CORN RESPONSE TO LONG-TERM APPLICATION OF $CuSO_4$, $ZnSO_4$ and Cu-ENRICHED PIG MANURE

by

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(ABSTRACT)

Three studies, two field and one laboratory, were performed to evaluate Cu or Cu and Zn from either sulfate sources or Cu-enriched pig manure. The studies were designed to investigate the effects of Cu and Zn in soils and corn (*Zea mays* L.).

The first field study consisted of continuing a long-term field experiment which was established in 1967 to evaluate corn response and changes in a Davidson clay loam soil to yearly additions of Cu and Zn sulfates. In 1983, the 17 annual additions of Cu and Zn resulted in cumulative totals of 280 kg Cu and 560 kg Zn ha^{-1}. These Cu and Zn additions, either alone or together, did not cause any grain or stalk yield decreases. The DTPA extractant effectively separated all soil treatment levels for both Cu and Zn. Copper concentrations in the blades and grain were not related to soil additions of Cu or Zn. However, Zn concentrations in blades and grain were directly related to each other, r=0.87**, to soil Zn treatment levels and to DTPA extractable Zn.

The second field experiment was designed to evaluate the effects of soil application of Cu-enriched pig manure and $CuSO_4$ on corn. The five treatments in each of three field locations consisted of a control, low and high Cu-enriched pig manure levels, and Cu, as $CuSO_4$, equivalent to Cu amounts in the manure. The soils varied in texture from clay loam to fine sandy loam, and ranged in CEC from 5 to 12.3 cmol(+) kg $^{-1}$. Copper-enriched pig manure, containing 1285 mg Cu kg^{-1}, was produced by pigs fed diets supplemented with 242 mg Cu kg^{-1}. After six years, 198 mg Cu kg^{-1} had been applied by the high treatments. The DTPA extractable Cu was not related to leaf nor grain Cu levels but was linearly related, r=0.95**, to applied Cu. No nutrient deficiencies or toxicities were observed.

The third study was a laboratory incubation of added Cu. The 15 soils ranged from 54 to 489 mg kg^{-1} in clay and from 5.4 to 7.4 in pH. Extractable Cu had simple correlations with five soil properties, clay, surface area, hydrous Al, hydrous Fe, and hydrous Mn. Three treatments, a control and 22 kg ha^{-1} Cu as $CuSO_4$ and as Cu-enriched pig manure (equivalent to 975 mt wet manure ha^{-1}), were applied to the soils at 33 k Pa moisture. Copper was extracted in the following order for the control and $CuSO_4$ treatments: $AlCl_3$ in 0.5M HCl > EDTA > DTPA. A different order of Cu extraction occurred for the Cu-enriched pig manure treatment such that EDTA > DTPA > $AlCl_3$ in 0.5M HCl. Extractable Cu decreased with time regardless of Cu source.

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Chapter I

INTRODUCTION

Both Cu and Zn are essential elements in animal and plant nutrition. Frequently, Cu and Zn are referred to as trace elements due to their low concentrations in living tissues. Yet, these elements are also considered heavy metals and, as such, pose a potential as environmental poisons. As our society looks more to soils for application of sewage sludges and manures there is an increased potential for Cu and Zn to be added in excess of crop needs. However, there is a lack of field data under controlled conditions to effectively set upper limits on a soils capacity to accept Cu and Zn. This dissertation combines three research projects that were designed to investigate the effects of Cu and Zn additions to soils and used corn as the evaluating crop. The objectives of the research presented in each chapter are as follows:

a. Chapter 2. To evaluate the effects of Cu and Zn, either alone or together, under field conditions on corn.

b. Chapter 3. To evaluate Cu-enriched pig manure effects on corn growth and to compare the availability of Cu from inorganic $CuSO_4$ to Cu from Cu-enriched pig manure.

c. Chapter 4. To evaluate Cu reversion and to evaluate Cu relations in various soils of Virginia.

Each project is presented as a self-contained chapter.

Copper in Human Health

Copper (Cu) has been recognized as an essential element in animals since 1928 (20). Copper is a component of many proteins, several of which were found to be enzymes with oxidative functions. Some of these enzymes are tyrosinase, laccase, ascorbic acid oxidase, cytochrome oxidase, uricase, and monoamine oxidase (20). Although Cu is highly variable within tissues, the liver, brain, kidneys, heart, and hair are tissues of relatively high Cu concentrations.

Copper is involved in numerous metabolic functions but its role in human metabolism is not fully understood. Adult humans require 2 mg Cu to satisfy their daily requirement (2, 20). Apparently, no upper limit has been set for human dietary consumption due to the variability in toxic quantities and the complexity of Cu itself in nutrition. About one-third of ingested dietary Cu is absorbed while the rest is excreted in the feces (2). Other researchers have found Cu absorption ranged from 40 to 70% (6, 17). Copper is mainly absorbed in the duodenum but the absorption mechanism remains elusive (17, 20). Once Cu is absorbed, it is passed to the blood plasma. From the plasma it is transported to the liver, which is the key organ in Cu metabolism (6, 20). Despite continued ingestion of dietary Cu, the liver Cu concentration does not increase with age (6). Apparently, the liver prevents Cu buildup through secretion of Cu proteins to the blood and excretion of Cu through the bile into the feces (2, 6). Fecal Cu excretion by normal adult humans range from 0.6 to 4.0 mg day^{-1} (17). Workers in Cu smelters excreted 5.3 to 16.3 mg Cu day^{-1} and Cu-poisoned (acute) persons 7 to 52 mg Cu day^{-1} (17).

Although the amount of Cu available for human consumption varies tremendously, there are few reported copper deficiencies or toxicities (1, 6). Copper can accumulate in the human system when the excretory capacity of the liver is exceeded (6). Normal liver concentrations in humans range from 6.5 to 200 mg Cu kg^{-1} dry liver weight (17). When excess Cu is ingested, most of the additional Cu is stored in the liver with the rest being excreted through the bile and diverted to the synthesis of ceruloplasmin (17). Copper toxicity has been observed in farm workers after years of exposure to Bordeaux mixture, a 1 to 2% solution of Cu sulfate neutralized with hydrated lime (5, 6, 8). These workers developed interstitial pulmonary lesions and associated nodular, fibrohayaline scars. Other known causes of Cu toxicosis are due to metallic Cu fragments in eyes (6), copper dust or salts on skin, breathing Cu fumes or dust, and direct ingestion of Cu salts and copper contaminated drinking water (1, 5). Surprisingly, oral ingestion of Cu salt is a common method of attempted suicide in India (2, 5). Contradictorily, exposure to high levels of copper does not always cause copper toxicosis. Chilean Cu miners have normal Cu concentrations in their livers and serum despite years of exposure to Cu sulfide and oxide dusts (6).

Acute toxicity of Cu to the gastrointestinal tract, renal, hematologic, cardiovascular, and neurologic systems seems to be limited to the ingestion of elemental Cu or salt form (5). Long-term (chronic) exposure to Cu compounds can cause toxicity problems. The general cure for chronic Cu intoxication is removal of the source of Cu (1, 5). There are no reports of Cu intoxication due to food consumption.

Copper in Swine

The evidence is strong that there are beneficial effects of supplemental dietary Cu, in excess of the minimal 7 mg kg^{-1} nutrient requirement, on growth rate in swine. The addition of Cu as Cu sulfate to pig rations has been recommended since 1955 (4). Copper additions to feed improved growth rate up to 9.7% and improved food conversion 7.9% without antibiotics (4). Additions of 250 mg kg^{-1} Cu sulfate to the rations of growing pigs was more effective in increasing live weight growth and efficiency of food conversion than a broad spectrum antibiotic (oxytetracycline). Young pigs responded to 257 mg kg^{-1} dietary Cu with increased weight gain but failed to improve the grain/feed ratio (12).

At the same time, hemoglobin and serum iron levels were reduced. Additions of iron reduced the Cu accumulation in the liver. Most researchers have found similar results except when iron and/or zinc interactions with Cu caused a Cu toxicity problem (20).

Land Application of Manure

Manure has been used for centuries as a fertilizer and conditioner of soils. The nutrient availability from solid swine manures (18% dry matter) have been estimated to be 2.3 kg N, 2.7 kg P_2O_5, and 2.7 kg K_2O per 907 kg of manure (13). Thus land application of manures appears to solve a disposal problem and provide valuable nutrients for crops. In some cases soils are naturally deficient in copper. Under this environmental condition, application of Cu-enriched manures has been recommended to prevent plant and animal copper deficiencies.

The disposal of manures becomes more complicated when the manure is Cu-enriched. The added Cu accumulates in the surface soil horizon and is almost immobile in soils (7, 11, 15). The relative immobility of metals indicates that the life of a waste application site is controlled by the cumulative addition of metals (21). Suggested maximum amounts of Cu that should be applied to agricultural soils are 140, 280, and 560 kg ha^{-1} when the soil CEC is <5, 5 to 10, >15 meq 100 g^{-1}, respectively. Switzerland and several other

European countries have set the limiting value of copper concentration in dry sludge to be 1000 mg Cu kg^{-1} (10).

Another possible problem of land applications of manures and sludges is the movement of heavy metals through the soil to contaminate ground waters. Elliott et al. (7) studied the movement of cations under a beef cattle feedlot. They found Cu and Zn accumulated in the upper 15 cm of the soil and did not represent a pollution problem to ground water. Surface applied of Cu, from either inorganic or organic sources, has generally remained in the upper soil horizons with little downward movement through the profile (3, 11, 15, 16, 19). Some movement of Cu in a sandy coastal plain soil to the B horizon has been found after heavy application of Cu-enriched pig manure (16).

Zinc in Human and Animal Health

The essential nature of Zn in human health was not recognized until the early 1960s despite having been known for years to be essential to microorganisms, plants and animals (18). Zinc deficiencies are the main areas of concern in micronutrient nutrition. Both human and animal metabolisms utilize Zn in more than 70 metalloenzymes (18). The daily human requirement for Zn is 15 to 20 mg and has been demonstrated as a limiting nutrient in many children and adults (18). Zinc toxicity in humans is uncommon and usually associated with direct ingestion of massive doses of Zn, as a sulfate or chloride, or direct inhalation of Zn fumes in industrial plants (18). There is some evidence that excess Zn may induce Cu deficiency and, thereby, might be useful in decreasing the Cu load in Wilson's disease (18).

Zinc deficiency has been observed in numerous species. Swine parakeratosis, Zn deficiency, is a major area of concern since it reduces weight gain, feed efficiency, causes lesions, and adversely effects reproduction (14).

The effects of excess dietary Zn are as dangerous, though much less common, than Zn deficiency (14). Zinc toxicity often causes loss of appetite, anemia, diarrhea, and paralysis (14). For swine, Zn toxicity symptoms include arthritis, congestion of the mesentery and gastritis, catarrhal enteritis, and internal hemorrhages. Death often follows these symptoms (14).

LITERATURE CITED

1. Arena, J.M. 1979. Poisoning. Toxicology symptons treatments. 4th ed. Charles C. Thomas, Springfield, IL.

2. Baselt, R.C. 1982. Disposition of toxic drugs and chemicals in man. 2nd ed. Biomedical Publications, Davis, CA.

3. Batey, T., C. Berryman, and C. Line. 1972. The disposal of copper-enriched pig manure slurry on grassland. J. Br. Grassld. Soc. 27:139-43.

4. Braude, R., M.J. Townsend, G. Harrington, and J.G. Rowell. 1962. Effects of oxytetracycline and copper sulphate, separately and together, in the rations of growing pigs. J. Agric. Sci. 58:251-256.

5. Cohen, S.R. 1979. Environmental and occupational exposure to copper. In J.O. Nriagu (ed.) Copper in the environment. Part II: health effects. John Wiley and Sons, New York, NY.

6. Committee on Medical and Biological Effects of Environmental Pollutants. 1977. Copper. National Academy of Sciences, Washington, DC.

7. Elliott, L.F., T.A. Travis, and T.M. McCalla. 1976. Soluble cations beneath a feedlot and an adjacent cropped field. Soil Sci. Soc. Am. J. 40:513-516.

8. Evans, G.W. 1979. Copper homeostasis and metabolism in mammalian systems. In J.O. Nriagu (ed.) Copper in the environment. Part II: health effects. John Wiley and Sons, New York, NY.

10. Gupta, S., and H. Haeni. 1981. Effect of copper supplied in the form of different Cu-saturated sludge samples and copper salts on the Cu-concentration and dry matter yield of corn grown in sand. p. 287-301. In P. L'Hermite and J. Dehandtschutter (ed.) Copper in animal wastes and sewage sludge. D. Reidel Pub. Co., Boston, MA.

11. Harmsen, K. 1977. Behaviour of heavy metals in soils. Centre for Agricultural Publishing and Documentation, Wageningen.

12. Hedges, J.D., and E.T. Kornegay. 1973. Interrelationship of dietary copper and iron as measured by blood parame-

ters, tissue stores and feedlot performance of swine. J. Anim. Aci., 37:1147-1154.

13. McCart, G.D., and S.J. Donohue. 1977. Use of manure in crop production. VPI&SU Ext. Pub. MA-208. p.1-2, Blacksburg, VA.

14. Miller, W.J., and M.W. Neathery. 1980. Manifestations of Zinc abnormailities in animals. p. 414-425. In Jerome O. Nriagu (ed.) Zinc in the environment Part II: Health effects. John Wiley and Sons, Inc., New York, NY.

15. Mullins, G.L., D.C. Martens, S.W. Gettier, and W.P. Miller. 1982. Form and availability of copper and zinc in a Rhodic Paleudult following long-term $CuSO_4$ and $ZnSO_4$ applications. J. Environ. Qual. 11:573-577.

16. 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. 11:316-320.

17. Owen Jr., C.A. 1982. Physiological aspects of copper. Copper in organs and systems. Noyes Publ., NJ.

18. Prasad, A.S. 1980. Manifestations of zink abnormalities. in human beings. In Jerome O. Nriagu (ed.) Zinc in the environment Part II: Health effects. John Wiley and Sons, Inc. New York, NY.

19. Sutton, A.L., D.W. Nelson, V.B. Mayrose, and D.T. Kelly. 1983. Effect of copper levels in swine manure on corn and soil. J. Environ. Qual. 12:198-203.

20. Underwood, E.J. 1971. Trace elements in human and animal nutrition. 3rd ed. Academic Press, New York, NY.

21. U. S. Environomental Protection Agency. 1983. Process design manual for land application of municipal sludge. U. S. Environmental Protection Agency. EPA-625/1-83-016. Cincinnati, OH.

Chapter II

THE EFFECTS ON CORN AND ON A RHODIC PALEUDULT OF 17 ANNUAL CU AND ZN SULFATE ADDITIONS

ABSTRACT

A long-term field experiment was established in 1967 and continued through 1983 to evaluate the response of corn (*Zea mays* L.) and the changes in a Davidson clay loam soil (clayey, kaolinitic, thermic Rhodic Paleudult) to yearly additions of Cu and Zn sulfates. By 1983, the 17-annual incremental additions of Cu and Zn resulted in cumulative totals up to 280 kg Cu and 560 kg Zn ha^{-1}. These Cu and Zn additions, either alone or together, did not cause any grain or stalk yield decreases. The DTPA extractant effectively separated all soil treatment levels for both Cu and Zn. The seven fold increase in DTPA Cu and 18 fold increase in DTPA Zn followed linear relationships to treatment levels with r values of 0.96** and 0.97**, respectively. Copper concentrations in the blades and grain were not related to soil additions of Cu or Zn. However, Zn concentrations in blades and grain were directly related to each other, r=0.87**, to soil Zn treatment levels and to DTPA Zn.

Additional index words: Zea mays L., DTPA extractable Cu, DTPA extractable Zn, Loading rates, Micronutrient tissue concentrations.

INTRODUCTION

Direct additions of copper and zinc to agricultural soil systems occur from many sources. Deliberate soil additions of Cu and Zn, as micronutrient fertilizers, are made to alleviate Cu and Zn deficiencies in plants (6, 19). Many soils, especially those with high organic matter or those which are highly weathered and deeply leached, require micronutrient fertilizers to attain high crop yields (6, 23). Both Cu and Zn are recommended for many crops, with corn (*Zea mays* L.) being the most widely Zn-fertilized crop in the United States (11). Soil application of either inorganic or organic sources of Cu and Zn are effective in alleviation of Cu and Zn deficiencies.

Often Cu and Zn are associated with soil inputs, such as manures, sewage sludges or fungicides. Uncontrolled additions of Cu and Zn to agricultural soils can also occur from hidden sources. Corrosion of Cu high-tension electric power lines and galvanized electrical transmission towers have increased both soil and plant Cu and Zn concentrations, respectively, in their vicinity (14, 16). Precipitation and particulate fallout have increased Cu and Zn concentrations 10 fold in soils of urban-industrial areas (21). Since these additions are not needed for soil improvement nor enhanced crop growth, the soil system would be acting as a recycling medium.

Copper and Zn added to soils accumulate in the Ap horizon with very little downward movement through the soil profile (3, 9, 14, 21, 22, 24, 25). Plants exhibited toxicity systems near a Zn smelter where total zinc concentration in the A horizon reached 2000 mg kg^{-1} (12). Corn grown in a cleared vineyard had toxicity symptoms when 1M ammonium acetate extractable Cu reached 200 mg kg^{-1} (2). Copper poisoning of sheep has been associated with the ingestion of herbage sprayed with Cu compounds (26). In addition, consumption of plants with high Cu levels, either by grazing or from dry feed, can cause toxic reactions in sheep, especially when associated with low levels of molybdenum (26). Consequently, the possibility of phytotoxicity in the rhizosphere or toxicity to animals being fed with plants high in Cu and/or Zn requires evaluation.

A long-term field study to evaluate Cu and Zn soil reactions and nutrient uptake by corn was initiated in 1967. No adverse effects were observed from 15 yearly additions of Cu and Zn sulfates (22). Broadcast applications of 172 kg Cu and 290 kg Zn ha^{-1} were accumulated in the Ap horizon with little downward movement. However, only Zn concentrations were increased in the corn leaves. The field experiment was continued on this soil for two additional years, the 16th and 17th years, respectively. Both years' results were similar therefore only results from the 17th year are reported herein. The purpose of this research was to evaluate this soil's Cu and Zn loading capacity under field conditions. A further aim was to study nutrient uptake by corn as it relates to soil applied and extractable Cu and Zn.

MATERIALS AND METHODS

A long-term field experiment was established in 1967 and continued through 1983 to evaluate corn response to applications of Cu and Zn sulfates. The initial properties of the Davidson clay loam soil are given in Table 1. Corn was monocropped on the experimental area, except in 1972, when soybeans [Glycine max (L.) Merr.] were grown on the site.

Field Experimentation

Ten treatments were annually applied to the soil during the 17-year period from 1967 through 1983. These treatments consisted of various levels of Cu and Zn sulfates, either alone or together, and a check (Table 2). Treatments 2, 4, and 5 were modified upward in 1979, 1980 and 1981 to evaluate corn response to higher levels of Cu and Zn.

The entire experimental area annually received an average of 1 kg B, 224 kg K, 93 kg Mg, 252 kg N, and 140 kg P ha^{-1} during the 3-year period from 1981 to 1983. Sources of the added nutrients were $Na_2B_4O_7$, KCl, $MgSO_4$, NH_4NO_3, and triple superphosphate. The Cu and Zn sulfates for the treatments and the amendments were broadcast in early April and disked into the soil to a depth of approximately 12 cm. In 1983 plant populations of the 'Pioneer 3369A' corn were thinned to 55 970 plants ha^{-1} on the experimental area.

Table 1. Selected properties of the Davidson soil used to evaluate Cu and Zn uptake by corn plants where these micronutrients were applied annually for 17 years.

pH	Organic Matter	Free Fe	CEC	Total		Particle-size		
				Cu	Zn	Sand	Silt	Clay
	--- g kg^{-1} --		cmol(+) kg^{-1}	mg kg^{-1}		----- % ------		
6.7	21	55	14	58	122	11	40	49

Table 2. Treatments applied on the Davidson clay loam soil over 17 years.

	Annual Application									
	1967-78		1979		1980		1981-83		17-Year Total	
Number	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
	kg ha^{-1}									
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	2.8	3.7	39.2	51.8	44.8	59.2	54.1	135.0	279.9	560.4
3	2.8	3.7	2.8	3.7	2.8	3.7	2.8	3.7	47.6	62.9
4	0.0	3.7	0.0	51.8	0.0	59.2	0.0	135.0	0.0	560.4
5	2.8	0.0	39.2	0.0	44.8	0.0	54.1	0.0	279.9	0.0
6	5.6	7.4	5.6	7.4	5.6	7.4	5.6	7.4	95.2	125.8
7	5.6	7.4	5.6	7.4	5.6	7.4	5.6	7.4	95.2	125.8
8	0.0	7.4	0.0	7.4	0.0	7.4	0.0	7.4	0.0	125.8
9	5.6	0.0	5.6	0.0	5.6	0.0	5.6	0.0	95.2	0.0
10	8.4	11.1	8.4	11.1	8.4	11.1	8.4	11.1	142.8	188.7

Sampling

Soil samples were obtained, prior to treatment applications, from the surface 0 to 20 cm depth in early April of each year. Each 5.0 by 7.6 m plot had six rows, two rows each for the harvest, tissue sampling and guard between plots. Ear leaf blade samples, from 10 plants/plot, were obtained at early silk from the two tissue sampling rows. Corn grain and stalks were harvested from 12.2 m of the center two harvest rows of each plot at plant maturity. Grain yields were calculated to 15.5% moisture content. Stalk yields were dried to 0% moisture and are so reported.

Laboratory Analysis

The plant tissue and grain samples were dried at 70° C and ground in a stainless steel mill to pass a 0.84 mm sieve. Dry 1-g samples were ashed at 450° C for two hours and the nutrient constituents of the ash dissolved in 25 ml of 0.5 M HCl. Solution concentrations of Ca, Cu, Fe, Mg, Mn, and Zn were determined by atomic absorption spectrophotometry. Potassium was determined by emission spectrophotometry, N by a microKjeldahl method and P by colorimetric procedure.

Soil samples were air dried and passed through a 2.0 mm sieve in preparation for analysis. The Mehlich 1 (0.05 M HCl in 0.0125 M H_2SO_4) extractable Ca, K, Mg, P, and Mn were determined on all soil samples (7). Soil pH in water (1:1 by volume), wet digested organic matter, and EDTA extractable

Zn were also determined for all soil samples according to procedures outlined by Donohue and Gettier (7). The DTPA-extractable Cu and Zn were determined by the procedure developed by Lindsay and Norvell (18).

Statistical Analysis

The randomized complete block experimental design was established in 1967. All data were evaluated by analysis of variance and LSD mean separation technique at the 0.05 level of probability. The relationship between treatment levels, DTPA Cu and Zn, EDTA extractable Zn, and tissue Cu and Zn levels were evaluated with regression analysis.

RESULTS AND DISCUSSION

The Davidson, a clay loam Piedmont soil, has an estimated maximum loading rate of 280 kg ha^{-1} for Cu and 560 kg ha^{-1} for Zn (27). These loading rates are based on U.S. EPA guidelines for soil disposal sites with a pH ≥6.5 and a moderate CEC of 5-15 cmol kg^{-1} (27). Earlier studies with this soil showed that 15 years of additions of Cu and Zn sulfates, which provided a cumulative total of 172 kg Cu and 290 kg Zn ha^{-1}, did not induce any detrimental effects on corn grain or silage yields (22). The 1982 and 1983 results for 16 and 17 years of annual Cu and Zn application, respectively, were similar and therefore only the 1983 data are given herein.

Soil Parameters

The soil pH and organic matter in the soil after 16 years exhibited some variability but were fairly uniform over the test area (Table 3). The Mn values show that the Davidson soil has a high level of extractable Mn. Actual Mn values were not determined above 16.1 mg kg^{-1}. The Mehlich 1 extractable Ca, K, Mg, and P did not exhibit any trends due to treatments (Table 3). The three main factors which have been found to interact with Cu and Zn availability, pH, organic matter and P, (5, 20) have been held constant within each year. Therefore, any observed effects on the corn should be due to the treatments.

Table 3. Effect of 16-annual applications of various levels of Cu and Zn on extractable nutrients, pH and organic matter on the clay loam soil, sampled in the spring of 1983.

Treatment Number	Total Micronutrient Application		Soil pH	Soil Organic Matter	Mehlich 1 Ext. Nutrients					EDTA Ext.
	Cu	Zn			Ca	K	Mg	P	Mn	Zn
	-- kg ha^{-1} --			g kg^{-1}	------------ mg kg^{-1} ------------					
1	0.0	0.0	6.7a†	22a	1197	120a	84a	17a	>16.1	2.5
2	225.8	425.4	6.6b	21a	1191	121a	86a	17a	>16.1	>6.1
3	44.8	59.2	6.8a	21a	>1200	112a	86a	18a	>16.1	4.9
4	0.0	425.4	6.7a	21a	>1200	116a	90a	17a	>16.1	>6.1
5	225.8	0.0	6.7a	22a	>1200	128a	88a	16a	>16.1	2.9
6	89.6	118.5	6.7a	21a	>1200	118a	83a	17a	>16.1	6.0
7	89.6	118.5	6.7a	21a	>1200	111a	84a	17a	>16.1	>6.1
8	0.0	118.5	6.7a	22a	>1200	110a	89a	16a	>16.1	>6.1
9	89.6	0.0	6.9a	21a	>1200	115a	90a	16a	>16.1	2.7
10	134.4	177.6	6.8a	21a	>1200	116a	87a	17a	>16.1	>6.1

†Column means followed by different letters are significantly different at the 0.05 probability level.

The DTPA extractant effectively separated all treatment levels for both Cu and Zn (Table 4). Generally, DTPA has been found to separate soil treatment levels of Cu and Zn from both organic and inorganic sources (10, 15, 24). The initial, 0 Cu applied, DTPA Cu level of 2.0 mg kg^{-1} is within the normal range of 0.2 to 3.2 mg Cu kg^{-1} reported for various soils (5, 10, 15). Application of 226 kg Cu ha^{-1} induced a seven fold increase in DTPA Cu to 14.8 mg kg^{-1}. This is well below the level of 96 mg Cu kg^{-1}, which was associated with metal toxicity in corn, Pioneer 3369-A, induced by a single $CuSO_4$ addition of 314 mg Cu kg^{-1} in a greenhouse study on a Typic Hapludult (15). The increase in DTPA Cu in the spring of 1983 (Table 4) followed a linear relationship:

$$\mathrm{DTPACu} = 1.41 + 4.67\times10^{-2}\mathrm{TCu} \qquad r = 0.95^{**}$$

where DTPACu is the DTPA extractable Cu and TCu is the Cu treatment level. A linear increase in DTPA Cu corresponding to treatment levels was reported earlier on this soil (22) and has been observed on various soils both in greenhouses and in fields (15, 24, 28).

The almost 18 fold increase in DTPA Zn in the spring of 1983 (Table 4) due to treatments followed the equation:

$$\mathrm{DTPAZn} = -0.275 + 5.11\times10^{-2}\mathrm{TZn} \qquad r = 0.96^{**}$$

where DTPAZn is the DTPA extractable Zn and TZn is the Zn treatment level. The untreated soil DTPA Zn level of 1.4 mg kg^{-1} is within the range of DTPA Zn values reported for uncontaminated soils (1, 5, 15). A single 827 mg $ZnSO_4$ kg^{-1}

Table 4. Effect of 16-annual applications of various levels of Cu and Zn as sulfates on DTPA extractable Cu and Zn in the Ap horizon of the clay loam soil.

Treatment Number	Total Micronutrient Application Cu	Total Micronutrient Application Zn	1983 DTPA Extractable Cu	1983 DTPA Extractable Zn
	----- kg ha^{-1} ----		--- mg kg^{-1} ---	
1	0.0	0.0	2.0f[†]	1.4e
2	225.8	425.4	14.8a	24.6a
3	44.8	59.2	3.5e	2.6de
4	0.0	425.4	2.2f	21.0b
5	225.8	0.0	11.1b	1.5e
6	89.6	118.5	5.4d	4.2cd
7	89.6	118.5	5.4d	4.3cd
8	0.0	118.5	2.2f	4.3cd
9	89.6	0.0	4.6d	1.5e
10	134.4	177.6	7.0c	5.7c

[†] Column means followed by different letters are significantly different at the 0.05 probability level.

treatment to a Typic Hapludult in pots raised the DTPA Zn to 244 g kg^{-1} which was associated with phytotoxic metal levels in corn (15). The DTPA Zn of 24.6 mg kg^{-1} for the high, 425 kg Zn ha^{-1}, treatment was considerably below this concentration. Linear increases in DTPAZn corresponding to treatment levels were reported earlier on this soil (22).

The long term trends to applied Cu and Zn sulfate were evaluated for six years (1971, 1979-83) of available data. The DTPA extractant was not used for the other years of the study. The DTPA extractant was linearly related with eight accumulative treatment levels for both Cu (Fig 1.) and Zn (Fig. 2) sulfates. The slopes of the Zn and Cu regression equations developed either for 1983 alone or for the long term are very similar. The DTPA extractant may be a good predictor of soils that are currently receiving, or have received at some time in the past, large amounts of Cu and Zn.

Cu and Zn in Corn Leaves

An imbalance of nutrients within the corn tissue could be an indication of the loading capacity of the soil being exceeded. Throughout the study, a fertility level suitable for high corn yields has been maintained. The fertility of the soil was reflected by all the measured macronutrients, i.e., Ca, K, Mg, N, and P (Table 5) being in the middle to upper parts of reported sufficiency ranges (8). None of the macronutrients showed any trend with the Cu and Zn treatments

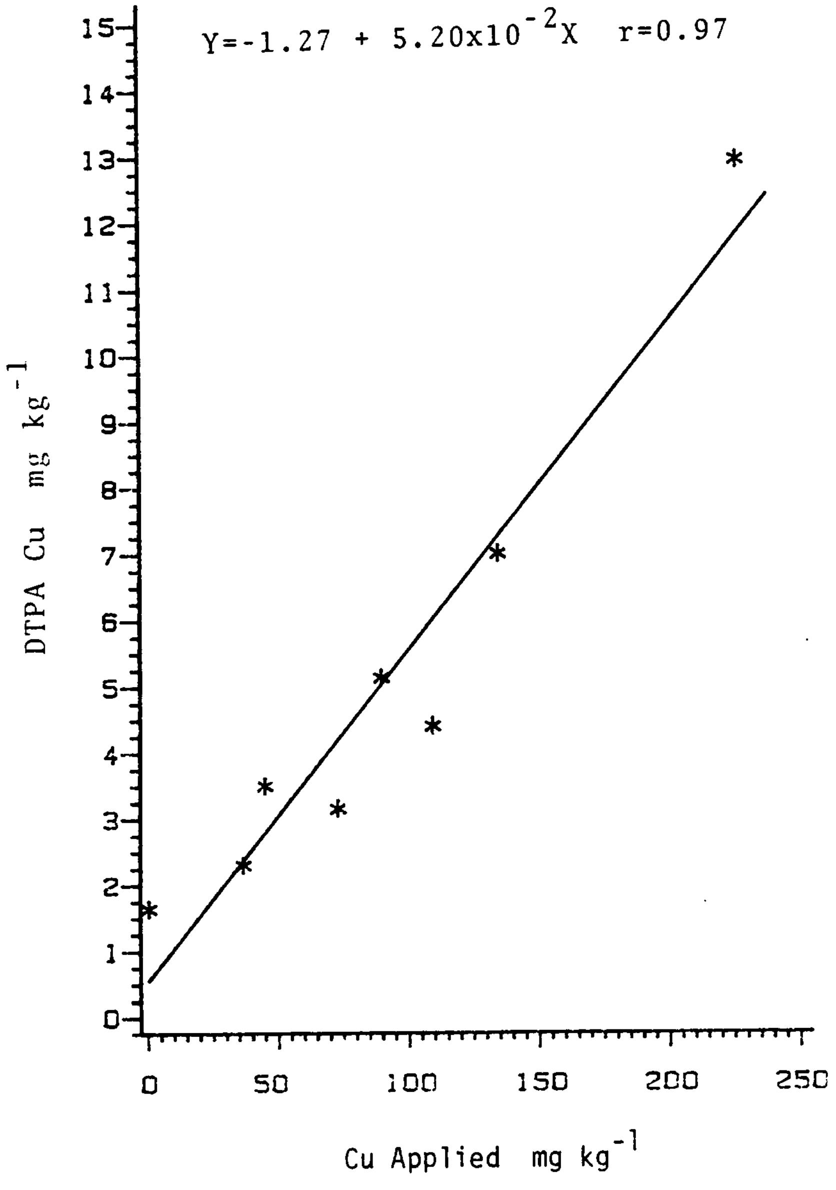

Figure 1. The relationship between DTPA extractable Cu and broadcast application of Cu, as $CuSO_4$, to a Davidson soil.

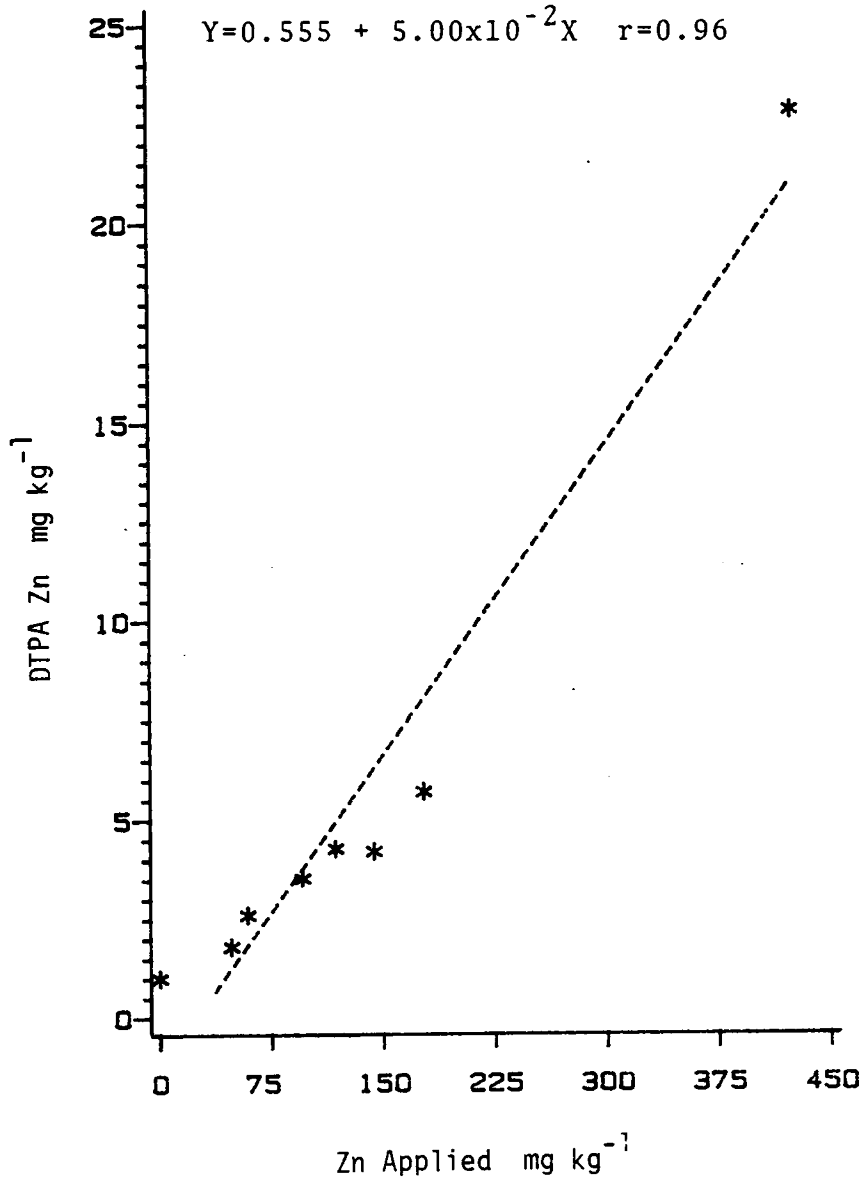

Figure 2. The relationship between DTPA extractable Zn and broadcast application of Zn, as $ZnSO_4$, to a Davidson soil.

Table 5. Effect of 17-annual applications of various levels of Cu and Zn on nutrient concentrations in ear leaf blades of corn on the clay loam soil.

Number	Total Micronutrient Application		Macronutrients				Treatment Micronutrients			
	Cu	Zn	Ca	K	Mg	P	Cu	Fe	Mn	Zn
	-- kg ha^{-1} --		----------- % -----------				-------- mg kg^{-1} -------			
1	0.0	0.0	0.80a[†]	3.7a	0.20a	0.39a	11.0a	117a	68a	20c
2	279.9	560.4	0.79a	3.7a	0.18a	0.38a	12.4a	106a	59bc	48a
3	47.6	62.9	0.71a	3.5a	0.17a	0.39a	12.2a	106a	63abc	24bc
4	0.0	560.4	0.73a	3.8a	0.17a	0.38a	14.8a	108a	57c	53a
5	279.9	0.0	0.74a	3.8a	0.18a	0.40a	14.1a	107a	66ab	20c
6	95.2	125.8	0.81a	3.7a	0.19a	0.39a	11.9a	108a	65ab	24bc
7	95.2	125.8	0.73a	3.6a	0.17a	0.39a	14.4a	114a	65ab	29b
8	0.0	125.8	0.72a	3.9a	0.19a	0.39a	16.6a	109a	62abc	27b
9	95.2	0.0	0.77a	3.7a	0.19a	0.39a	11.8a	114a	66ab	20c
10	142.8	188.7	0.85a	3.9a	0.20a	0.40a	13.3a	115a	65ab	29b

[†] Column means followed by different letters are significantly different at the 0.05 probability level.

(Table 5) nor were any visual deficiency or toxicity symptoms observed.

The micronutrients measured in the corn ear leaf blades, i.e., Cu, Fe, Mn and Zn, (Table 5) were all within reported sufficiency ranges (8). The Fe levels remained unaffected by the treatments (Table 5). This is an expected result as the Davidson soil has a very high free Fe concentration of 55 g kg^{-1}. An inverse relationship between Zn and Mn has begun to develop (Table 5). There may be competition between these metals at the absorption sites in plant roots and/or in the translocation mechanisms within the plant.

Copper concentrations in leaf blades ranged from 11.0 to 16.6 mg kg^{-1} but did not correlate with Cu additions to the soil (Table 5). The same lack of relationship was reported from 15 annual Cu and Zn treatments (22). This is an expected result since Cu is only partially mobile within the plant and tends to accumulate in the roots (19, 29). Lexmond (17) suggested that Cu toxicity and translocation of Cu from roots to the aerial portions of corn are essentially unrelated phenomena and, therefore, critical toxicity levels for leaf Cu might not be attainable.

Zinc concentrations in the blades varied directly with treatment and DTPA Zn levels such that:

$$LZn = 18.66 + 7.21 \times 10^{-2} TZn \qquad r=0.73^{**}$$

$$LZn = 19.96 + 1.28 DTPAZn \qquad r=0.90^{**}$$

where LZn is the ear leaf blade Zn concentration and TZn is the Zn treatment level. This relationship indicates that a 14 kg Zn ha^{-1} broadcast application would result in a 1 mg kg^{-1} increase in blade Zn concentration. A direct relationship between cumulative Zn levels has been reported earlier on this soil (22) and agrees with the general finding that soil applied Zn is readily plant available (4, 13). The highest Zn tissue concentration, representing an almost 3 fold increase to 50.5 mg Zn kg^{-1} (Table 5), was within normal levels of 20 to 60 mg kg^{-1} (8). No toxicities were expected as Zn toxicity has not been in evidence at blade concentrations of 285 mg kg^{-1} (4).

Yield Components

Yield losses and increased heavy metal concentration in the tissue are two primary areas of concern when Cu and Zn are being added to the soil. The 17-annual incremental additions of Cu and Zn resulting in cumulative totals of 280 kg Cu ha^{-1} and 560 kg Zn ha^{-1}, either alone or together, did not cause any grain or stalk yield decreases (Table 6). There were no discernible interactions between Cu and Zn within the plant resulting from incremental additions to the soil of these elements. The previous research with lower levels of Cu and Zn on this soil had similar results (22).

Both Cu and Zn concentrations within the grain were within normal levels of 3 to 20 and 20 to 60 mg kg^{-1}, respectively (8) (Table 6). The Cu concentration in grain was not affected by soil treatments or Zn tissue concentrations. However, Zn concentrations in the grain were increased 6.0 mg kg^{-1} by the cumulative level of 560 kg Zn ha^{-1} (Table 6). A linear relationship was found such that:

$$GZn = 14.96 + 1.47\times10^{-2}TZn \qquad r=0.93^{**}$$

$$GZn = 15.23 + 2.60\times10^{-1}DTPAZn \qquad r=0.88^{**}$$

where GZn is the Zn concentration in the grain and TZn is the treatment Zn level. There was a direct relationship between the grain Zn and blade Zn:

$$GZn = 11.88 + 1.79\times10^{-1}LZn \qquad r=0.87^{**}$$

where GZn is the Zn concentration in the grain and LZn is the Zn concentration in the ear leaf blade. The linear equations developed with six years of data (1971, 1979-83) indicate that the grain consistently accumulates Zn at lower level than the blades (Fig. 3.).

In summary, yearly additions of Cu and Zn sulfates to a cumulative total of 280 and 560 kg ha^{-1}, respectively, either alone or together, did not cause any toxicity symptoms or yield reductions in corn. The DTPA extractable Cu and Zn were linearly related to broadcast soil treatment levels. Copper concentrations in blades and grain were unaffected by treatment levels and did not have discernible interactions with other elements. However, Zn concentrations in blades and grain were directly related to each other and soil Zn treatment levels.

Table 6. Grain Cu and Zn concentrations and grain yield after 17-annual applications of various levels of Cu and Zn to the clay loam soil.

Treatment Number	Total Micronutrient Application Cu	Total Micronutrient Application Zn	Grain Micronutrient Concentration Cu	Grain Micronutrient Concentration Zn	Grain Yield	Stalk Yield
	-- kg ha^{-1} --		-- mg kg^{-1} --		--- kg ha^{-1} --	
1	0.0	0.0	1.8a[†]	15.2cd	11 880a	8 900a
2	279.9	560.4	1.9a	21.2a	11 920a	8 800a
3	47.6	62.9	1.8a	16.3bc	11 180a	9 250a
4	0.0	560.4	2.0a	21.2a	12 030a	8 760a
5	279.9	0.0	1.9a	14.5d	11 540a	8 740a
6	95.2	125.8	2.0a	17.4b	11 540a	9 330a
7	95.2	125.8	1.7a	16.4bc	11 460a	8 780a
8	0.0	125.8	1.8a	16.5b	11 290a	8 360a
9	95.2	0.0	1.9a	14.7d	12 100a	8 930a
10	142.8	188.7	1.8a	17.4b	11 810a	9 300a

[†] Column means followed by different letters are significantly different at the 0.05 probability level.

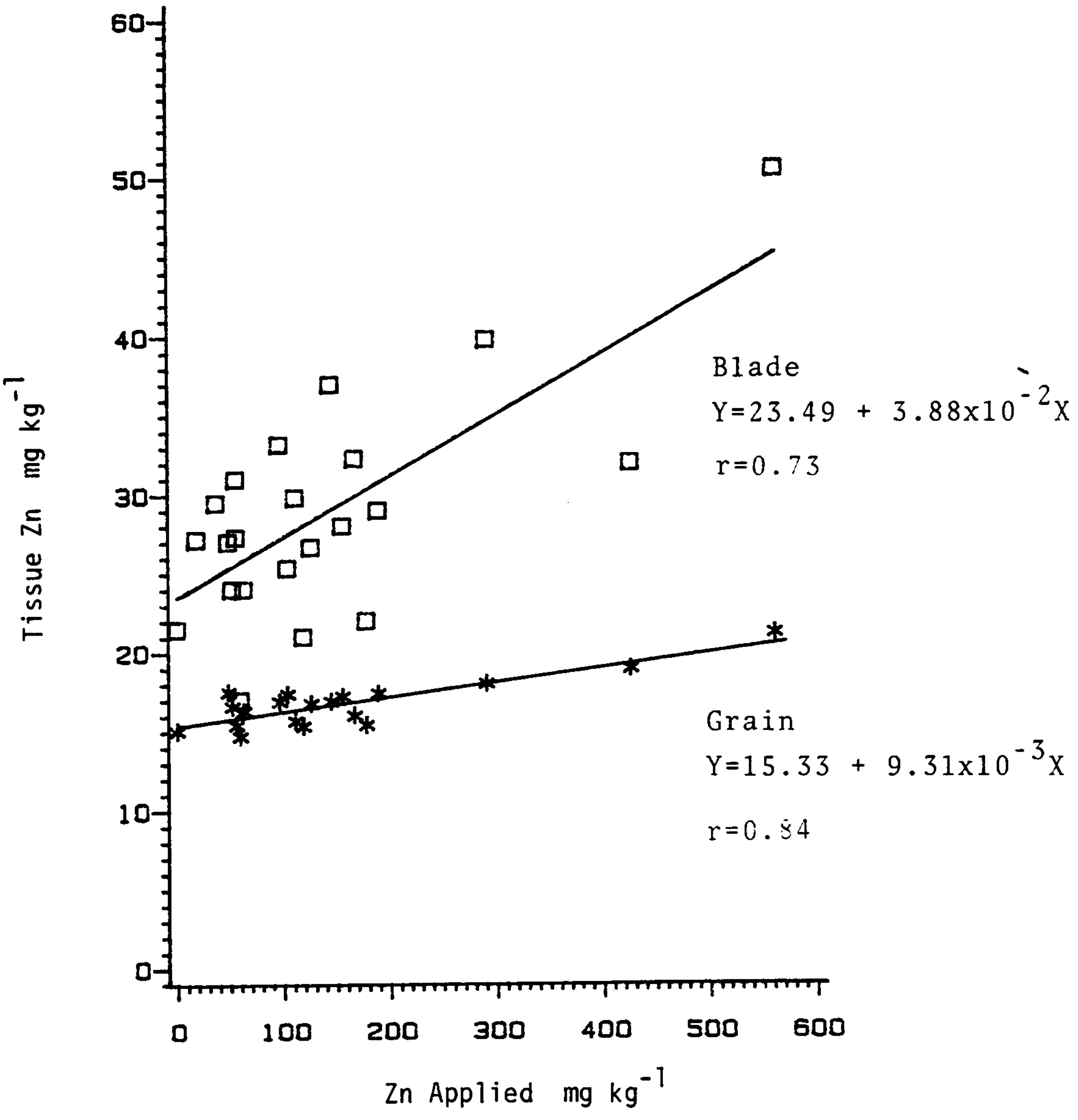

Figure 3. The effects of long-term broadcast application of Zn, as $ZnSO_4$, on corn blade and Zn concentrations.

LITERATURE CITED

1. Alley, M.M., C.I. Rich, G.W. Hawkins, and D.C. Martens. 1978. Correction of Mn deficiency of soybeans. Agron. J. 70:35-38.

2. Aubert, H., and M. Pinta. 1977. Trace elements in soils. Elsevier Scientific Publishing Company, New York, NY.

3. Chang, A.C., J.E. Warneke, A.L. Page, and L.J. Lund. 1984. Accumulation of heavy metals in sewage sludge-treated soils. J. Environ. Qual. 13:87-91.

4. Cox, F.R., and J.I. Wear. 1977. Diagnosis and correction of zinc problems in corn and rice production. Southern Cooperative Series Bull. 222, North Carolina State University, Raleigh, NC.

5. Dolar, S.G., and D.R. Keeney. 1971. Availability of Cu, Zn, and Mn in soils. I. Influence of soil, pH, organic matter, and extractable phosphorus. J. Sci. Fd. Agric. 22:273-278.

6. Donald, C.M., and J.A. Prescott. 1975. Trace elements in Australian crop and pasture production, 1924-1974. p. 7-37. In D.J.D. Nicholas and A.R. Egan (ed.) Trace elements in soil-plant-animal systems. Academic Press, Inc., New York, NY.

7. Donohue, S.J., and S.W. Gettier. 1982. Laboratory procedures, Extension Soil Testing and Plant Analysis Laboratory, Agronomy Department, VPI&SU Ext. Pub. 452-881. 26 p.

8. Donohue, S.J., and G.W. Hawkins. 1979. Sampling instructions and nutrient sufficiency ranges for plant tissue analysis. VPI&SU Agronomy Mimeo MA-211. 4 p.

9. Emmerich, W.E., L.J. Lund, A.L. Page, and A.C. Chang. 1982. Movement of heavy metals in sewage sludge-treated soils. J. Environ. Qual. 11:174-178.

10. Follett, R.H., and W.L. Lindsay. 1971. Changes in DTPA-extractable zinc, iron, manganese, and copper in soils following fertilization. Soil Sci. Soc. Am. Proc. 35:600-602.

11. Giordano, P.M., and J.J. Mortvedt. 1980. Zinc uptake and accumulation by agricultural crops. p. 401-414. In Jerome O. Nriagu (ed.) Zinc in the environment Part II:

Health effects. John Wiley and Sons, Inc., New York, NY.

12. Harmsen, K. 1977. Behaviour of heavy metals in soils. Centre for Agricultural Publishing and Documentation, Wageningen.

13. Iyengar, S.S., D.C. Martens, and W.P. Miller. 1981. Distribution and plant availability of soil zinc fractions. Soil Sci. Soc. Am. J. 45:735-739.

14. Jones, R., and M.S.E. Burgess. 1984. Zinc and cadmium in soils and plants near electrical transmission (hydro) towers. Environ. Sci. Technol. 18:731-734.

15. Korcak, R.F., and D.S. Fanning. 1978. Extractability of cadmium, copper, nickel, and zinc by double acid versus DTPA and plant content at excessive soils levels. J. Environ. Qual. 7:506-512.

16. Kraal, H., and W. Ernst. 1976. Influence of copper high tension lines on plants and soils. Environ. Pollut. 11:131-135.

17. Lexmond, T.M. 1980. The effect of soil pH on copper toxicity to forage maize grown under field conditions. Neth. J. Agric. Sci. 28:164-183.

18. Lindsay, W.L., and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci. Soc. Am. J. 42:421-428.

19. Loneragan, J.F. 1975. The availability and absorption of trace elements in soil-plant systems and their relation to movement and concentrations of trace elements in plants. p. 109-134. In D.J.D. Nicholas and A.R. Egan (ed.) Trace elements in soil-plant-animal systems. Academic Press, Inc., New York, NY.

20. Martens, D.C. 1968. Plant availability of extractable boron, copper, and zinc as related to selected soil properties. Soil Sci. 106:23-28.

21. Miller, W.P., W.W. McFee, and J.M. Kelly. 1983. Mobility and retention of heavy metals in sandy soils. J. Environ. Qual. 12:579-584.

22. Mullins, G.L., D.C. Martens, S.W. Gettier, and W.P. Miller. 1982. Form and availability of copper and zinc in a Rhodic Paleudult following long-term $CuSO_4$ and $ZnSO_4$ applications. J. Environ. Qual. 11:573-577.

23. Murphy, L.S., and L.M. Walsh. 1972. Correction of micronutrient deficiencies with fertilizers. p.347-387. In J.J. Mortvedt et al. (ed.) Micronutrients in agriculture. Soil Science Society of America, Madison, WI.

24. Sposito, G., J.P. LeClaire, C.S. LeVesque, and N. Senesi. 1984. Methodologies to predict the mobility and availability of hazardous metals in sludge amended soils. Univ. of California Water Resources Center Contribution 189. 94p., Davis, CA.

25. Sutton, A.L., D.W. Nelson, V.B. Mayrose, and D.T. Kelly. 1983. Effect of copper levels in swine manure on corn and soil. J. Environ. Qual. 12:198-203.

26. Underwood, E.J. 1971. Trace elements in human and animal nutrition. 3rd ed. Academic Press, New York, NY.

27. U. S. Environomental Protection Agency. 1983. Process design manual for land application of municipal sludge. U. S. Environmental Protection Agency. EPA-625/1-83-016. Cincinnati, OH.

28. Walsh, L.M., W.H. Erhardt, and H.D. Seibel. 1972. Copper toxicity in snapbeans (Phaseolus vulgaris L.). J. Environ. Qual. 1:197-200.

29. Woolhouse, H.W., and S. Walker. 1981. The physiological basis of copper tolerance in higher plants. p. 235-262. In J.F. Loneragan et al. (ed.) Copper in soils and plants. Academic Press, New York, NY.

Chapter III

CORN RESPONSE TO SIX ANNUAL CU-ENRICHED PIG MANURE APPLICATIONS TO THREE SOILS

ABSTRACT

Three long-term field experiments were established in the spring of 1978 and continued through 1983 to evaluate the response of corn (*Zea mays* L.) grown on three soils receiving annual applications of Cu-enriched pig manure or copper sulfate. The soils varied in texture from clay loam to fine sandy loam, and ranged in CEC from 5 to 12.3 cmol(+) kg^{-1}. Five treatments in each field experiment consisted of a control, low and high Cu-enriched pig manure levels, and $CuSO_4$ levels equivalent to the amounts of Cu in the manure. After six years, 638 mt ha^{-1} of wet Cu-enriched pig manure containing 198 mg Cu kg^{-1} had been applied by the high treatment. The Cu-enriched pig manure, containing 1285 mg Cu kg^{-1}, was produced by pigs fed diets supplemented with 242 mg Cu kg^{-1}. In the sixth year, leaf blade Cu concentrations were increased 29% by the high Cu treatments but remained in the middle of the Cu sufficiency range. Grain Cu was 73 to 84% lower than blade Cu and was unaffected by the treatments. The DTPA extractable Cu was not related to leaf nor grain Cu levels. However, the DTPA extractable Cu was linearly related, r=0.92 to 0.95, to applied Cu in the three soils. No nutrient deficiencies or toxicities were observed.

Additional index words: *Zea mays* L., DTPA extractable Cu, Loading rates, Micronutrient tissue analysis.

INTRODUCTION

Copper can enter the environment through many channels. One channel is the use of copper sulfate ($CuSO_4$) as a feed additive (9). Since 1955, the addition of $CuSO_4$ has been recommended as a feed additive for growing pigs (Sus scrofa L.) (2). Up to 250 mg Cu kg^{-1} has been proven effective in increasing pig live-weight growth (2, 5, 17), increasing efficiency of food conversion (2), and reducing the need for certain antibiotics (2, 17). Most of the supplemental Cu is subsequently excreted. Copper concentrations in such manures have been in the range of 600-2370 mg Cu kg^{-1} in the dry matter (1, 9, 15). Thus Cu concentrations in pig manure can be similar to or exceed Cu concentrations found in many sewage sludges (8, 21, 24). The recycling of manures is becoming a standard farm technique. The effects on soils and crops from the addition of Cu from Cu-enriched pig manure needs to be evaluated.

When Cu, from either organic or inorganic sources, has been surface applied there has been little downward movement through the soil profile (1, 14, 15, 18, 24). The elevated Cu levels in the Ap horizon have been detected with numerous techniques including extraction with DTPA (14, 15, 24), EDTA (1), 1N HCl (18), 4N HNO_3 (24) and total digestion (9). In general, there has been a linear increase in extractable Cu with added Cu. However, extractable Cu has not correlated with plant uptake on soils high in Cu (11).

Information relating elevated levels of extractable Cu to plant availability is severely limited. Although high levels of soil Cu are definitely toxic there is not a consensus on how much Cu can be safely added to a soil system. The U.S. EPA has set guidelines tied to a soil's CEC and recommends that a pH ≥6.5 be maintained at disposal sites (20). Problems other than Cu toxicity have often been encountered when evaluating these guidelines with short term toxicity experiments. High levels of organic matter rich in Cu can cause restricted plant growth due to adverse soluble salt effects (10) and smothering (1).

Information on the use of organic wastes containing heavy metals has been targeted as a top priority need by individuals in waste management (7). In keeping with these goals, a long term experiment was established in 1978 to study land application of Cu-enriched pig manure. The data obtained from 1978-80 have been reported previously (15). The purpose of this investigation was 1) to evaluate Cu-enriched pig manure effects on corn growth, 2) to compare the availability of inorganic Cu to Cu-enriched pig manure, and 3) to evaluate the DTPA soil test for prediction of Cu uptake by corn.

MATERIALS AND METHODS

In the spring of 1978 three experiments were established in soils of the three physiographic regions of Virginia, the Appalachian, Piedmont, and Coastal Plain. The soils varied in texture from clay loam to fine sandy loam and in CEC from 5 to 12.3 cmol(+) kg^{-1} (Table 7). Corn (Zea mays L.) was monocropped on the experimental areas except for one growing season of soybeans (Glycine max (L.) Merr. 'Essex') on the Bertie soil in 1978. Five treatments, a control, low and high Cu-enriched pig manure levels, and $CuSO_4$ equivalent to the manure Cu content, were applied to each of the three soils. The low Cu-enriched pig manure plots received 33.6 mt ha^{-1} from 1978 to 1982 and received none in 1983. The low $CuSO_4$ plots continued to receive Cu levels equivalent to 33.6 mt of Cu-enriched pig manure ha^{-1}. The high Cu-enriched pig manure plots received 67.2 mt ha^{-1} from 1978 to 1980, 134.4 in 1981 and 1982, and 168 in 1983.

Manure Collection

Pigs were fed diets supplemented with 250 mg kg^{-1} Cu as $CuSO_4$ in protected pens on a concrete slab. Elemental analysis of the feed indicates that the feed was appropriate for pig nutrition and contained 281.4, 225.2, and 283.5 mg Cu kg^{-1} in 1981, 1982, and 1983, respectively. All pigs had a three day equilibration period on the Cu-enriched rations.

Table 7. Properties of soils in 1978 at initiation of the Cu-enriched pig manure experiments.

Soil Classification and Location	Soil Series	Soil Texture	Soil pH	Organic Matter	Cation Exchange Capacity
				g kg^{-1}	cmol(+) kg^{-1}
fine-loamy, siliceous thermic Aquic Paleudults Coastal Plain	Bertie	fine sandy loam	6.2	1.7	5.0
fine, mixed, mesic Aquic Hapludalfs Appalachian	Guernsey	silt loam	5.7	1.8	10.4
clayey, mixed mesic Typic Rhodudults Piedmont	Starr-Dyke Complex	clay loam	5.9	1.4	12.3

The manure produced during this time was discarded. After the equilibration period the manure was bulked daily, then partitioned between the plastic barrels used for manure transport to the field. The manure for each field location was collected separately.

Field Fertilization

Fertilizer was applied at recommended rates based on annual soil and tissue analyses. The fertilizer value of the applied manure was calculated at 18% dry matter to be 2.5 kg N, 1.3 kg P, and 1.3 kg K 10^{-3}kg (12). The calculated manure fertilizer value was subtracted from the recommended fertilizer level and the difference, if any, was made up with conventional fertilizer N, P, and K sources as NH_4NO_3, triple superphosphate, and KCl, respectively. All soils received supplemental Zn as $ZnSO_4$ and the Bertie soil received additional Mn as $MnSO_4$.

Sampling

Subsamples of feed and manure were obtained in triplicate for each of the manure batches used at each location while manure production was in process. Each year soil samples, 10 cores per plot, were pulled from 0-20 cm in the spring prior to application of treatments or fertilizers. At early silk, 10 ear leaf blades were obtained from the two outside rows of the four rows per plot. The corn ears from

6.1 m of the inner two plot rows were harvested at plant maturity. Stalks, at plant maturity, were obtained at the Starr-Dyke location.

Laboratory Analyses

Soil samples were air-dried and ground to pass a 2.0 mm sieve in preparation for analysis. The Mehlich-1 (0.05 M HCl in 0.0125 M H_2SO_4) extractable Ca, K, Mg, Mn and P were determined on the samples as outlined by Cox (4) but without charcoal. Water soil pH (1:1 v/v) and wet digested organic matter were determined according to procedures outlined by the Council on Soil Testing and Plant Analysis (3). Manure for nutrient analysis was handled in the same manner as the blades and grain except the manure was dried at 105°C for 24 hours. The % solids were then determined as dry manure divided by wet manure times 100. The blades and grain samples were dried at 70°C. The manure, blades, and grain were ground in a stainless steel mill to pass a 0.84 mm sieve. Dry 1 g samples were ashed at 450°C for two hours and the nutrient constituents of the ash were dissolved in 25 ml of 0.5 M HCl. Elemental solution concentrations, except N and P, were determined by atomic absorption spectrophotometry. Nitrogen was determined by a microKjeldahl method and P by a colorimetric procedure.

Statistical Analysis

Each location was arranged in a randomized complete block design with four replications. All data were evaluated by analysis of variance and LSD mean separation technique at the 0.05 level of probability. The relationships between applied Cu levels from 1978 to 1983 and DTPA Cu and tissue Cu levels were evaluated with regression analyses.

Results and Discussion

Soils vary in their capacity to accept Cu-enriched wastes in an environmentally safe manner (10, 20). Therefore, three experimental areas were chosen for their diversity in soil properties and geographic location (Table 7). The U.S EPA guidelines suggest a safe Cu loading rate of 280 kg Cu ha^{-1} for soils with a pH $\geq$6.5 and a CEC within 5 to 15 cmol(+) kg^{-1} (20). After the three soils were limed to about pH 6.5, they were all considered to have 280 kg Cu ha^{-1} loading rates. Earlier studies on these soils indicated no detrimental effect on corn from application of 66 kg Cu ha^{-1} as Cu-enriched pig manure or $CuSO_4$ (15).

Corn Nutrition

Fertility levels suitable for high corn yields were maintained at all three locations (Table 8). The control and the two $CuSO_4$ treatments received the same rates of fertilizer, except for Cu, within each location (Table 9). The fertilizer regime was appropriate as shown by analysis of the corn ear leaf blades at early silk, an oft suggested diagnostic plant part for corn. The macronutrients, i.e., Ca, K, Mg, N, and P (Table 10), were within accepted nutrient sufficiency ranges (6). The macronutrients exhibited no trends with Cu treatment levels but were related to manure additions.

Table 8. Effect of five-annual applications of $CuSO_4$ or Cu-enriched pig manure on extractable nutrients, pH and organic matter on three soils sampled in the spring of 1983.

Cu Treatment		Soil	Soil Organic	Dilute $HCl-H_2SO_4$ Ext. Nutrients					EDTA Ext.
Amount	Source	pH	Matter	Ca	K	Mg	P	Mn	Zn
kg ha^{-1}			g kg^{-1}	------------ mg kg^{-1} ----------------					
		Field Experiment - Guernsey							
0.0	Control	6.9bc†	2.2ab	840c	129a	>120	55	15.2a	5.0ab
48.3	Pig Manure	7.0ab	2.4ab	1080ab	127a	>120	>60	15.2a	5.8ab
48.3	$CuSO_4$	7.1a	2.1b	888bc	142a	>120	53	14.8a	4.7b
137.5	Pig Manure	6.7d	2.5a	1110a	157a	>120	>60	16.0a	6.1a
137.5	$CuSO_4$	6.8cd	2.5ab	819c	125a	>120	58	14.8a	4.8ab
		Field Experiment - Bertie							
0.0	Control	6.3a	2.4a	750b	59b	90ab	>60	3.8b	4.7a
49.7	Pig Manure	6.5a	2.4a	861a	66b	97a	>60	4.8a	5.1a
49.7	$CuSO_4$	6.2a	2.4a	708b	65b	81ab	>60	3.7b	4.7a
133.2	Pig Manure	6.5a	2.5a	924a	84a	97a	>60	4.5ab	5.5a
133.2	$CuSO_4$	6.3a	2.3a	690b	65b	79b	>60	3.9ab	4.2a
		Field Experiment - Starr-Dyke Complex							
0.0	Control	6.6b	1.8b	900c	103a	>120	20c	15.9	2.8b
48.0	Pig Manure	6.9a	1.9b	1107b	100a	>120	36b	>16.1	5.1a
48.0	$CuSO_4$	6.7b	1.9b	903c	105a	>120	21c	>16.1	3.1b
132.4	Pig Manure	7.0a	2.2a	1200a	120a	>120	59a	>16.1	5.6a
132.4	$CuSO_4$	6.6b	2.0ab	933c	112a	>120	22c	>16.1	3.2b

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†Column means for each experiment followed by different letters are significantly different at the 0.05 probability level.

>Indicates readings above the maximum calibration for the element.

Table 9. Cumulative amounts of amendments applied as inorganic salts from 1978 through 1983 for the five treatments of the field experiments designed to determine the plant availability of Cu in pig manure.

Treatments	Macronutrients				Micronutrients			Pig Manure
	K	N	P	B	Cu	Mn	Zn	
	------------- mg kg^{-1} -----------------							mt ha^{-1}
	Field Experiment - Guernsey							
Control	780[†]	1232	204	3.3	---	--	34	---
Moderate Manure Rate	397	682	86	3.3	---	--	34	168.0
Moderate $CuSO_4$ Rate	780	1232	204	3.3	60.4	--	34	---
High Manure Rate	93	----	86	3.3	---	--	34	638.4
High $CuSO_4$ Rate	780	1232	204	3.3	197.8	--	34	---
	Field Experiment - Bertie							
Control	767	1064	159	2.2	---	84	34	---
Moderate Manure Rate	344	639	37	2.2	---	84	34	168.0
Moderate $CuSO_4$ Rate	767	1064	159	2.2	61.6	84	34	---
High Manure Rate	---	32	---	2.2	---	84	34	638.4
High $CuSO_4$ Rate	767	1064	159	2.2	192.6	84	34	---
	Field Experiment - Starr-Dyke Complex							
Control	1045	1260	366	3.3	---	--	17	---
Moderate Manure Rate	540	708	111	3.3	---	--	17	168.0
Moderate $CuSO_4$ Rate	1045	1260	366	3.3	58.5	--	17	---
High Manure Rate	---	41	---	3.3	---	--	17	638.4
High $CuSO_4$ Rate	1045	1260	366	3.3	185.0	--	17	---

[†] Inorganic nutrient sources were muriate of potash, ammonium nitrate, triple superphosphate, sodium borate, and copper, manganese, and zinc sulfates.

Table 10. Effect of six-annual applications of Cu as either $CuSO_4$ or pig manure on corn ear leaf blades on three soils.

Cu Treatment		Leaf Blades								
		Macronutrient					Micronutrient			
Amount	Source	Ca	K	Mg	N	P	Cu	Fe	Mn	Zn
kg Cu ha^{-1}		--------------- % -----------------					------- mg kg^{-1} --------			
		Field Experiment - Guernsey								
0.0	Control	0.43ab†	3.0abc	0.26a	3.0ab	0.39bc	8.3b	101a	57c	31a
48.3	Pig Manure	0.38b	3.1a	0.20a	2.9b	0.42ab	8.6b	96b	59bc	27a
60.4	$CuSO_4$	0.39ab	2.8c	0.22a	2.9b	0.39bc	8.6b	94b	51d	29a
197.8	Pig Manure	0.46a	3.1ab	0.24a	3.1a	0.46a	11.0a	98ab	67a	26a
197.8	$CuSO_4$	0.40ab	2.9bc	0.24a	3.0ab	0.37c	9.4b	98ab	63ab	31a
		Field Experiment - Bertie								
0.0	Control	0.47a	2.7a	0.31a	2.6ab	0.43bc	8.8d	88ab	15ab	29a
49.7	Pig Manure	0.46a	2.6a	0.30a	2.4b	0.48ab	10.0c	79b	12b	28a
61.6	$CuSO_4$	0.49a	2.6a	0.34a	2.5ab	0.41c	10.7bc	93a	14b	28a
192.6	Pig Manure	0.45a	2.7a	0.27a	2.7a	0.54a	12.1a	87ab	19a	27a
192.6	$CuSO_4$	0.50a	2.6a	0.30a	2.6a	0.41a	11.2ab	89ab	16ab	28a
		Field Experiment - Starr-Dyke Complex								
0.0	Control	0.60ab	2.7a	0.27a	3.1bc	0.37a	9.6b	192a	75a	23ab
48.0	Pig Manure	0.62a	2.7a	0.29a	3.2b	0.40a	10.8ab	197a	78a	22ab
58.5	$CuSO_4$	0.60ab	2.8a	0.30a	3.0c	0.38a	9.9b	185a	73a	22ab
185.0	Pig Manure	0.53b	2.8b	0.21b	3.4a	0.43a	12.1a	190a	80a	21b
185.0	$CuSO_4$	0.66a	2.6a	0.29a	3.3ab	0.39a	11.1ab	207a	79a	24a

†Column means for each experiment followed by different letters are significantly different at the 0.05 level of probability.

Even though no supplemental N has been applied to the high Cu-enriched pig manure plots (HMCu) on the Guernsey and none for five years on the Bertie and Starr-Dyke soils, the corn was healthy. The HMCu has supplied, together with natural fertility, the N needs of the corn. The blades of corn from the HMCu on the Starr-Dyke soil had 0.3% more N than the control (Table 10). Increased leaf N levels up to 1% due to swine primary lagoon effluent application of 21.6 ha-cm have been reported (23). Nitrogen content in the blades followed soil texture such that decreasing blade N accompanied decreasing clay content. This was probably a result of N losses through leaching and volatilization. Others have found that applications of pig manure above 90 t ha^{-1} provided excessive N to the soil and resulted in considerable leaching losses (19).

The HMCu contributed more P to the corn than was needed for growth. Consequently, P levels in blades (Table 10) of the HMCu, 0.43 to 0.54%, were on the high side of the sufficiency range, 0.25 to 0.45% (6). Elevated P levels in corn leaves have been observed following application of swine lagoon effluents (23) and anaerobic liquid swine manure from pits (18). Phosphorus may cause nutrient imbalances within the plant before toxic levels of Cu are reached in the Cu-enriched manure treatments as these experiments are continued.

The micronutrients measured in the blades, i.e., Cu, Fe, Mn, and Zn, were all within reported sufficiency ranges (6). Supplemental Mn was deemed necessary on the Bertie soil due to low levels of available Mn (Table 8). In addition, supplemental Zn was added to all three soils to alleviate possible Zn deficiency (Table 9). Although there were some differences in blade Fe, Mn, and Zn concentrations, they were not related to Cu treatments (Table 10). There were no observed nutrient deficiency or toxicity symptoms.

In the sixth year, Cu concentrations in the leaf blades were increased an average of 29% by the HMCu (Table 10). The highest Cu concentration in the blades (12.1 mg kg^{-1}) is in the middle of the Cu sufficiency range (6 to 20 mg kg^{-1}) (6). Consequently, harvested corn would have no use restrictions due to Cu content. The Cu from Cu-enriched pig manure was more available than Cu from $CuSO_4$ in the Guernsey soil at the high Cu rates (Table 10). The Bertie soil had the lowest free Fe and Mn, 0.3% and 20 mg kg^{-1}, respectively, of the three soils. In this soil, both the low and high Cu treatments resulted in increased blade Cu concentrations. Increased leaf Cu concentrations were reported earlier for these soils (15). For other studies the application of 24 kg Cu ha^{-1} for three years as Cu-enriched pig manure increased corn leaf Cu concentrations from 8.1 to 12.3 mg kg^{-1} (9). However, application of Cu from Cu-enriched pig manure at lower rates, 15 kg ha^{-1}, to a Crosby silt loam for four years did not increase leaf Cu concentrations (18). This would indicate low availability of soil applied Cu or low mobility within the plant.

Grain and Yield

Favorable early season weather promoted early vegetative corn growth. However, lack of rain at the time of tasseling caused relatively low grain yields, especially on the Starr-Dyke soil (Table 11). Due to the low grain yields on the Starr-Dyke soil, the stalk was harvested as an indicator of corn growth. The HMCu improved the stalk yield, relative to the control, on the Starr-Dyke soil (Table 11). Visual observations confirmed that the pig manure treatments enhanced early vegetative growth on all three soils. Enhanced corn growth due to manure applications has often been observed and can be attributed to generally improved soil tilth and moisture relations (19).

Grain yields were not affected by treatments on the Bertie and Starr-Dyke soils, and were improved on the Guernsey. Increased or similar yields due to addition of the Cu-enriched pig manure have been reported earlier on these soils (15) and other soils (9, 18). The high $CuSO_4$ treatment of 198 kg Cu ha^{-1} is approximately 88 mg Cu kg^{-1} on a concentration basis, assuming a bulk density of 1.5 Mg m^{-3} and a soil volume of 15 cm. The 88 mg Cu kg^{-1} is considerably below the level of 200 mg Cu kg^{-1}, as $CuSO_4$, found to decrease

Table 11. Effect of six-annual applications of Cu as either $CuSO_4$ or pig manure on Cu and Zn concentrations in corn grain and on corn grain yields on three soils.

Cu Treatment		Micronutrient		Grain Yield	Stalk Yield
Amount	Source	Cu	Zn		
kg Cu ha^{-1}		--- mg kg^{-1} ---		--- kg ha^{-1} ----	
		Field Experiment - Guernsey			
0.0	Control	1.63a[†]	18.1a	6 493bc	---
48.3	Pig Manure	1.60a	18.3a	5 512c	---
60.4	$CuSO_4$	1.75a	18.1a	9 134a	---
197.8	Pig Manure	1.78a	17.1a	6 658bc	---
197.8	$CuSO_4$	1.60a	17.1a	8 096a	---
		Field Experiment - Bertie			
0.0	Control	2.35a	23.0a	6 365a	---
49.7	Pig Manure	2.42a	22.5a	7 124a	---
61.6	$CuSO_4$	2.26a	21.5a	6 306a	---
192.6	Pig Manure	2.65a	23.2a	6 293a	---
192.6	$CuSO_4$	2.00b	20.9a	6 799a	---
		Field Experiment - Starr-Dyke Complex			
0.0	Control	2.35a	21.8a	724a	6 603b
48.0	Pig Manure	2.43a	20.5a	801a	7 810ab
58.5	$CuSO_4$	2.43a	21.5a	641a	6 806ab
185.0	Pig Manure	1.93a	19.5a	1 693a	8 416a
185.0	$CuSO_4$	2.28a	21.7a	1 077a	7 291ab

[†] Column means for each experiment followed by different letters are significantly different at the 0.05 level of probability.

bush bean growth in pots (22). However, a single Cu application of 200 kg ha^{-1}, as $CuSO_4$, to a Loopodzolgrond soil at KCl pH 5.9 resulted in depressed corn yields (11).

The grain Cu and Zn concentrations were not affected by Cu additions of up to 198 kg ha^{-1} from either the inorganic, $CuSO_4$, or the organic, Cu-enriched pig manure sources (Table 11). The grain Cu levels were within normal levels of 1 to 5 mg kg^{-1} (6). Grain Cu levels ranged from 73 to 84% lower than blade Cu levels. This is consistent with the concept that the Cu accumulates in the roots and is not readily translocated within the plant.

Soil Cu

Soil samples were collected in the spring prior to treatment application. Consequently, the soil samples reflect five rather than six years of treatments. The application of 470 mt ha^{-1} of manure has added many elements to the soil in addition to Cu (Table 12). The manure has increased, to a greater or lesser extent, the extractable Ca, K, Mg, P, and Zn relative to the control treatment and two $CuSO_4$ treatments (Table 8). The manure did not induce any abnormally high extractable soil nutrients and increased organic matter concentrations in only the Starr-Dyke soil (Table 8).

The DTPA extractant effectively separated all treatment levels of Cu (Table 13). There was no difference between Cu

Table 12. Nutrient concentrations and per cent solids in pig manure used for three field experiments during six growing seasons.

Growing Season	Crop	Micronutrient B	Micronutrient Cu	Micronutrient Fe	Micronutrient Mn	Micronutrient Zn	Macronutrient Ca	Macronutrient K	Macronutrient Mg	Macronutrient Na	Macronutrient P	Solids
		------ mg kg^{-1} --------					---------- % -----------					%
		Field Experiment - Guernsey										
1978	Corn	16.6†	1398	1210	222	160	3.4	1.4	0.83	0.53	1.8	25.2
1979	Corn	12.9	899	1382	172	119	6.4	0.8	0.84	0.26	1.7	25.7
1980	Corn	17.9	1157	1121	159	353	3.9	1.5	0.83	0.52	1.9	21.2
1981	Corn	24.1	1410	1000	189	328	4.2	1.6	0.88	0.99	2.9	20.6
1982	Corn	17.2	1390	1613	220	334	3.3	2.9	0.86	0.32	1.4	23.0
1983	Corn	15.8	1550	1181	520	478	4.0	1.4	0.69	0.36	1.5	23.2
		Field Experiment - Bertie										
1978	Soybeans	18.4	1396	1142	227	186	3.4	1.6	0.84	0.73	1.8	26.7
1979	Corn	16.8	1273	1082	147	101	4.7	1.4	0.94	0.33	1.9	25.9
1980	Corn	16.0	1331	910	141	246	4.1	1.5	0.77	0.34	1.9	20.5
1981	Corn	23.5	1140	1210	179	283	3.7	1.3	0.82	0.89	2.5	19.3
1982	Corn	17.7	1154	1036	225	315	2.7	3.2	0.77	0.39	1.1	24.5
1983	Corn	12.8	1454	1309	281	297	3.4	1.3	0.67	0.33	1.2	24.4
		Field Experiment - Starr-Dyke Complex										
1978	Corn	15.1	1313	916	200	170	3.0	1.4	0.75	0.62	1.7	26.7
1979	Corn	16.9	1125	1303	166	121	5.5	1.2	0.80	0.25	1.8	24.7
1980	Corn	18.9	1335	817	128	232	3.9	1.7	0.80	0.52	1.9	19.6
1981	Corn	19.5	1152	1030	203	295	4.2	1.5	0.81	0.88	2.7	22.6
1982	Corn	19.3	1273	1256	232	363	3.0	3.6	0.81	0.45	1.4	22.0
1983	Corn	14.3	1375	1239	418	446	3.4	1.4	0.66	0.34	1.5	22.7

†Each value represents the average of triplicate determinations for two subsamples.

Table 13. Effect of five-annual applications of Cu as either $CuSO_4$ or pig manure on DTPA extractable Cu in the Ap horizon.

Cu Treatment		Concentration of DTPA Extractable Cu
Amount	Source	
kg Cu ha^{-1}		mg kg^{-1}
	Field Experiment - Guernsey	
0.0	Control	1.5c†
48.3	Pig Manure	8.8b
48.3	$CuSO_4$	9.4b
137.5	Pig Manure	22.6a
137.5	$CuSO_4$	24.7a
	Field Experiment - Bertie	
0.0	Control	0.8d
49.7	Pig Manure	5.1c
49.7	$CuSO_4$	5.3c
133.2	Pig Manure	8.1b
133.2	$CuSO_4$	14.1a
	Field Experiment - Starr-Dyke Complex	
0.0	Control	1.9c
48.0	Pig Manure	7.3b
48.0	$CuSO_4$	5.9b
132.4	Pig Manure	17.8a
132.4	$CuSO_4$	15.3a

†Column means for each experiment followed by different letters are significantly different at the 0.05 level of probability.

source at the low Cu rate in the three soils. At the high Cu rate, source made a difference in the Bertie soil but not in the Guernsey or Starr-Dyke soils. The HMCu had 6 mg kg^{-1} less DTPA Cu than the comparable $CuSO_4$ treatment in the Bertie soil. The Bertie soil had 68% sand and its clay fraction was dominated by 14 A intergrade and kaolinite minerals (15). Copper is strongly held by organic matter (16). Thus Cu may be leaching below the Ap horizon bound to soluble organic compounds. Although not measured, the leaching of Cu observed earlier in the Bertie soil to lower levels of applied Cu-enriched pig manure (15) has most likely continued.

The DTPA Cu is linearly related to applied Cu (Fig. 4). The regression lines were calculated using the two years (1981 and 1982) that DTPA Cu was determined. The average of the four replications was used and no difference between Cu source was assumed. This is a valid assumption for the Guernsey and Starr-Dyke soils. Since there was evidence of Cu movement in the Bertie soil below the 20 cm depth, the HMCu were not included for the Bertie soil's regression analysis. There were no relationships between DTPA Cu and blade or grain Cu levels. This is consistent with earlier results to lower Cu levels in these soils (15).

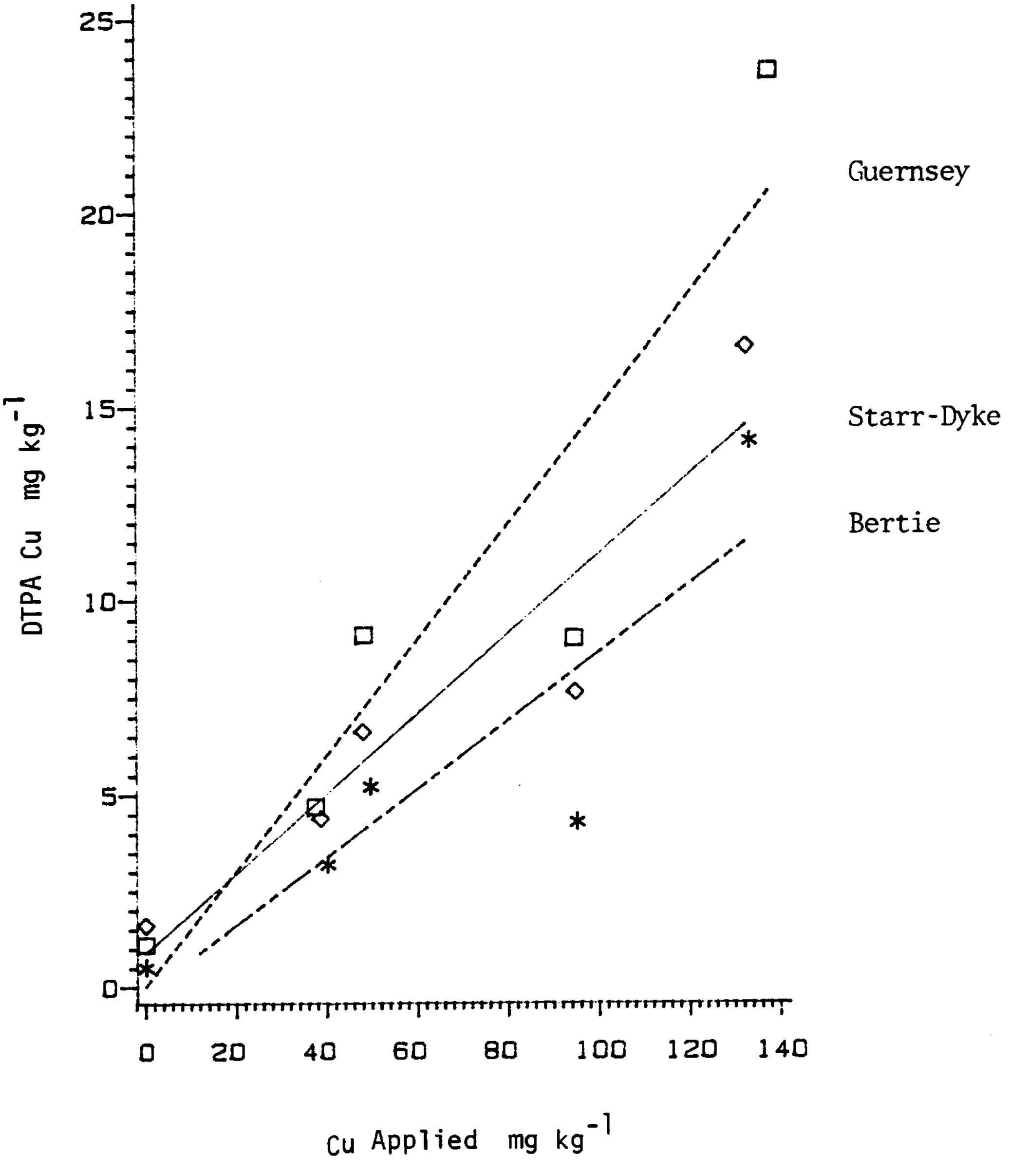

Figure 4. The relationship of DTPA extractable Cu to applied Cu, as either Cu-enriched pig manure or $CuSO_4$, in the Ap horizon of three soils.

LITERATURE CITED

1. Batey, T., C. Berryman, and C. Line. 1972. The disposal of copper-enriched pig manure slurry on grassland. J. Br. Grassld. Soc. 27:139-43.

2. Braude, R., M.J. Townsend, G. Harrington, and J.G. Rowell. 1962. Effects of oxytetracycline and copper sulphate, separately and together, in the rations of growing pigs. J. Agric. Sci. 58:251-256.

3. Council on Soil Testing and Plant Analysis. 1980. Handbook on reference methods for soil testing. 1111 Plant Sci. Bldg., Univ. of Georgia, Athens, GA.

4. Cox, F.R. 1968. Development of a yield response prediction and manganese soil test interpretation for soybeans. Agron. J. 60:521-524.

5. Hedges, J.D., and E.T. Kornegay. 1973. Interrelationship of dietary copper and iron as measured by blood parameters, tissue stores and feedlot performance of swine. J. Anim. Sci. 37:1147-1154.

6. Jones, J.B., Jr., amd H.V. Eck. 1973. Plant analysis as an aid in fertilizing corn and grain sorghum. p. 349-364. In L.M. Walsh and J.B. Beaton (ed.) Soil testing and plant analysis. Soil Science Society of America, Inc., Madison, WI.

7. Kelly, W.D., T.W. Simpson, R.B. Reneau, Jr., G.D. McCart, and D.C. Martens. 1984. Perception of technical personnel on research and education needs for land application of sewage sludge. Water, Air and Soil Pollution 22:181-185.

8. Korcak, R.F., and D.S. Fanning. 1978. Extractability of cadmium, copper, nickel, and zinc by double acid versus DTPA and plant content at excessive soils levels. J. Environ. Qual. 7:506-512.

9. Kornegay, E.T., J.D. Hedges, D.C. Martens, and C.Y. Kramer. 1976. Effect on soil and plant mineral levels following application of manures of different copper contents. Plant Soil. 45:151-162.

10. Kuo, S. 1981. Effects of drainage and long-term manure application on nitrogen, copper, zinc, and salt distribution and availability in soils. J. Environ. Qual. 10:365-368.

11. Lexmond, T.M. 1980. The effect of soil pH on copper toxicity to forage maize grown under field conditions. Neth. J. Agric. Sci. 28:164-183.

12. McCart, G.D., and S.J. Donohue. 1977. Use of manure in crop production. VPI&SU Ext. Publication MA-208. Blacksburg, VA.

14. Mullins, G.L., D.C. Martens, S.W. Gettier, and W.P. Miller. 1982. Form and availability of copper and zinc in a Rhodic Paleudult following long-term $CuSO_4$ and $ZnSO_4$ applications. J. Environ. Qual. 11:573-577.

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. 11:316-320.

16. Petruzzelli, G., G. Guidi, and L. Lubrano. 1978. Organic matter as an influencing factor on copper and cadmium adsorption by soils. Water, Air, and Soil Pollution 9:263-269.

17. Prince, T.J., V.W. Hays, and G.L. Cromwell. 1979. Effects of copper sulfate and ferrous sulfide on performance and liver copper and iron stores of pigs. J. Anim. Sci. 49:507-513.

18. Sutton, A.L., D.W. Nelson, V.B. Mayrose, and D.T. Kelly. 1983. Effect of copper levels in swine manure on corn and soil. J. Environ. Qual. 12:198-203.

19. Sutton, A.L., D.W. Nelson, V.B. Mayrose, J.C. Nye, and D.T. Kelly. 1984. Effects of varying salt levels in liquid swine manure on soil composition and corn yield. J. Environ. Qual. 13:49-59.

20. U. S. Environmental Protection Agency. 1983. Process design manual for land application of municipal sludge. U. S. Environmental Protection Agency. EP-625/1-83-016 Cincinnati, OH.

21. Valdares, J.M.A.S., M. Gal, U. Mingelgrin, and A.L. Page. 1983. Some heavy metals in soils treated with sewage sludge, their effects on yield, and their uptake by plants. J. Environ. Qual. 12:49-57.

22. Walsh, L.M., W.H. Erhardt, and H.D. Seibel. 1972. Copper toxicity in snapbeans (*Phaseolus vulgaris* L.). J. Environ. Qual. 1:197-200.

23. Wilhelm, R.G., D.C. Martens, D.L. Hallock, E.R. Collins, Jr., and E.T. Kornegay. 1980. Characterization and agricultural utilization of swine waste lagoon effluents. Water, Air and Soil Pollution 14:443-450.

24. Williams, D.E., J. Vlamis, A.H. Pukite, and J.E. Corey. 1984. Metal movement in sludge-treated soils after six years of sludge addition: 1. cadmium, copper, lead, and zinc. Soil Sci. 137:351-359.

Chapter IV

EFFECT OF CU-ENRICHED PIG MANURE AND CUSO$_4$ ON EXTRACTABLE CU, EH AND PH OF 15 VIRGINIA SOILS.

ABSTRACT

An incubation study on reversion of extractable Cu in 15 Virginia soils was performed for 64 days. The soils ranged from 54 to 489 mg kg^{-1} in clay and from 5.4 to 7.4 in pH. Simple correlations were obtained between the three extractants used to measure available Cu, $AlCl_3$, DTPA, and EDTA, and five soil properties, clay, surface area, hydrous Al, hydrous Fe, and hydrous Mn.

Treatments to each soil consisted of a control, 22 kg ha^{-1} Cu as $CuSO_4$ and as Cu-enriched pig manure, (equivalent to 975 mt ha^{-1}). The incubation was conducted at an elevated temperature of 35° C and with the soils at 33 k Pa moisture. The $CuSO_4$ was acidifying, increased soluble salt levels by about 20 S m^{-1}, and initially decreased the Eh. The Cu-enriched pig manure additions increased the soluble salt levels by about 200 S m^{-1}, buffered the pH at about 8.4, and reduced the Eh levels about 200 mv with the Eh rising after day 8 to 16. The Eh was inversely related to pH, R^2=49 to 92, depending on soil. The acid extractant, $AlCl_3$, extracted far less Cu in the Cu-enriched manure treatment than in the $CuSO_4$ treatment. Copper was extracted in the following order for the control and $CuSO_4$ treatments: $AlCl_3$ > EDTA > DTPA. The Cu-enriched pig manure followed a different order of Cu extraction such that EDTA > DTPA > $AlCl_3$. Extractable Cu decreased with time despite Cu source.

Additional index words: Acid extractant, DTPA, EDTA, Soluble salts

INTRODUCTION

Reversion of Cu from available to unavailable forms has major importance both from the consideration of toxicity mitigation and residual value of Cu fertilizers. Copper availability has been shown to decrease with time (6, 14) but slowly enough that applied Cu should have multi-year residual effect in acid soils (3, 6). An indirect indication of the rapid rate of Cu reversion after application from either inorganic or organic sources is the Cu accumulation in the A horizon (8, 10, 16, 18, 19, 21).

Movement of Cu between physiochemical forms within the soil is a partially understood phenomenon. Early research indicated that pH, Eh and ligand concentrations greatly affected Cu transformations (22). Copper is strongly bound to organic matter (5, 7, 12, 13), is absorbed on the surfaces of layer silicates, and hydrous oxides Al, Fe, and Mn (1). Copper attraction to organic matter and clays increases as the pH increases. In non-calcareous soils, Cu is readily co-precipitated by Al and Fe hydroxides (11). Associations between Cu and Mn oxides and, to a lesser extent, Fe oxides have often been observed (11, 13, 14). Proposed Cu adsorption maxima for soil constituents are Mn oxides > organic matter > Fe oxides > clay minerals (13).

Less information is available on the effects of pH and Eh on Cu availability. Increases in pH and Eh can cause reduction of Mn^{4+} to Mn^{2+} (15). Both high pH and Eh, either together or separately, increased the Cu associated with Mn oxides and hydroxides and oxalate fractions and decreased exchangeable Cu, and Cu associated with organic matter (20). Under waterlogged conditions, low Eh and pH solublized Cu but the soluble cation was soon associated with exchangeable and organic fractions. Availability of Cu depends on the rate with which it is complexed by organic matter but precipitation reactions are important, even in slightly acidic soils (4).

The purpose of this study was 1) to evaluate Cu relations in soils from the three physiographic regions of Virginia, and 2) to evaluate Cu reversion to unavailable forms.

MATERIALS AND METHODS

Fifteen soils spanning the three physiographic provinces of Virginia were selected for this study (Table 14). Selected properties of the soils are given in Table 15. These soils were chosen for their range in soil properties. The Cu-enriched pig manure was collected from feces of pigs fed diets enriched with 250 mg kg^{-1} Cu as $CuSO_4$. The manure consisted of 0.23 kg solids kg^{-1} and was frozen until needed. The frozen manure was thawed, dried to a constant weight at 70° C in a gravity convection oven, and ground to pass a 0.84 mm sieve for improved handling and mixing with the soils. Dry 1-g samples were ashed at 450° C for two hours and the nutrient constituents of the ash dissolved in 25 ml of 0.5 M HCl. Solution concentrations of Cu, Mn, and Zn were determined by atomic absorption spectrophotometry. The manure was dried at 105° C to determine amounts of moisture and solids (Table 16).

The following procedures were outlined and in previous research (9). Clay content was determined by the pipette method and organic matter content by the Walkley-Black procedure. Surface area (SA) was determined using the ethylene glycol monoethyl ether adsorption procedure. Ammonium oxalate extractable Al (AMOXAl) and Fe (AMOXFe) were estimated by NH_4-oxalate extraction adjusted to pH 3.25 under UV light at 85° C. The hydrous Mn content (HYHCLMn) was deter-

Table 14. The soil series and taxonomic class of the 15 soils studied.

Soil Series	Taxonomic Class
Christian	Typic Hapludults, clayey kaolinitic mesic
Craven	Aquic Hapludults, clayey mixed thermic
Dragston	Typic Ochraquults, fine-loamy siliceous thermic
Dyke	Typic Rhodudults, clayey mixed mesic
Emporia	Typic Hapludults, fine-loamy siliceous thermic
Fauquier	Ultic Haplidalf, fine mixed mesic
Kempsville	Typic Hapludults, fine-loamy siliceous thermic
Lodi	Typic Hapludults, clayey kaolinitic mesic
Myatt	Typic Ochraquults, fine-loamy siliceous thermic
Portsmouth	Typic Umbraquults, fine-loamy siliceous thermic
Rains	Typic Hapludults, fine-loamy siliceous thermic
Rumford	Typic Hapludults, coarse-loamy siliceous thermic
Slagle	Aquic Hapludults, fine-loamy siliceous thermic
Starr	Fluventic Dystrochrepts, fine-loamy mixed thermic
Tarboro	Typic Udipsamments, mixed mesic

Table 15. Initial properties and soil moisture of the 15 soils studied in the incubation experiment.

Soil Series	pH	OM	SA	Clay	Soil Moisture Air Dry	 33 k Pa	AMOX-Al	AMOX-Fe	HYHCL-Mn
		g kg^{-1}	m^2g^{-1}	mg kg^{-1}	------------	g kg^{-1}	------------	------------	mg kg^{-1}
Christian	5.7	19	35	164	8.0	130	4.5	27.8	499.7
Craven	5.5	38	35	121	11.8	118	2.4	3.3	73.6
Dragston	6.9	11	15	96	7.9	58	4.4	5.2	3.2
Dyke	6.9	15	92	454	72.6	188	16.9	66.2	3699.1
Emporia	6.5	20	20	97	5.8	116	3.6	6.7	12.5
Fauquier	7.3	30	95	255	26.0	235	17.3	87.6	1880.1
Kempsville	6.0	10	11	71	4.0	64	2.3	4.1	56.7
Lodi	7.4	26	37	168	12.0	187	5.4	12.6	314.1
Myatt	6.8	11	12	79	3.9	52	1.7	2.4	2.5
Portsmouth	5.8	74	.	.	18.0	147	.	.	.
Rains	6.0	24	28	156	7.9	111	3.2	4.5	4.5
Rumford	6.6	10	12	54	1.9	48	2.8	4.3	51.8
Slagle	6.6	34	33	88	11.7	138	4.6	4.1	179.2
Starr	6.1	21	108	489	43.6	208	17.0	77.1	2984.8
Tarboro	5.4	4	18	64	8.0	37	4.6	8.0	48.2

Table 16. Properties of the Cu-enriched pig manure.

	Total			Dry Manure	
Solids	Cu	Mn	Zn	Solids	Moisture
%	------mg kg^{-1}------			---------%----------	
23	974.6	170.1	227.6	94	6.6

mined by hydroxylamine hydrochloride extraction. The Al, Fe, and Mn contents were determined by atomic absorption spectrophotometry.

Incubation Experiment

An elevated temperature of 35° C, to facilitate chemical reactions, and a high humidity environment, to prevent soil drying between sampling, were deemed desirable. Incubators were constructed using TY05 blood serum styrofoam boxes. The incubator bottom was flooded with approximately 3 cm water to maintain a high humidity environment. The temperature regime was accomplished with a 100 watt Hartz aquarium heater placed within a 4 l glass, water filled, wax (parafilm) sealed jar. This low cost incubator maintained temperatures within plus or minus 0.5° C. Soil moisture at 33 k Pa was determined for the fifteen soils by pressure plate analysis.

Three treatments were evaluated, a control, 224 mt ha^{-1} dry manure (975 mt ha^{-1} wet manure), and 21.86 kg Cu ha^{-1} as $CuSO_4$ (Cu equivalent to the Cu-enriched manure treatment). For each soil, three 100 g subsamples of air dry soil received no amendments (the control), 10 g air dry manure or 37 mg $CuSO_4$. Each amendment was thoroughly mixed with the dry soil. Appropriate amounts of deionized water were next added to attain a moisture regime of 33 k Pa. The soils were placed within plastic cups 44 mm high and 114 mm in diameter. The manure treatments received water to raise 110 g soil to 33 k

Pa to compensate for water retention of the added manure. The cups were covered with plastic wrap and placed within the incubator.

Sampling and Analysis

Sampling was initiated immediately upon mixing amendments and water into the soils and prior to placing into incubator. Samples were obtained from 2^0 to 2^6 days. The sampling procedure was to mix the soil with a spatula and remove 14.10 g soil for analysis. Containers were weighed before and after sampling. More deionized water was added, if needed, to return each soil to 33 k Pa. The cups were rotated within the incubator at each sampling.

To 6 g soil in a 25 ml centrifuge tube, 12 ml deionized water was added. Samples were shaken for 6 hours and filtered through Whatman 42 filter paper. The filtrate was analyzed for soluble salts, pH and Eh. Duplicate analyses were determined on separate soil subsamples for the following three extractable Cu determinations: 1) 0.90 g soil shaken in 4.5 ml of 0.5 M HCl + 0.025M $AlCl_3$ for 5 min., 2) 2.24 g soil shaken in 4.5 ml of 0.005 M DTPA + 0.01M $CaCl_2$ + TEA (pH 7.3) for two hours, and 3) 0.09 g soil shaken in 4.5 ml 0.05M di-Na EDTA (pH 7.0) for one hour. Samples were centrifuged at 2 000 rpm for five min., filtered and refrigerated until analyzed by atomic absorption spectrophotometry.

Statistical Analysis

Simple correlations and regression analyses were performed to evaluate the relationships between the dependent variables (extractable Cu) and the independent variables (soil properties, extractable Cu, and time).

RESULTS AND DISCUSSION

The 15 soils selected for this incubation study had large differences in seven measured soil properties, especially in their content of hydrous Mn (HYHCLMn) and clay (Table 15). These seven properties were evaluated with respect to their relationship to the initial values of extractable Cu by three extractants, $AlCl_3$, EDTA, and DTPA. The studied soils were low, ≤ 38 g kg^{-1}, in organic matter concentration (Table 17). Consequently, a large proportion of the Cu would be held by the inorganic soil fractions. Correlation analysis indicates that these extractants were related to clay, SA, AMOXAl, AMOXFe, and HYHCLMn soil fractions (Table 15). The following linear correlations were found between HYHCLMn and ALCLCu or EDTACu:

$$\text{ALCLCu} = 0.521 + 1.02\text{x}10^{-3}\text{HYHCLMn} \qquad R^2=91^{**}$$

$$\text{EDTACu} = 0.212 + 8.90\text{x}10^{-4}\text{HYHCLMn} \qquad R^2=96^{**}$$

Copper is known to have a strong affinity with Mn oxides across a wide range in pH (12). The affinity of Cu for Mn oxides is stronger than for Fe oxides (11). The DTPA extractant was highly correlated with clay such that:

$$\text{DTPACu} = -0.137 + 2.36\text{x}10^{-3}\text{Clay} \qquad R^2=95^{**}$$

Copper can be specifically adsorbed to layer silicates and associated hydrous oxide impurities (11).

Table 17. Correlation coefficients between extractable Cu of three extractants and selected soil properties.

Soil Property	simple r		
	$AlCl_3$	DTPA	EDTA
Soluble Salts	-0.046ns	-0.117ns	-0.059ns
pH	0.283ns	0.189ns	0.288ns
Eh	-0.253ns	-0.166ns	-0.257ns
OM	0.706ns	0.080ns	0.068ns
Clay	0.941**	0.977**	0.946**
SA	0.860**	0.873**	0.874**
AMOXAl	0.869**	0.866**	0.898**
AMOXFe	0.803**	0.803**	0.830**
HYHCLMn	0.951**	0.963**	0.981**

**--Significant at the 0.01 level of probability.
ns--Not significant.

Soluble Salt and pH Changes

Additions of Cu from either the $CuSO_4$ or manure source caused many changes in the soils. The soluble salt (SS) concentrations were elevated by the treatments (Fig. 5). Addition of 21.9 kg Cu ha^{-1} induced 0.01 to 0.02 S m^{-1} increases in SS. These salt levels remained relatively constant for the duration of the incubation. The manure addition produced large increases of 0.15 to 0.20 S m^{-1} in SS and, although somewhat variable, remained high for the duration of the incubation (Fig. 5). Soluble salt levels of 1.10 S m^{-1} have been observed in manure slurries (10). The high SS was associated with elevated Na and NH_4-N levels and could cause restricted plant growth. Considerable NO_3-N levels were observed in leachate of soil columns amended with sewage sludge after only two weeks (5).

The pH of the soils ranged from 4.4 to 7.7 (Table 18). Addition of $CuSO_4$ was acidifying and kept the pH depressed for the duration of the incubation. The manure addition had the opposite effect on all 15 soils (Table 18). All pH levels were immediately raised above 7.0 and peaked at 8.2 to 8.5. Manure has been generally observed to increase the pH of acid to neutral soils (10, 15). Application of 476 mt ha^{-1} (air dry) sewage sludge to soil columns increased soil pH from about 4.5 to 7.1 at the end of a 25 month leaching study (5). The added sludge had a strong buffering effect regardless of the initial pH of the soil. Addition of freeze dried manure

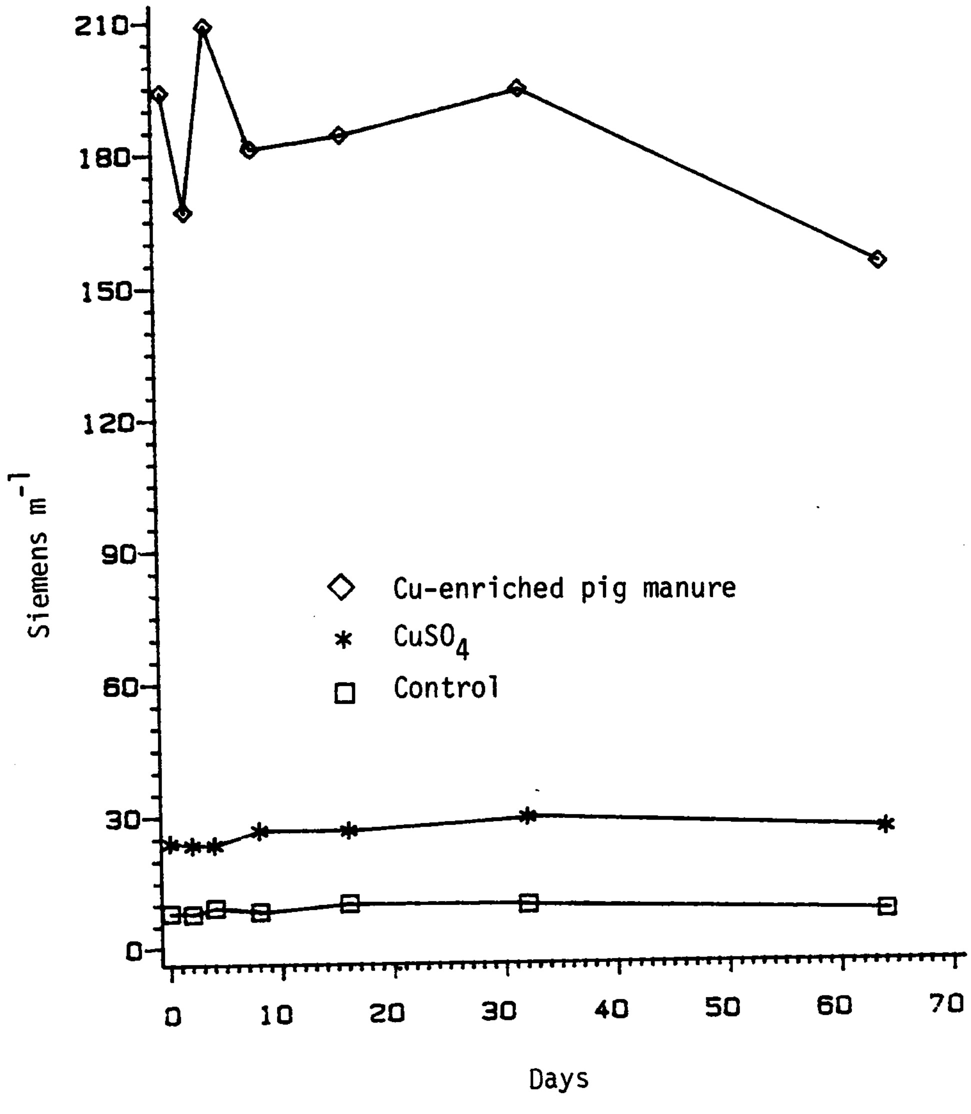

Figure 5. Effects of added $CuSO_4$ and Cu-enriched pig manure on soluble salt levels in a Dragston soil.

Table 18. Effect on pH of $CuSO_4$ and Cu-enriched pig manure over time in the 15 incubated soils.

	Control		+Cu		+Manure	
Soil Series	Day 0	Day 64	Day 0	Day 64	Day 0	Day 64
	pH					
Christian	5.2	5.1	5.0	5.1	7.1	7.7
Craven	5.2	5.3	5.3	5.3	7.1	6.9
Dragston	6.2	6.1	5.4	5.2	7.3	8.3
Dyke	6.2	4.5	5.0	5.5	7.6	7.6
Emporia	6.0	5.9	5.2	4.9	7.2	8.4
Fauquier	7.7	6.1	7.3	5.9	7.7	7.6
Kempsville	5.1	5.1	4.8	4.8	7.1	8.2
Lodi	7.5	6.0	6.7	5.9	7.7	8.3
Myatt	6.1	5.6	5.3	5.2	7.3	8.4
Portsmouth	5.4	5.6	5.2	5.2	7.2	7.5
Rains	5.6	5.6	5.5	5.1	7.1	7.5
Rumford	4.9	5.1	4.6	4.6	7.2	8.5
Slagle	5.7	5.4	5.6	5.2	7.3	8.0
Starr	5.4	5.1	5.0	5.1	7.4	7.7
Tarboro	4.4	4.7	4.3	4.2	7.2	8.5

to three soils immediately increased their pH to above 7.0 and the pH increased over the 29 day laboratory incubation (15). The pH changes were attributed to the high initial pH (7.8) of the manure slurry and to consumption of protons during the reduction process in the soils.

Relations of Eh and pH Changes

The incubation of the soils was conducted in an environment designed to be conducive to oxidizing conditions. The 33 k Pa moisture regime (approximately field capacity) did not waterlog the soils. Incubation cups were large enough to allow a shallow depth of soil, ≤2 cm, and a large air volume. Even though the cups were covered, oxidizing conditions should have prevailed. The cups were opened periodically for sampling and, coupled with mixing of the soil while sampling, would have had ample air exchange. An indication of the redox conditions in soils is for oxidized soils to have redox potentials of >400 mv, moderately reduced 100 to 400 mv, reduced -100 to 100 mv, and highly reduced -300 to -100 mv (2). To compare the observed Eh values with this scale, the Eh values were adjusted to pH 7 by using a conversion factor of -59 mv pH^{-1} and are notated as Eh_7 (2). On this scale all the control and $CuSO_4$ treatments would be considered to be moderately reduced and the Cu-enriched pig manure treatments to be reduced.

The addition of $CuSO_4$ generally decreased the Eh relative to the control. By the end of the incubation the Eh values had recovered or exceeded the control treatment Eh levels.

The addition of Cu-enriched pig manure greatly decreased the Eh values for all soils. The Eh was variable but generally reached a minimum by day 8 to 16 and then slowly increased for the duration of the incubation. Without the moderating influences of soil, the Eh of a digested sewage sludge was about 50 mv and, subjected to aeration, increased rapidly (within six days) to > 300 mv (17). Manure additions to three soils immediately reduced Eh by about 100 mv, reached a minimum at day four and then increased for the duration of a 29 day incubation experiment (15).

The Nerstian equation predicts a linear response between pH and Eh. Linear regression analysis of all soils across treatments and time indicated an inverse linear relationship between Eh and pH (Table 19). The average equation for all soils:

$$Eh = 535.0 - 56.9pH$$

shows a slope fairly close to the -59 mv pH^{-1} often used to adjust Eh readings to pH 7.

Treatment Effects on Extractable Cu

Copper was extracted in the following order for the control and $CuSO_4$ treatments in all soils: $AlCl_3$ > EDTA >

Table 19. The relationship between Eh (in mv) and pH for 15 soils.

Soil Name	Linear Equation†	R^2
Christian	Eh = 543.7 - 56.8pH	78
Craven	Eh = 544.0 - 59.0pH	86
Dragston	Eh = 537.0 - 56.2pH	75
Dyke	Eh = 511.9 - 52.0pH	77
Emporia	Eh = 543.1 - 59.2pH	85
Fauquier	Eh = 448.0 - 43.4pH	49
Kempsville	Eh = 561.6 - 59.2pH	77
Lodi	Eh = 511.1 - 50.7pH	60
Myatt	Eh = 522.8 - 54.9pH	82
Portsmouth	Eh = 543.5 - 60.7pH	87
Rains	Eh = 597.6 - 68.1pH	90
Rumford	Eh = 542.4 - 59.4pH	87
Slagle	Eh = 548.9 - 59.9pH	88
Starr	Eh = 520.6 - 53.5pH	83
Tarboro	Eh = 549.8 - 61.1pH	92

†All linear equations were significant at the 0.1 level of probability.

DTPA. The Cu-enriched pig manure treatment followed a different order of Cu extraction where EDTA > DTPA > $AlCl_3$. This could be the result of the manure buffering the soil at a higher pH and thereby reducing the effectiveness of the acid extractant. These results would indicate that a chelating agent remains more effective at extracting Cu over a wider range of pH and organic matter than an acid extractant. The relationship between DTPA and EDTA follows a linear relationship:

$$\text{DTPACu} = -1.08 + 0.754\ \text{EDTACu} \quad r=97^{**}.$$

Either extractant could be used as an indicator of soils high in Cu.

Despite the acidifying nature of the $CuSO_4$ treatment, extractable Cu levels decreased in all soils (Table 20). Precipitation reactions involving Cu have been observed in acid soils (4, 14). The general trend was for extractable Cu values to rapidly decrease to about day 8 and then to decrease at a much reduced rate for the duration of the incubation. The Cu-enriched pig manure treatment followed the same pattern (Table 20). However, the analyses were greatly hampered by manure induced physical and biotic changes. The initial manure odor was replaced by a musty smell by day two. The soil had a sponge-cake type texture and a white mold/fungal growth. By day four the soils were very spongy and exhibited hydrophobic tendencies. By day 32 the soils were highly hydrophobic and meaningful extractable analyses

Table 20. Effect of time and added Cu, from either CuSO$_4$ or Cu-enriched pig manure, on available Cu as measured by three extractants.

Extractant	Control		+CuSO$_4$		+Manure	
	Day 0	Day 64	Day 0	Day 64	Day 0	Day 64
	----------------	mg kg^{-1}	----------------			
			Christian			
AlClCu	0.8	0.5	104.4	90.2	15.4	.
DTPACu	0.16	0.14	82.3	61.8	54.1	47.0
EDTACu	0.4	0.4	95.9	74.0	74.0	.
			Craven			
AlClCu	0.5	0.2	182.7	81.4	16.9	.
DTPACu	0.09	0.04	75.8	38.7	53.5	35.2
EDTACu	0.2	0.1	99.8	54.1	77.1	.
			Dragston			
AlClCu	0.7	0.6	111.1	86.8	21.4	.
DTPACu	0.13	0.09	81.6	46.9	62.5	49.6
EDTACu	0.4	0.6	98.9	60.2	81.9	.
			Dyke			
AlClCu	4.8	4.8	102.4	78.7	20.0	.
DTPACu	1.08	0.89	61.2	35.1	71.4	43.8
EDTACu	3.9	3.8	100.0	71.9	83.7	.
			Emporia			
AlClCu	0.5	0.3	134.1	75.5	17.8	.
DTPACu	0.07	0.06	80.8	50.0	55.6	.
EDTACu	0.3	0.2	99.1	62.2	77.1	.
			Fauquier			
AlClCu	2.1	2.0	76.0	84.1	18.8	.
DTPACu	0.37	0.32	58.1	29.1	51.5	34.9
EDTACu	1.6	1.7	78.6	66.6	77.1	.
			Kempsville			
AlClCu	0.4	0.3	116.5	96.3	18.4	17.6
DTPACu	0.07	0.06	97.4	71.0	76.9	54.0
EDTACu	0.2	0.1	112.1	82.3	82.8	.
			Lodi			
AlClCu	0.9	0.7	95.6	84.1	18.2	13.7
DTPACu	0.17	0.11	73.5	40.2	45.2	41.0
EDTACu	0.6	0.7	85.4	60.5	74.1	.

Table 20 continued. Effect of time and added Cu, from either $CuSO_4$ or Cu-enriched pig manure, on available Cu as measured by three extractants.

	Control		$+CuSO_4$		+Manure	
Extractant	Day 0	Day 64	Day 0	Day 64	Day 0	Day 64
	---------------	mg kg^{-1}	---------------			
			Myatt			
AlClCu	0.4	0.2	113.2	96.3	18.4	.
DTPACu	0.05	0.03	99.9	61.2	61.9	52.3
EDTACu	0.2	0.1	105.7	76.1	80.7	.
			Portsmouth			
AlClCu	0.8	0.6	130.0	66.6	15.2	13.5
DTPACu	0.13	0.08	81.9	37.4	54.0	30.0
EDTACu	0.4	0.2	112.7	69.1	78.3	.
			Rains			
AlClCu	1.7	1.2	103.7	86.8	17.2	15.4
DTPACu	0.29	0.22	71.0	40.8	58.3	40.5
EDTACu	0.6	0.4	94.6	60.1	81.9	.
			Rumford			
AlClCu	0.3	0.2	115.2	94.9	17.9	.
DTPACu	0.05	0.2	90.8	54.0	60.6	50.4
EDTACu	0.2	0.1	102.1	65.4	81.3	.
			Slagle			
AlClCu	0.6	0.4	139.5	77.4	18.3	13.1
DTPACu	0.09	0.06	71.8	41.6	55.1	35.3
EDTACu	0.3	0.2	98.3	61.4	78.2	.
			Starr			
AlClCu	3.2	2.9	96.3	70.6	17.9	14.2
DTPACu	0.96	1.02	69.3	41.2	65.9	34.0
EDTACu	2.6	2.5	96.6	65.6	79.5	.
			Tarboro			
AlClCu	0.4	0.2	111.8	92.2	18.9	.
DTPACu	0.06	0.04	103.5	72.1	61.9	45.9
EDTACu	0.2	0.1	105.1	76.2	86.1	.

became difficult. Addition of a surfactant, as a wetting agent, to the extracting reagents may improve the analyses. The overall observed trend was for extractable Cu values to rapidly decrease to about day eight and then to continue to decrease, but at a much reduced rate, for the duration of the incubation.

LITERATURE CITED

1. Baker, D.E. 1974. Copper: soil, water, plant relationships. Fed. Proc. 33:1188-1193.

2. Bohn, H.L. 1971. Redox potentials. Soil Sci. 112:39-45.

3. Cook, R.L., and J.F. Davis. 1957. The residual effect of fertilizer. Adv. Agron. 9:205-216.

4. Dolar, S.G., and D.R. Keeney. 1971b. Availability of Cu, Zn, and Mn in soils. II. Chemical extractability. J. Sci. Fd. Agric. 22:279-286.

5. Emmerich, W.E., L.J. Lund, A.L. Page, and A.C. Chang. 1982. Movement of heavy metals in sewage sludge-treated soils. J. Environ. Qual. 11:174-178.

6. Follett, R.H., and W.L. Lindsay. 1971. Changes in DTPA-extractable zinc, iron, manganese, and copper in soils following fertilization. Soil Sci. Soc. Am. Proc. 35:600-602.

7. Gerritse, R.G., R. Vriesema, J.W. Dalenber, and H.P DeRoos. 1982. Effect of sewage sludge on trace element mobility in soils. J. Environ. Qual., 11:359-364.

8. Harmsen, K. 1977. Behaviour of heavy metals in soils. Centre for Agricultural Publishing and Documentation, Wageningen.

9. Jin, J. 1985. Boron chemistry in selected virginia soils and hydroxy aluminum and iron systems. Dissertation. Va. Polytechnic Inst. and State Univ., Blacksburg, VA.

10. Kuo, Shiou. 1981. Effects of drainage and long-term manure application on nitrogen, copper, zinc, and salt distribution and availability in soils. J. Environ. Qual. 10:365-368.

11. McBride, M.B. 1981. Forms and distribution of copper in solid and solution phases of soil. p. 25-45. <u>In</u> J.F. Loneragan et al. (ed.) Copper in soils and plants. Academic Press, Inc. New York, NY.

12. McLaren, R.G., and D.V. Crawford. 1973. Studies on soil copper I. The fractionation of copper in soils. Soil Sci. 24:173-442.

13. McLaren, R.G., and D.V. Crawford. 1973. Studies on soil copper II. The specific adsorption of copper by soils. Soil Sci. 24:443-452.

14. Miller, W.P. 1981. Sequential extraction of Cu from soil components and Cu amended soils. Ph.D. Dissertation. Va. Polytechnic Inst. and State Univ., Blacksburg, VA.

15. Miller, W.P., D.C. Martens, and L.W. Zelazny. 1985. Effects of manure amendment on soil chemical properties and hydrous oxides. Soil Sci. Soc Am. J. 49:856-861.

16. Miller, W.P., W.W. McFee, and J.M. Kelly. 1983. Mobility and retention of heavy metals in sandy soils. J. Environ. Qual. 12:579-584.

17. Molina, J.A.E., O.C. Braids, T.D. Hinesly, and J.B. Cropper. 1971. Aeration-induced Changes in liquid digested sewage sludge. Soil Sci. Soc. Am. Proc. 35:60-63.

18. Mullins, G.L., D.C. Martens, S.W. Gettier, and W.P. Miller. 1982. Form and availability of copper and zinc in a Rhodic Paleudult following long-term $CuSO_4$ and $ZnSO_4$ applications. J. Environ. Qual. 11:573-577.

19. 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. 11:316-320.

20. Sims, J.L., and W.H. Patrick, Jr. 1978. The distribution of micronutrient cations in soil under conditions of varying redox potential and pH. Soil Sci. Soc. Am. J. 42:258-262.

21. Sutton, A.L., D.W. Nelson, V.B. Mayrose, and D.T. Kelly. 1983. Effect of copper levels in swine manure on corn and soil. J. Environ. Qual. 12:198-203.

22. Viets, F.G., Jr. 1962. Chemistry and availability of micronutrients in soils. Agricultural and Food Chemistry. 10:174-178.

Chapter V

SUMMARY AND CONCLUSIONS

The research was performed to evaluate Cu or Cu and Zn from either sulfate sources or Cu-enriched pig manure. The two field studies investigated the effects of soil application of Cu and Zn, either alone or together, on corn. A laboratory incubation study was undertaken to evaluate Cu relations and reversion in a variety of Virginia soils. The results from the three studies conducted should have a wide range of applicability due to the diverse nature of the selected soils.

Application of 638 mt ha^{-1} Cu-enriched pig manure containing 198 mg Cu kg^{-1} did not induce Cu toxicity in corn. The Cu-enriched manure treatments supplied much of the N, P, and K requirements for high yields of corn. Grain yields either were not affected or were slightly increased by the Cu-enriched pig manure. In general, application of Cu, from either $CuSO_4$ or Cu-enriched pig manure, slightly increased blade Cu concentrations but did not affect Cu levels in the grain. Continued field studies under these controlled conditions is needed to accumulate information on acceptable Cu levels in agricultural soils. This information is not currently available to agencies determining acceptable Cu loading levels in soils.

The DTPA extractant was effective at separating Cu and Zn treatment levels but was not related to Cu concentrations in the aerial portions of corn. However, DTPA was an effective predictor of Zn in the aerial portions of corn. Laboratory studies indicated that more Cu was extracted by $AlCl_3$ in 0.5M HCl than EDTA which extracted more than DTPA. By increasing the pH of 15 soils through the addition of Cu-enriched pig manure this Cu extraction order was altered such that EDTA > DTPA > $AlCl_3$ in 0.5M HCl. Based on the laboratory studies, EDTA should be similar to DTPA in separating Cu treatment levels.

Laboratory incubation of 15 Virginia soils indicated that 22 kg Cu ha^{-1}, as $CuSO_4$, was acidifying and slightly increased soluble salt levels. Addition of a high rate of Cu-enriched pig manure (975 mt ha^{-1} wet manure) created reducing conditions, buffered the soils at a pH of 8.4, and increased the soluble salt concentrations 200 S m^{-1}. Despite the very different effects of the two Cu sources, extractable Cu, as measure by three extractants, decreased with time. Consequently, the availability of applied Cu would be expected to decrease with time.

Additions of Cu and Zn sulfates for 17 years to a cumulative total of 280 kg Cu and 560 kg Zn ha^{-1}, respectively, did not adversely effect corn growth on a Davidson soil. The 17 annual additions of Cu and Zn, either alone or together, did not cause any grain or stalk yield decreases. Copper concentrations in the blades and grain were not related to soil additions of Cu or Zn. However, Zn concentrations in the blades and grain were directly related to each other, r=0.87**, to soil Zn treatment levels, and to DTPA Zn. This experiment needs to be continued to determine the levels at which Cu and Zn will prove toxic to corn.

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