

Zublena, J. P. 1991. Soil Facts: Nutrients removed by Crops in North Carolina. North Carolina Coop. Ext. Serv. Bull. AG−439−16. Available at: www.soil.ncsu.edu/publications/soilfacts/AG−436−16. Accessed 11 June 2004.