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AVAILABILITY AND DISTRIBUTION OF COPPER IN
SOILS FOLLOWING EIGHT ANNUAL APPLICATIONS OF
$CuSO_4$ OR Cu-ENRICHED SWINE MANURE

by

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(ABSTRACT)

Three field studies were established in 1978 and
continued through 1985 in order to determine the effects of
long-term Cu applications, as either CuSO$_4$ or Cu-enriched
swine manure, at high application rates on corn (Zea mays
L.) production. The studies were conducted on Bertie fine
sandy loam (Aquic Hapludult), Guernsey silt loam (Aquic
Hapludalf), and Starr clay loam (Fluventic
Dystrochrept)-Dyke clay (Typic Rhodudult) soils in the
Coastal Plain, Ridge and Valley, and Piedmont physiographic
regions of Virginia, respectively. A laboratory incubation
study was also conducted using the same soils to determine
the effects of soil pH on the changes in forms of soil Cu
present over a 256-day period following addition of CuSO$_4$ or
Cu-enriched swine manure.

Application of 336 kg Cu ha^{-1}, as CuSO$_4$, or up to 264
kg Cu, as Cu-enriched swine manure, from 1978 through 1985

had no adverse affects on corn grain yields or on Cu concentrations in corn ear leaves or grain. Copper concentrations in ear leaf tissue were increased only slightly (≤ 2.1 mg kg^{-1}) by the eight annual Cu additions with no increase in Cu content of grain being observed. Copper concentrations in both ear leaves and grain remained within normal levels.

DTPA extractable Cu was linearly related to the cumulative amount of Cu applied to the three soils ($r = 0.93$ to 0.98, $\alpha = 0.01$). Extractable soil Cu showed no relationship with Cu levels in grain or leaf tissue. Analysis of subsoil samples indicated little downward movement of the applied Cu due to leaching. Fractionation of the Cu in the three soils indicated increases in all fractions resulting from Cu treatments.

Results of the laboratory incubation study showed that the distribution of applied Cu among soil fractions was dependent on soil pH, length of time following Cu additions, and to some degree on the source of applied Cu. Levels of Ex-Cu and HOAc-Cu decreased with time and increasing pH. No consistent differences in Pyro-Cu were observed due to pH or length of incubation. Concentrations of Ox-Cu parallelled the levels of Fe and Mn in the soils and generally increased with time. Soils at low pH tended to contain less Ox-Cu than soils at higher pH levels.

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CHAPTER I

INTRODUCTION

Copper is an essential element required in small quantities for the normal development and growth of both animals and plants. However, at higher concentrations Cu, like other heavy metals, can be toxic. In view of the greater disposal of sewage sludge and animal manures on agricultural land, the potential environmental hazards associated with the accumulation of heavy metals in soils is increasingly becoming the subject of concern. The amount of Cu that can be safely added to soils can not be accurately predicted due to the lack of knowledge about the factors controlling the mobility and plant availability of applied Cu. This dissertation represents the product of two related research projects designed to evaluate the environmental impacts of long-term Cu applications as $CuSO_4$ or Cu-enriched swine manure and to add to the understanding of the chemical processes controlling the distribution of Cu in soils. Each research project is presented as a self contained chapter, the specific objectives of which are as follows:

   a. Chapter 2. To evaluate the effects of long-term applications of Cu as either Cu-enriched swine manure or $CuSO_4$ on corn growth and composition, and

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to determine the form and mobility of Cu in field soils following eight annual applications of Cu.

b. Chapter 3. To determine the changes in the forms of Cu present in soils following applications of Cu, as either $CuSO_4$ or Cu-enriched swine manure, as influenced by soil pH.

LITERATURE REVIEW

Copper has played an important role in human civilization since its use in crude hammered artifacts of the stone age. In more recent times, Cu has been used for a wide range of purposes due to its chemical qualities, ductility, malleability, and conductive properties.

Copper in Soils

The average concentration of Cu in the earth's crust is 55 mg kg^{-1}, while the concentrations found in the Ap horizons of most soils range from 10 to 80 mg kg^{-1} (42). The concentrations of Cu in soils generally reflect the Cu content of the soil forming minerals with relatively uniform distribution of Cu through the soil profile (60). Minor redistribution of soil Cu can occur through weathering and nutrient cycling by plants (30). In highly acidic or reducing environments, Cu exhibits greater mobility and is therefore more subject to movement within the soil profile than it is in oxidizing environments at higher pH levels (30, 60). Mafic rocks can contain appreciable concentrations of Cu, up to 100 mg Cu kg^{-1}, presumably associated with the high percentage of ferro-magnesian minerals and sulfides in these rocks. The form of Cu in these minerals is uncertain but may involve either subsitution of Cu for Fe^{2+}, Mn^{2+}, and Mg^{2+} in the crystal lattices or the inclusion of micro-crystals of CuS (42).

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Copper released by weathering processes may be adsorbed on soil components or precipitated as CuS and is subsequently incorporated into sedimentary rocks. Copper is generally excluded from carbonate rocks due to the inability of Cu to substitute into the carbonate structure.

The most common primary minerals containing Cu are very insoluble simple and complex sulfides, covelline (CuS), chalcocite (Cu_2S), and chalcopyrite ($CuFeS_2$), with strong covalent bonds formed between reduced Cu (Cu^+) and sulfide (S^{2-}) anions. Significant amounts of Cu may also be found in moderately soluble hydroxycarbonates (malachite, $Cu_2(OH)_2CO_3$ and azurite, $Cu_3(OH)_2(CO_3)_2$), silicates (chrysocolla, $CuSiO_3$), oxides (tenorite, CuO), and secondary minerals. In highly reduced environments, substantial levels of Cu may also exist in the metallic form (42).

In addition to the Cu released from native soil minerals, Cu commonly enters agricultural soils from a variety of sources including industrial emissions (71), sewage sludge (16, 36, 63, 67), pesticides (71), fertilizers (58), and animal manures (4, 19, 59). Copper has the ability to react with a wide variety of soil constituents and can therefore exist in a number of forms: i) ionic and complexed forms in the soil solution, ii) on exchange sites, iii) on specific adsorption sites, iv) occluded in Al, Fe, and Mn oxides and hydroxides, v) in biological residues, and vi) in the lattice structure of primary and secondary clay

minerals (48, 52). The distribution and availability of Cu to plants is controlled by the physical and chemical properties of the soil such as type and amount of clay, amount of organic matter, CEC, pH, redox potential, and concentration of sesquioxides as well as the concentration of Cu present (4, 38).

Only very small quantities of Cu generally exist in the soil solution at any one time due to strong adsorption of Cu by organic matter (61, 68), clay minerals (23, 25, 39), and Al, Fe, and Mn oxides (33, 34). Several researchers (32, 53) have observed Cu to be adsorbed by soils and soil components in excess of their CEC with much of the adsorbed Cu not being displaceable by neutral salts. These specifically adsorbed Cu ions are held more strongly than those held by simple electrostatic forces due to the formation of covalent bonds via O atoms (e.g. Cu-O-Al or Cu-O-Fe bonds) or OH groups to the structural cations of clay minerals and sesquioxides (1, 38).

Adsorption isotherms determined by McLaren and Crawford (54) indicate that clay minerals have a low affinity for Cu compared to amorphous oxides and organic matter. This difference among soil components with respect to their ability to adsorb Cu probably reflects the concentration and nature of functional groups present and the available surface area of the various constituents. In addition, Jenne (34) points out that due to the predominance of

organic and oxide coatings in most soils and sediments there are few "clean" clay surfaces that are in contact with the soil solution containing Cu which therefore limits the direct influence of clay minerals on Cu immobilization. The coatings, however, can be highly active in Cu adsorption. Organic and oxide coatings can also act to decrease the availability of Cu by occluding the Cu adsorbed on clay and oxide surfaces. Occlusion of adsorbed Cu has been shown to be especially important in environments with widely fluctuating redox conditions (1, 33, 34).

The adsorption of Cu by sesquioxides and clay minerals has been shown to be pH dependent (17, 25, 50, 53, 64). McLaren and Crawford (53) found increased adsorption of Cu by soils and individual soil components from a solution containing 5 μg ml^{-1} as pH was increased. Kishk and Hasson (39) had similar results and attributed the increases in adsorption to an increase in pH dependent charge and less competition with Al^{3+} and H^{+} ions for adsorption sites at higher pH levels.

Increasing pH results in a greater hydrolysis of the Cu to hydroxy-Cu forms. According to Farrah and Pickering (23) clay surfaces show an apparent preference for Cu-hydroxy, $Cu(OH)^{+}$, ions resulting in a strong pH dependent selectivity. As well, $Cu_2(OH)_2^{2+}$ and $Cu(OH)_2$ have been proposed as possible preferentially adsorbed species (3, 55). Electron spin resonance studies of Cu adsorbed on clay

minerals performed by McBride (47) and Clementz et al. (18) supports the hypothesis of preferential adsorption of Cu hydrolysis species. They showed that at low pH levels Cu was adsorbed as the $Cu(H_2O)_6^{2+}$ ion which tumbled freely at interlayer and external surface positions and could therefore be displaced easily by other cations. As the pH is increased, the layer silicate apparently promotes hydrolysis of Cu^{2+} and the Cu becomes much less exchangable.

The importance of organic matter in the retention of Cu by soils has been known for many years. In most soils, organically bound Cu represents the major reserve of potentially available Cu (48, 52, 61, 74). McBride (48) indicates that organic solids in soils act to hold Cu^{2+} in a kinetically available but thermodynamically stable (insoluble) form. Organic matter forms highly stable complexes with Cu by direct bonding of Cu^{2+} ions to two or more functional groups (primarily carboxylic, carbonyl, and phenolic) (9, 10, 68). The high stability of these Cu-organic complexes arises, in part, from the unique ability of Cu to form rigid inner-sphere complexes with soil humic substances over a wide pH range (9, 10). At low pH levels, other divalent transition metals (Mn^{2+}, Fe^{2+}, Co^{2+}, and Ni^{2+}) are bound by outer-sphere complexation with the metals retaining their inner hydration sphere (9, 10). However, as the pH increases the ability of these metals to form inner-sphere complexes is enhanced.

Despite the high affinity for metals and ubiquitous occurrence of organic matter, Jenne (34) has argued that the importance of organics in the retention of Cu in soils and sediments is over emphasized. He points out that in reducing environments organic matter may exercise a dominant influence in heavy metal adsorption, while in highly oxidized soils, hydrous oxides of Fe and Mn may be of primary importance due to their wide spread occurrence as coatings on clay minerals and disproportionately large surface areas.

Due to the high affinity of soil humic substances for Cu, a major portion of the Cu in soil solution exists as soluble organic complexes (17, 37, 49, 50, 68). At high pH levels, organic complexation of soluble Cu may be nearly complete due to the increased solubility of organics (17, 31, 49). The bioavailability of these Cu-organic complexes depends on the molecular weight of the complex and the concentration of Cu. Because of their lower molecular weight and greater concentration of acidic functional groups which adsorb Cu, metal complexes with fulvic acids generally exhibit a greater availability and mobility compared with humic acid complexes (68). In natural soils, the mobility of Cu-organic complexes can be reduced by adsorption of the complexes by clay minerals (1, 68). Stevenson and Fitch (68) summarized the effects of the formation of Cu-organic matter complexes on Cu availability and mobility as follows:

i) Copper concentrations in soils solution can be decreased through the formation of insoluble complexes with humic substances or complexation with organics adsorbed on clay minerals; ii) at high pH levels, complexation can serve to maintain Cu in soluble forms; iii) in Cu amended soils where large amounts of Cu are present, complexation may reduce the concentration of Cu^{2+} to nontoxic levels; and iv) natural complexing agents may be involved in the mobility and transport of Cu through the soil profile.

Copper in Plants

The presence of Cu in plant tissues was first demonstrated in the early 1800's, however, not until the 1930's was the essentiality of Cu clearly established using nutrient solution cultures (66). Copper is required in very small amounts for normal plant growth and is therefore considered one of the seven essential micronutrients. Concentrations of Cu from 5 to 20 mg kg^{-1} in plant tissues is adequate for the normal growth of most plants (35, 45, 75), while concentrations of less than 4 mg kg^{-1} are considered deficient and those above 20 mg kg^{-1} are generally thought to be toxic. Jones and Eck (35) reported a critical Cu concentration of 5 mg kg^{-1} for corn (Zea mays L.) ear leaves at the early silk stage of growth and a sufficiency range of 1 to 5 mg Cu kg^{-1} for corn grain at plant maturity.

In plants, Cu is a vital part of many important enzymes and proteins. Copper containing enzymes have been implicated in a number of physiological processes including carbohydrate metabolism (photosynthesis, respiration, and carbohydrate distribution), N metabolism (N_2 fixation and protein synthesis and degradation), cell wall metabolism (lignin synthesis), water relations, seed production (especially pollen viability), and disease resistance (15).

Absorption rates of Cu by plant roots are among the lowest for the essential elements (27, 45). The exact absorption process is yet to be understood but evidence suggests that it is active in nature. Graham (27) indicates that at normal Cu concentrations a linear relationship exists between absorption rate and external Cu concentration, while at higher concentrations the relationship is curvilinear. Much of the Cu adsorbed by plants is held in immobile forms in the roots through complexation by cell wall proteins and other insoluble proteins (27, 75). This immobilization of Cu results in little translocation of the adsorbed Cu and has been proposed as one of the mechanisms which allow many plant species to tolerate high concentrations of Cu in soils (27, 45, 75). The lack of correlation between Cu concentrations in plant leaf tissue and extractable soil Cu, which is commonly observed on Cu amended soils, is also attributable to the limited amount of Cu translocation from plant roots.

Translocation of Cu within plants reportedly involves complexation of Cu^{2+} by certain amino acids or other soluble proteins in the xylem and phloem saps (45, 75). Once complexed, Cu is able to move freely in the translocatory streams. The rate of Cu movement in plants is controlled by both the adsorption processes occurring in the roots and leaves and the concentration of soluble proteins which facilitate the translocatory process. Differences in levels of certain amino acids in the phloem and xyloem saps has been proposed as a possible explanation for the observed genotypic variations in the ability to translocate Cu (45).

In soils which received repeated Cu applications, the concentration of Cu will eventually reach toxic levels. Due to the general lack of knowledge about the chemistry of the applied Cu in soils and the inability to estimate plant absorption of Cu from Cu amended soils, the amount of applied Cu that will be toxic to plants cannot be accurately predicted. The U.S.E.P.A. currently bases its guidelines for the land disposal of heavy metal-rich waste on soil CEC and recommends that the pH of the soil be maintained at 6.5 or above to minimize the solubility of the added metals (72). These guidelines have yet to be tested adequately with long-term experiments under field conditions.

Copper in Swine Production

Copper is an essential element for the growth and health of all animals but can be toxic at higher levels. In swine, Cu is an essential part of a number of metalloenzymes which are known to be involved in hemoglobin synthesis; red blood cell maturation and maintenance; development and integrity of the skeletal, cardiovascular, and central nervous systems; pigmentation; reproduction; and function of the immune system (56). Most scientists (12, 56) consider dietary Cu levels from 5 to 10 mg Cu kg^{-1} to be adequate to prevent Cu deficiency in swine.

In 1945, Braude (11) first reported that swine apparently exhibited a craving for Cu in excess of their minimum dietary requirements after observing that pigs continously licked Cu rings located in their pens. In a subsequent study, Braude demonstrated that this craving was specific for Cu by placing plates of six different metals (Al, brass, Cu, Mg, Ni, and Tn) within easy access of confined pigs (12). He found that only the Cu and brass (which contains Cu) plates were licked by the pigs. The importance of these observations was not understood until 1955 when claims of improved growth in pigs resulting from the addition of $CuSO_4$ at a rate of 250 mg kg^{-1} to normal swine rations appeared in the literature (5, 6). Since then, the response of swine to Cu-supplemented diets has been studied extensively (7, 12, 13, 14, 20, 62, 76). In a

review of the available literature, Braude (12) reported that an average increase in daily live weight gain of 8.1% and a 5.4% increase in the efficiency of feed utilization resulted from the addition of 250 mg Cu kg^{-1} to the diets of growing swine. The actual response to the Cu-supplemented diet has been shown to be dependent on a number of factors including dietary levels of other nutrients (Fe, Mg, and Zn), protein source, and growth stage of the pigs (12, 20 , 28, 29, 56).

Prior to the introduction of Cu-supplemented diets in swine production, antibiotics were widely used as feed additives to improve swine performance. Therefore, a number of researchers (12, 13, 14, 46) conducted studies to compare the effectiveness of $CuSO_4$ with that of antibiotics. The majority of these studies indicated that the response to $CuSO_4$ was equal or superior to that produced by antibiotics. Research has also shown that under some conditions there may be an additive effect when $CuSO_4$ and antibiotics are given together (12, 46, 56).

The mechanism responsible for the stimulatory effect of Cu has yet to be determined but several theories have been proposed. Most of the suggested mechanisms involve changes in the microbial populations in the digestive tract of the pig caused by the high Cu levels. The most common mechanisms proposed include: i) regulation of the populations of disease causing microbes in the small

intestine by the supplemental Cu, ii) increased reproduction of desirable bacteria in the gastointestinal tract, and iii) reaction of Cu with microbially produced toxins (HS and NH_4) in the pigs intestine leading to greater absorption and utilization of proteins and nutrients (12). Attempts to evaluate these hypotheses have yielded inconclusive results (12, 56, 65).

Land Application of Cu-Enriched Swine Manure

Copper supplementation of swine rations has been used as an economical alternative to the use of certain antibiotics to improve growth and efficiency of feed utilization in swine for the past three decades. In recent years, increasing opposition to the use of high levels of Cu in swine production has been voiced, primarily out of concern about the potential environmental hazards associated with the repeated application of the resulting Cu-rich manure on agricultural soils (4, 19). In order to prevent a build-up of excessive levels of Cu in the soil, many European countries now limit the amount of Cu which can be added to swine rations.

Since as much as 95% of the dietary Cu is subsequently excreted in the wastes (12, 56), the Cu concentration in manure collected from swine feed Cu-enriched diets can be as high or higher than that found in most sewage sludges (40, 73, 78). Copper concentrations in these manures generally

are in the range of 600 to 2370 mg Cu kg^{-1} dry matter (8, 41, 59) and, unlike most sewage sludges, much of the Cu in the manure is in readily soluble forms (57).

Relatively few long-term field studies have been conducted to determine the effects of Cu-enriched swine manure applications on plant growth and composition. Batey et al. (8) found that three annual applications of 12.2 kg Cu ha^{-1} as a Cu-enriched pig manure slurry to a perennial ryegrass-white clover pasture increased the Cu concentration in the herbage, but not to toxic levels. Much of the increase in Cu concentration was attributed to direct foliar contamination during application of the manure. Decreased yields were only observed when foliage was smothered by the applied manure. They further concluded that the greatest risk to grazing livestock occurs from direct ingestion of forage contaminated with the slurry. Similar conclusions were made by McGrath (51) after applying Cu-rich manure to a number of grass and clover pastures. Allaway (2) indicates that Cu phytotoxicity will generally inhibit plant growth before concentrations in plant tissues reach levels that would be toxic to most grazing animals.

Sutton (69), working on a Crosby silt loam, found that four annual applications of 15.3 kg Cu ha^{-1} resulted in neither the loss of corn grain yield nor increased Cu concentrations in plant tissues. Application of 102.9 kg Cu ha^{-1} as Cu-rich pig manure over a three year period on a

Groseclose silt loam resulted in the doubling of the Cu
levels in corn roots and slight increases in the ear leaves,
but did not influence grain yield or Cu concentration (41).
Mullins et al. (59) reported similar results following
repeated applications of Cu, as either $CuSO_4$ or Cu-enriched
swine manure, to three Virginia soils with a wide range of
physical and chemical properties. They observed slight
increases in the Cu concentrations in corn ear leaves and
grain with no decrease in grain yield resulting from three
Cu additions. After applying up to 138 kg Cu ha^{-1}, as Cu-
enriched manure or $CuSO_4$, to the same soils over a six year
period, there were still no adverse effects on corn grain
yield or composition (26). A number of researchers (8, 43,
70) have pointed out factors other than the elevated levels
of Cu that can restrict plant growth following continued
applications of manure at high rates. These factors include
smothering of plants when the manure is applied to growing
plants and build up of excessive levels of N, P, and soluble
salts in the soil.

Analyses of subsoil samples taken from sites where Cu-
enriched manure (8, 59, 69), sewage sludge (22, 78), or
soluble Cu salts (58, 59) have been surface applied indicate
that there is little downward movement of the applied Cu
through the soil profile over considerable periods of time.
This lack of leaching indicates the speed and strength in
which applied Cu is bound by various soil components

including clay minerals, Fe and Mn hydrous oxides, and organic matter (32, 33, 34, 38, 53, 68). Due to the rapid immobilization of the applied Cu in the soil, Cu contamination of ground water resources is generally not considered a major threat in most soils until very high levels of Cu are added.

By performing soil Cu fractionation procedures before and after applications of Cu, as either $CuSO_4$ or Cu-enriched swine manure, Mullins (59) demonstrated that both plant available and unavailable fractions increased as a result of the Cu additions. These results agree well with reports that a portion of the applied Cu reverts to plant unavailable forms with time, probably due to the conversion to insoluble hydroxide and oxide forms (36, 41, 59).

Copper concentrations in soils which received repeated applications of Cu-rich wastes will eventually reach toxic levels. Using greenhouse pot experiments, Walsh et al. (77) found that $CuSO_4$ applied at a rate of 88 mg Cu kg^{-1} was sufficient to cause a reduction in the growth of bush beans. Lexmond (44) reported that a single application of 200 kg Cu ha^{-1}, as $CuSO_4$, was toxic to corn grown on a Loopodzolgrond soil with a 1 $\underline{M}$ KCl pH of 5.9. Levels of Cu in soils amended with either organic or inorganic Cu sources have been evaluated using a number of analytical procedures including EDTA (8), DTPA (24, 26, 58, 78), Mehlich-1 (40), 1 $\underline{M}$ HCl (69), and 4 $\underline{M}$ HNO_3 (78) extractions and total soil

digests (41). Results of these analyses have frequently indicated linear increases in extractable Cu with applied Cu. However, Cu extracted from soils to which large amounts of Cu have been added has not generally been closely correlated with Cu concentrations in plant tissues. This lack of correlation reflects limited translocation of absorbed Cu from the plant roots which has been demonstrated by several scientists (21, 41).

Due to the lack of knowledge about the chemistry of the applied Cu in soil and the inability to estimate plant absorption of the added Cu, the maximum safe Cu loading rates for soils can not be accurately predicted. The U.S.E.P.A. currently bases its guidelines for the land application of heavy metal rich wastes on soil CEC and recommends that the pH of the soil be maintained at 6.5 or above to minimize solubility of the added metals (72). These guidelines have yet to be tested adequately with long-term field experiments.

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CHAPTER II

CORN RESPONSE TO EIGHT ANNUAL APPLICATIONS OF Cu-ENRICHED SWINE MANURE TO THREE SOILS

ABSTRACT

The use of high levels of Cu in swine (Sus scrofa domesticus L.) production to stimulate growth and improve feed efficiency is presently being questioned due to the potential environmental hazards associated with the repeated disposal of the resulting Cu-rich manure on agricultural land. Three field studies were established in 1978 and continued through 1985 to determine the effects of long-term applications of Cu, as either $CuSO_4$ or Cu-enriched swine manure, at high rates on corn (Zea mays L.) production. The studies were conducted on Bertie fine sandy loam (Aquic Hapludult), Guernsey silt loam (Aquic Hapludalf), and Starr clay loam (Fluventic Dystrochrept)-Dyke clay (Typic Rhodudult) soils in the Coastal Plain, Ridge and Valley, and Piedmont physiographic regions of Virginia, respectively, and having CEC levels ranging from 5.0 to 12.3 cmol(+) kg^{-1}. Based on this range of CEC values and pH levels above 6.5, the U.S.E.P.A. predicts the maximum safe Cu loading rate for these soils to be 280 kg Cu ha^{-1}. After eight years, 890.4 mt ha^{-1} of wet Cu-enriched manure had been applied to these soils. The manure contained up to 1550 mg Cu kg^{-1} and was

produced by pigs fed diets containing an average of 250 mg
Cu kg^{-1}. Application of 336 kg Cu ha^{-1} as $CuSO_4$ or up to
264 kg Cu ha^{-1} as Cu-rich manure from 1978 through 1985 had
no adverse effects on corn grain yield or on Cu
concentrations in corn ear leaves or grain. Copper
concentrations in ear leaf tissue were increased by 2.1 mg
kg^{-1} or less by the eight annual Cu additions with no
increase in Cu content of grain being observed in either
1984 or 1985. The Cu levels in all plant tissue remained
within normal ranges. Diethylenetriaminepenta-acetic acid
(DTPA) extractable Cu was linearly related to the amount of
Cu applied to the three soils with correlation coefficients
ranging from 0.93 to 0.98 (α = 0.01). Extractable Cu showed
no relationship to Cu levels in grain or leaf tissue. Small
increases, up to 1.6 mg Cu kg^{-1}, in extractable Cu in the B
horizons of the Bertie and Starr-Dyke soils indicate little
downward movement of the applied Cu. Larger increases in
extractable Cu were observed in the Guernsey subsoil and are
probably attributable to downward movement caused by deep
plowing. Fractionation of the Cu in the three soils shows
increases in all fractions resulting from Cu treatments.
This indicates that a substantial portion of the applied Cu
has reverted to forms not available to plants.

Additional index words: <u>Zea</u> <u>mays</u> L., DTPA extractable Cu, soil Cu fractionation, micronutrient concentrations, manure disposal.

INTRODUCTION

Supplementing the diets of growing swine (<u>Sus scrofa</u> <u>domesticus</u> L.) with from 125 to 250 mg Cu kg^{-1} has been shown to stimulate growth and to increase feed efficiency (1, 5, 9, 16). Feeding swine Cu-enriched rations has become a common practice in many European countries (2, 5) and appears to have originated as an economical alternative to the use of certain antibiotics as growth stimulants or as a disease control measure (2, 6). The use of high levels of Cu in swine production has received considerable opposition in the United States due to the concern about the potential environmental hazards associated with the repeated disposal of the resulting Cu-rich manure on agricultural land (1, 7, 9). Since as much as 95% of the dietary Cu is subsequently excreted in the wastes (31), the Cu concentration in the manure collected from swine fed Cu-enriched diets can be as high or higher than that found in most sewage sludges (23, 43, 47). Copper concentrations in these manures generally are in the range of 600-2370 mg Cu kg^{-1} dry matter (3, 24, 35) and, unlike most sewage sludges, much of the Cu in the manure is in readily soluble forms (33).

Relatively few long-term field studies have been conducted to determine the effects of Cu-enriched swine manure applications on plant growth and composition. Batey et al. (3) found that three annual applications of 12.2 kg

Cu ha^{-1} as a Cu-enriched pig manure slurry to a perennial
ryegrass-white clover pasture increased the Cu concentration
in the herbage, but not to toxic levels. Much of the
increase in Cu concentration was attributed to direct foliar
contamination during application of the manure. Decreased
yields were only observed when foliage was smothered by the
applied manure. They further concluded that the greatest
risk to grazing livestock occurs from the direct ingestion
of forage contaminated with the slurry. Similar conclusions
were made by McGrath (27) after applying Cu-rich manure to a
number of grass and clover pastures. Sutton (39), working
on a Crosby silt loam, found that four annual applications
of 15.3 kg Cu ha^{-1} resulted in neither the loss of corn
grain yield nor increased Cu concentrations in plant
tissues. Application of 102.9 kg Cu ha^{-1} as Cu-rich pig
manure over a three-year period on a Groseclose silt loam
resulted in the doubling of the Cu levels in corn roots and
slight increases in the ear leaves, but did not influence
grain yield or Cu concentration (24). Mullins et al. (35)
reported similar results following repeated applications of
Cu as either $CuSO_4$ or Cu-enriched swine manure to three
Virginia soils with a wide range of physical and chemical
properties. They observed slight increases in the Cu
concentrations in corn ear leaves and grain with no decrease
in grain yield resulting from three Cu additions. After
applying up to 138 kg Cu ha^{-1} as $CuSO_4$ or Cu-rich manure to

the same soils over a six-year period, there were still no adverse effects on corn grain yield or composition (14). A number of researchers (3, 25, 40) have pointed out factors other than the elevated levels of Cu that can restrict plant growth following continued applications of manure at high rates. These factors include smothering of plants when the manure is applied to growing plants and building up of excessive levels of N, P, and soluble salts in the soil.

Levels of Cu in soils amended with either organic or inorganic Cu sources have been evaluated using a number of analytical procedures including EDTA (3), DTPA (13, 34, 35, 47), Mehlich-1 (23), 1 $\underline{M}$ HCl (39), and 4 $\underline{M}$ HNO_3 (47) extractions and total soil digests (24). Results of these analyses have frequently indicated linear increases in extractable Cu with applied Cu. However, Cu extracted from soils to which large amounts of Cu have been added has not generally been closely correlated with Cu concentrations in plant tissues. This lack of correlation reflects limited translocation of absorbed Cu from the plant roots which has been demonstrated by several scientists (11, 24).

Analyses of subsoil samples taken from sites where Cu-enriched manure (3, 34, 39), sewage sludge (12, 47), or soluble Cu salts (34, 35) have been surface applied indicate that there is little downward movement of the applied Cu through the soil profile over considerable periods of time. This lack of leaching indicates the speed and strength in

which applied Cu is bound by various soil components including clay minerals, Fe and Mn oxides, and organic matter (18, 30).

By performing soil Cu fractionation procedures before and after applications of Cu as either $CuSO_4$ or Cu-enriched swine manure Mullins (35) demonstrated that both plant available and unavailable fractions increased as a result of the Cu additions. These results agree well with reports that the applied Cu reverts to plant unavailable forms with time, probably due to the conversion to insoluble hydroxide and oxide forms (20, 24, 34).

Copper concentrations in soils that received repeated applications of Cu-rich wastes will eventually reach toxic levels. Using greenhouse pot experiments, Walsh et al. (45) found that $CuSO_4$ applied at a rate of 88 mg Cu kg^{-1} was sufficient to cause a reduction in the growth of bush beans. Lexmond (26) reported that a single application of 200 kg Cu ha^{-1}, as $CuSO_4$, was toxic to corn grown on a Loopodzolgrond soil with a 1 $\underline{M}$ KCl pH of 5.9. Due to the lack of knowledge about the chemistry of the applied Cu in soil and the inability to estimate plant absorption of the added Cu, the maximum safe Cu loading rates for soils can not be accurately predicted. The U.S.E.P.A. currently bases its guidelines for the land application of heavy metal rich wastes on soil CEC and recommends that the pH of the soil be maintained at 6.5 or above to minimize solubility of the

added metals (42). These guidelines have yet to be tested adequately with long-term field experiments.

The need for more information concerning the environmental impacts of land application of organic wastes having elevated heavy metal contents has been pointed out by scientists working in waste management (21). In order to supply some of the needed experimental data, this long-term field study was established in 1978 to evaluate the potential environmental hazards associated with the repeated application of Cu as either $CuSO_4$ or Cu-enriched swine manure at high rates on agricultural land. The experimental results obtained for the period through 1983 have been reported previously (14, 35). The specific objectives of this study for the period 1984-1985 were: 1) to evaluate the effects of long-term applications of Cu-enriched swine manure and $CuSO_4$ on corn growth and composition, 2) to determine the form and availability of Cu present in soils following eight annual applications of Cu, and 3) to evaluate the DTPA soil test procedure for use in predicting Cu absorption by plants grown on Cu amended soils.

MATERIALS AND METHODS

Field experiments were initiated in 1978 on three soils in Virginia having diverse physical and chemical properties selected to ensure wide applicability of the experimental results. The soils used were a Bertie fine sandy loam (fine-loamy, mixed, thermic Aquic Hapludult), a Guernsey silt loam (fine, mixed, mesic Aquic Hapludalf), and a Starr clay loam (fine-loamy, mixed, thermic Fluventic Dystrochrept)-Dyke clay (clayey, mixed, mesic Typic Rhodudult) complex located in the Coastal Plain, Ridge and Valley, and Piedmont physiographic regions of Virginia, respectively. The soils ranged in CEC from 5.0 to 12.3 cmol $(+)$ kg^{-1} (Table 1) which indicates all three soils would have a maximum safe Cu loading rate of 280 kg ha^{-1} as prescribed by the U.S.E.P.A (42).

Field Experimentation

The treatments imposed at each experimental site are described below. Treatment 1 acted as a control and received no Cu applications. Treatment 2 received 33.6 mt Cu-enriched swine manure ha^{-1} year^{-1} from 1978 through 1982, no copper application in 1983, and in 1984 Cu as $CuSO_4$ was added at a rate sufficient to bring the total amount of applied Cu to the maximum safe Cu loading rate (280 kg ha^{-1}) for these soils with an additional 56 kg Cu ha^{-1} as $CuSO_4$ being applied in 1985. Treatment 3 received $CuSO_4$

Table 1. Soil properties prior to the initiation of field experiments in 1978.

Location	Soil Series	Soil Texture	Soil pH	Organic Matter	Cation Exchange Capacity
				g kg^{-1}	cmol(+) kg^{-1}
Blacksburg-Ridge & Valley	Guernsey	silt loam	5.7	18	10.4
Holland-Coastal Plain	Bertie	fine sandy loam	6.2	17	5.0
Orange-Piedmont	Starr-Dyke complex	clay loam	5.9	14	12.3

applications each year to equal the Cu in the manure and $CuSO_4$ applied to treatment 2. Copper-enriched swine manure was applied to treatment 4 at the rates of 67.2 mt ha^{-1} $year^{-1}$ from 1978 through 1980, 134.4 mt ha^{-1} $year^{-1}$ in 1981 and 1982, 168 mt ha^{-1} $year^{-1}$ in 1983 and 1984, and 84 mt ha^{-1} in 1985. Copper, as $CuSO_4$, was added to treatment 5 at rates equivalent to the Cu supplied in the manure applied to treatment 4. The treatments were arranged in a randomized complete block design with four replications at each location.

The nutrient requirements of the plants grown during this study were met by applications of NH_4NO_3, KCl, triple superphosphate, $Na_2B_4O_7$, and $MgSO_4$ at rates recommended based on the results of annual soil and plant analyses (Table 2). Estimated amounts of nutrients supplied by manure applications were subtracted from the recommended fertilizer application rates. All plots received additional Zn applications as $ZnSO_4$, and the plots located on the Bertie fine sandy loam also received Mn applications as $MnSO_4$. The soil pH at all locations was maintained at or above 6.5 by applications of dolomitic limestone. All amendments were incorporated into the soil by disking and plowing prior to planting. During the 1984 growing season, corn (Zea mays L.), 'Pioneer 3192', was grown at populations of 54530, 49400, and 55030 plants ha^{-1} at the Orange, Holland, and Blacksburg locations, respectively. Corn

Table 2. Cumulative amounts of amendments applied to the five treatments in field experiments conducted from 1978 through 1985*.

| Treatment | | Macronutrients | | | Micronutrients | | | | Hog |
No.	Description	K	N	P	B	Cu	Mn	Zn	Manure
		----------------kg ha^{-1}--------------							mt ha^{-1}
					Blacksburg location				
1	control	1128	1736	265	5.5	-----	28	90	-----
2	manure + CuSO$_4$	745	1186	147	5.5	287.7	28	90	168.0
3	Cu equal to no. 2	1128	1736	265	5.5	336.0	28	90	----
4	high manure rate	93	----	110	5.5	-----	28	90	890.4
5	Cu equal to no. 4	1128	1736	265	5.5	264.0	28	90	-----
					Holland location				
1	control	1115	1568	220	4.4	-----	140	90	-----
2	manure + CuSO$_4$	692	1143	98	4.4	286.3	140	90	168.0
3	Cu equal to no. 2	1115	1568	220	4.4	336.0	140	90	-----
4	high manure rate	----	34	---	4.4	-----	140	90	890.4
5	Cu equal to no. 4	1115	1568	220	4.4	255.5	140	90	-----
					Orange location				
1	control	1393	1764	427	4.4	-----	28	73	-----
2	manure + CuSO$_4$	888	1212	172	4.4	288.0	28	73	168.0
3	Cu equal to no. 2	1393	1764	427	4.4	336.0	28	73	-----
4	high manure rate	----	293	---	4.4	-----	28	73	890.4
5	Cu equal to no. 4	1393	1764	427	4.4	253.0	28	73	-----

*Inorganic sources of nutrients were muriate of potash, ammonium nitrate, triple superphosphate, sodium borate, and copper and zinc sulfates.

populations were 61650, 57360, and 51380 plants ha^{-1} at these locations in 1985. Weed control was achieved through the use of chemical herbicides during both 1984 and 1985.

Manure Collection and Analysis

The manure used during this study was collected from sows and gilts fed diets containing approximately 250 mg Cu kg^{-1} as $CuSO_4$ while confined in protected pens on a concrete slab. Manure collection was started following a three-day period in which the swine were equilibrated on the Cu-enriched rations. After the equilibration period, the manure was collected daily and placed in plastic cans for transport to the experimental sites. After manure collection was completed, samples were taken from the manure to be used at each location. The Cu-enriched feed used to produce the manure was also sampled. Triplicate subsamples of each manure and feed sample were dried at 105°C for 24 hours to determine percent dry matter. The dried samples were ground to 0.84 mm size using a stainless steel Wiley mill. One gram subsamples were then ashed at 450°C for two hours and the ash dissolved in 25 ml of 0.5 $\underline{M}$ HCl, and the mixture filtered prior to analysis for Ca, Cu, Fe, K, Mg, Mn, and Zn by atomic absorption spectrophotometry. Nitrogen concentration in the feed and manure was determined by a micro-Kjeldahl procedure (28). Phosphorus was analyzed by a molybdivanadophosphoric acid procedure described by Kitson

and Mellon (22) and B by the Azomethine-H procedure of
Parker and Gardner (36).

Soil Sampling and Analysis

Each spring, prior to application of treatments and
fertilizers, soil samples consisting of 10 cores per plot
were taken from the 0-15 cm depth. In 1985, samples were
also taken from the top 15 cm of the B horizon at each
location. Soil samples were air-dried and crushed to pass a
2 mm sieve prior to being analyzed. Samples were extracted
using the Mehlich-1 (0.05 $\underline{M}$ HCl and 0.0125 $\underline{M}$ H_2SO_4)
procedure (8). The extracts were analyzed for Ca, K, Mg,
Mn, and P. Extractable soil Cu and Zn were determined by
the DTPA (diethylenetriaminepenta-acetic acid) extraction
procedure (8). Extractable soil Zn was also determined by
the EDTA (ethylenediaminetetra-acetic acid) extraction
procedure described by Trierweiler and Lindsay (41). Soil
pH was determined in water with a 1:1 soil:solution ratio
after a one-hour equilibration period. Soil organic matter
was determined by a chromic acid oxidation procedure and
particle size distribution by the pipette method. Cation
exchange capacity was evaluated by saturation with 0.5 $\underline{M}$ Ca
acetate (pH 7.0) followed by displacement with neutral 0.5 $\underline{M}$
Mg acetate.

The forms of Cu present in the 1985 surface soil
samples were evaluated using a fractionation procedure
modified from that given by McLaren and Crawford (29). The

solution and exchangeable, specifically absorbed, organically bound, oxide occluded, and residual Cu fractions were extracted using the procedure described below.

1) Solution and exchangeable Cu (Ex-Cu): Five grams of soil were weighed into a 100-mL plastic centrifuge tube to which 50 mL of 0.05 $\underline{M}$ $CaCl_2$ were added. The suspension was then shaken at 180 cycles min^{-1} in a 30°C water bath for 24 h.

2) Specifically adsorbed Cu (HOAc-Cu): The residue from the 0.05 $\underline{M}$ $CaCl_2$ extraction was shaken with 50 mL of 2.5% acetic acid for 24 h in a 30°C water bath (17).

3) Organically bound Cu (Pyro-Cu): Copper adsorbed by soil organic matter was determined by shaking a new 0.5 g subsample with 50 mL of 0.1 $\underline{M}$ $K_4P_2O_7$ for 16 h at 30°C. Pyro-Cu was calculated by subtracting the Ex-Cu and HOAc-Cu fractions from that extracted by the 0.1 $\underline{M}$ $K_4P_2O_7$.

4) Oxide occluded Cu (Ox-Cu): The residue from the potassium pyrophosphate extraction was washed with 15 mL of deionized water. The residue was then transferred into a 50-mL beaker and evaporated to dryness in a 85°C oven. Twenty-five milliliters of a solution containing 0.1 $\underline{M}$ oxalic acid and 0.175 $\underline{M}$ ammonium oxalate (pH = 3.25) were added to the dried residue. After being covered with plastic, the samples were placed in an 85°C water bath and exposed to ultraviolet light for two h (32).

5) Residual Cu (Res-Cu): Total soil Cu was determined by dissolving a 0.5 g sample with an Aqua regia-HF mixture in a Parr digestion bomb as described by Bernas (4). Res-Cu was calculated by subtracting the sum of the Cu in the other fractions from the amount of total soil Cu. All extracts from the fractionation procedure were analyzed for Cu by atomic absorption spectrophotometry.

Plant Tissue Sampling and Analysis

At the early silk stage of growth, 10 ear leaves were taken from the two outside rows in each of the four row plots. Corn grain was harvested from 6.1 m of the center two rows at plant maturity with grain yields being adjusted to 155 g moisture kg^{-1}.

In preparation for analysis, all plant tissue was dried at 70°C and ground in a stainless steel Wiley mill to pass a 0.84 mm sieve. Elemental analysis of the leaf samples was accomplished using the procedures described previously for the analysis of the manure and feed. One-half gram subsamples of the grain were digested in a HNO_3-$HClO_4$ mixture prior to determination of Cu and Zn concentrations by atomic absorption spectrophotometry.

Statistical Analysis

All data were evaluated statistically by analysis of variance and LSD mean separation procedures using a 0.05 level of probability. The relationships between DTPA

extractable Cu, the cumulative amounts of Cu applied from 1978 through 1985, and Cu concentrations in plant tissues were evaluated using simple linear regression analyses.

RESULTS AND DISCUSSION

Analysis showed that the Cu concentration in the swine feed used to produce the Cu-enriched swine manure used in the three field studies ranged from 178 to 300 mg kg^{-1} with an average of 251 mg Cu kg^{-1} for the period 1978-1985 (Table 3). The Cu concentrations in the feed were generally in the upper portion of the range considered to have growth stimulating effects (5, 31). Zinc, which is commonly added to Cu-enriched rations to lessen the risk of Cu toxicity in the swine (10, 15, 31), averaged 65 mg kg^{-1} in the feed during the eight year period. The swine manure used in the field experiments contained a considerable amount of plant nutrients (Table 4). The average composition of the manure was 19 mg B kg^{-1}, 241 mg Mn kg^{-1}, 311 mg Zn kg^{-1}, 39 g Ca kg^{-1}, 17 g K kg^{-1}, 7.9 g Mg kg^{-1}, 20 g P kg^{-1}, and 224 g solids kg^{-1}. The Cu concentration in the manure ranged from 899 to 1550 mg kg^{-1} and averaged 1310, 1292, and 1269 mg kg^{-1} for the Blacksburg, Holland, and Orange locations, respectively. The concentrations of Cu and solids in the manure are comparable with those in manure collected from other pigs fed similar Cu-enriched diets (3, 24).

A high fertility status was maintained on all soils as evidenced by the high levels of Mehlich-1 extractable nutrients in the soil samples taken in the spring of 1985 (Table 5) and the nutrient concentrations in ear leaf tissue

Table 3. Average nutrient concentrations in swine feed used
for production of Cu-enriched manure used in field
experiments over the eight-year period from 1978
through 1985.

Growing	Micronutrients				Macronutrients			
Season	B	Cu	Mn	Zn	Ca	K	Mg	P
	---------mg kg^{-1}--------				--------g kg^{-1}--------			
1978	10.2*	260.8	33.0	47.2	8.7	7.9	1.7	7.5
1979	11.1	178.1	23.0	38.7	8.6	7.1	1.5	5.1
1980	5.9	223.6	26.1	45.7	8.5	6.7	1.5	5.0
1981	12.7	281.4	40.2	68.0	9.8	8.1	---	6.3
1982	9.6	225.2	33.8	65.6	9.2	---	1.3	---
1983	9.5	283.5	61.0	71.2	8.9	7.5	1.7	4.7
1984	12.6	254.0	41.9	56.7	10.8	8.4	1.7	5.0
1985	9.3	300.8	39.0	129.2	11.0	6.0	1.5	8.1

*Each value represents the average elemental composition
on a dry matter basis from triplicate determinations.

Table 4. Nutrient concentrations and percent solids in
swine manure applied to three field experiments
over the eight-year period from 1978 through 1985.

Growing Season	Micronutrients				Macronutrients				solids
	B	Cu	Mn	Zn	Ca	K	Mg	P	
	-------mg kg^{-1}------				-----------g kg^{-1}----------				
Blacksburg location									
1978	16.6*	1398	222	160	34	14	83	18	252
1979	12.9	899	172	119	64	8	84	17	257
1980	17.9	1157	159	353	39	15	83	19	212
1981	24.1	1410	189	328	42	16	88	29	206
1982	17.2	1390	220	334	33	29	86	14	230
1983	15.5	1550	520	478	40	14	69	15	232
1984	28.1	1455	241	290	41	19	78	23	193
1985	27.5	1224	213	497	32	20	70	35	185
Holland location									
1978	18.4	1396	227	186	34	16	84	18	267
1979	16.8	1273	147	101	47	14	94	19	259
1980	16.0	1331	141	246	41	15	77	19	205
1981	23.5	1140	179	283	37	13	82	25	193
1982	17.7	1154	225	315	27	32	77	11	245
1983	12.8	1454	281	297	34	13	67	12	244
1984	22.0	1290	283	365	44	16	81	24	208
1985	21.3	1296	234	556	32	17	72	27	198
Orange location									
1978	15.1	1313	200	170	30	14	75	17	267
1979	16.9	1125	166	121	55	12	80	18	247
1980	18.9	1335	128	232	39	17	80	19	196
1981	19.5	1152	203	295	42	15	81	27	226
1982	19.3	1273	232	363	30	36	81	14	220
1983	14.3	1375	418	446	34	14	66	15	227
1984	25.7	1425	268	317	43	18	87	25	208
1985	27.0	1150	251	606	30	19	65	25	189

*Each value represents the average of elemental composition
on a dry matter basis from triplicate determinations.

Table 5. Effect of seven annual applications of Cu as either $CuSO_4$ or Cu-enriched swine manure on extractable nutrients, pH, and organic matter in three soils sampled in the spring of 1985.

| Cu Treatment | | Soil pH | Soil Organic Matter | Mehlich-1 Ext. Nutrients | | | | | EDTA ext. |
Amount	Source			Ca	K	Mg	P	Mn	Zn
kg ha^{-1}			g kg^{-1}	--------------mg kg^{-1}--------------					
			Guernsey silt loam						
0	control	6.7a*	20cd	828	155	>120	53	>16.1	>6.1
280	manure+CuSO$_4$	6.6a	21bc	945	>157	>120	>60	>16.1	>6.1
280	CuSO$_4$	6.6a	19d	735	155	>120	55	>16.1	>6.1
245	manure	6.5a	24a	>1200	>157	>120	>60	>16.1	>6.1
245	CuSO$_4$	6.7a	22ab	855	155	>120	56	>16.1	5.8
			Bertie fine sandy loam						
0	control	6.2a*	21b	606bc	73b	88b	>60	4.8b	5.5a
280	manure+CuSO$_4$	6.2a	20b	705b	72b	96b	55	5.4b	6.1a
280	CuSO$_4$	6.2a	21b	576c	74b	82b	>60	4.3b	5.8a
234	manure	6.3a	26a	1143a	116a	117a	>60	8.1a	6.1a
234	CuSO$_4$	6.1a	21b	591c	70b	83b	>60	4.3b	6.1a
			Starr-Dyke clay loam						
0	control	6.6a	16b	849	145	>120	18	>16.1	6.0
280	manure+CuSO$_4$	6.7a	17b	1032	141	>120	30	>16.1	>6.1
280	CuSO$_4$	6.6a	16b	849	132	>120	15	>16.1	5.9
235	manure	6.7a	23a	>1200	>157	>120	>60	>16.1	>6.1
235	CuSO$_4$	6.7a	16b	1008	131	>120	16	>16.1	5.8

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

in both 1984 (Table 6) and 1985 (Table 7). Nutrient concentrations in the ear leaf tissue were within accepted sufficiency ranges (19). Ear leaf composition, except Cu concentration, was not related to Cu application. Concentrations of P, Mn, and Zn were related to manure applications, however. Leaf tissue from plots that received the high levels of manure tended to contain higher concentrations of P and Mn and lower concentrations of Zn (Tables 6 and 7). Other researchers (39, 46) have also reported elevated P levels in corn leaves following application of swine wastes and indicated that a build up of excessive quantities of N and P represents one of the hazards associated with repeated applications of large amounts of manure.

The observed increases in Mn concentrations on the Guernsey and Starr-Dyke soils (Tables 6 and 7) were probably due to the Mn applied in the manure and the reduction of insoluble soil Mn to plant available forms. Limited amounts of available Mn in the Bertie soil (Table 5) made supplemental Mn applications necessary. The lower levels of Zn in corn leaves from soils that received manure applications indicates a greater adsorption of Zn by these soils and more probably a dilution effect resulting from the greater vegetative growth by plants on the manure treated soils which was noted at the time the samples were taken. Enhanced corn growth resulting from manure applications is

Table 6. Effect of seven annual applications of Cu as either $CuSO_4$ or Cu-enriched swine manure on nutrient concentrations in ear leaves of corn grown on three soils.

Cu Treatment		Macronutrients					Micronutrients		
Amount	Source	Ca	K	Mg	N	P	Cu	Mn	Zn
kg ha^{-1}		--------------g kg^{-1}-----------					----mg kg^{-1}---		
		Guernsey silt loam							
0	control	3.6c*	21a	1.9a	30.6a	3.0b	8.9c	47b	34a
280	manure+CuSO$_4$	4.3ab	20ab	1.8a	30.8a	3.1b	10.4ab	49b	31a
280	CuSO$_4$	4.2ab	20ab	1.9a	33.2a	3.1b	10.7a	50b	35a
245	manure	4.4a	20ab	1.9a	32.0a	3.6a	10.1b	68a	25b
245	CuSO$_4$	3.9bc	19b	2.0a	32.4a	3.1b	10.7a	52b	32a
		Bertie fine sandy loam							
0	control	4.7b	21ab	2.6a	27.2a	3.2bc	6.5c	18ab	41a
280	manure+CuSO$_4$	5.2a	22a	2.6a	27.2a	3.4ab	7.1bc	14b	32bc
280	CuSO$_4$	4.7b	19b	2.7a	27.6a	3.0c	7.6b	23a	37ab
234	manure	4.5b	23a	2.0b	29.2a	3.8a	7.6b	23a	26c
234	CuSO$_4$	4.5b	22a	2.6a	27.7a	3.3bc	8.6a	23a	41a
		Starr-Dyke clay loam							
0	control	4.8a	18a	1.9a	32.0ab	2.6b	10.5ab	69b	26a
280	manure+CuSO$_4$	5.0a	17a	2.1a	30.3b	2.5b	10.5ab	72b	21b
280	CuSO$_4$	4.6a	17a	1.9a	31.8ab	2.5b	10.9ab	70b	25a
235	manure	4.9a	18a	2.0a	32.5a	3.4a	10.2b	87a	19b
235	CuSO$_4$	5.0a	17a	2.0a	30.1b	2.6b	11.1a	74b	26a

*Nutrient concentrations reported on a dry matter basis. Column means for each soil followed by different letters are significantly different at the 0.05 probability level.

Table 7. Effect of eight annual applications of Cu as either $CuSO_4$ or
Cu-enriched swine manure on nutrient concentrations in ear
leaves of corn grown on three soils.

Cu Treatment		Macronutrients					Micronutrients		
Amount	Source	Ca	K	Mg	N	P	Cu	Mn	Zn
kg ha^{-1}		---------------g kg^{-1}-----------					----mg kg^{-1}----		
		Guernsey silt loam							
0	control	4.1b*	22a	4.9a	31ab	2.5bc	9.3b	62bc	45a
336	manure+CuSO$_4$	4.2b	22a	4.4a	31ab	2.6b	10.2ab	65b	42ab
336	CuSO$_4$	3.8b	23a	4.4a	29c	2.4c	9.5ab	54c	38b
264	manure	5.6a	22a	5.3a	33a	3.0a	10.7a	101a	41ab
264	CuSO$_4$	3.8b	22a	5.0a	30bc	2.5bc	10.5a	64bc	39ab
		Bertie fine sandy loam							
0	control	4.1a	20a	5.5ab	29a	2.7ab	9.1a	25a	45a
336	manure+CuSO$_4$	4.6a	20a	5.2ab	30a	2.9a	9.9a	26a	50a
336	CuSO$_4$	4.4a	20a	5.7a	30a	2.6b	10.0a	26a	44a
256	manure	4.4a	20a	4.9b	31a	3.0a	10.1a	23a	37b
256	CuSO$_4$	4.2a	20a	5.2ab	30a	2.7ab	9.2a	28a	48a
		Starr-Dyke clay loam							
0	control	5.7a	22a	6.0a	27a	2.3b	8.8a	78b	25a
336	manure+CuSO$_4$	6.0a	22a	5.8ab	28a	2.4b	9.8a	84b	24a
336	CuSO$_4$	6.0a	23a	5.5ab	28a	2.3b	9.3a	85b	26a
253	manure	6.3a	23a	5.2b	29a	2.9a	9.2a	101a	17b
253	CuSO$_4$	6.2a	22a	6.0a	28a	2.5b	9.0a	88b	25a

*Nutrient concentrations reported on a dry matter basis. Column means
for each soil followed by different letters are significantly different
at the 0.05 probability level.

frequently observed and is generally attributed to improved soil physical properties and moisture relations (40). Increases in organic matter content ranging from 4 to 7 g kg^{-1} were observed in this study following seven annual applications of the Cu-rich manure (Table 5) and are probably responsible for such improvements in the physical properties and water relations in these soils. The N concentrations in the leaves from the manure treated soils were equal to those from the control plots (Tables 6 and 7) even though no inorganic sources of N had been applied to the manure amended soils for several years (Table 2).

Copper concentration in the ear leaf samples from the 1984 growing season were increased slightly by Cu applications on the Guernsey and Bertie soils, but not on the Starr-Dyke soil. The Cu concentrations in all leaf tissue were well within the accepted normal range of 6 to 20 mg kg^{-1} (19). The Cu levels in the leaves from the soils treated with the high rate of manure were below those found in tissue from soils to which an equivalent amount of Cu as $CuSO_4$ had been applied. This may represent a lower availability of Cu from the manure compared with that from the $CuSO_4$ or more probably a dilution effect as described previously.

In 1985, even though the total amount of applied Cu had reached 336 kg ha^{-1}, increases in Cu concentration in leaf tissue were observed only on the Guernsey soil treated with

245 kg Cu ha^{-1} as either $CuSO_4$ or Cu-enriched swine manure. No difference was observed between the sources of Cu with respect to Cu concentrations in ear leaves. These results are consistent with the finding that most of the Cu in the manure exists in a soluble form (33).

Copper concentration in corn grain was not increased by any Cu treatment on any of the three soils in either 1984 (Table 8) or 1985 (Table 9) despite the fact that the predicted maximum safe loading rate (280 kg Cu ha^{-1}) for these soils (42) had been exceeded by $CuSO_4$ additions and closely approached by application of Cu-enriched swine manure. Compared with the control treatment, the manure applications resulted in a decrease in the Cu concentration in the grain from the Bertie and Starr-Dyke soils in 1984. A similar reduction in Zn concentration in the grain from the manure treated Starr-Dyke soil was also observed. In 1985, the Cu concentration in the grain from the manure treated Bertie soil was lower than that in grain from plots that received an equivalent amount of Cu as $CuSO_4$ but was not different from that in the grain taken from the control plot. Manure application did not influence Zn levels in the 1985 grain samples. These results are consistent with the reports that Cu is accumulated in corn roots with little internal translocation. Similar small or negligible increases in Cu concentration in corn ear leaves and grain have been reported in a number of other studies following Cu

Table 8. Effect of seven annual applications of Cu as either $CuSO_4$ or Cu-enriched swine manure on concentrations of Cu and Zn in grain and on grain yields of corn grown on three soils.

Cu Treatment		Grain Micronutrient Concentration		Grain Yield
Amount	Source	Cu	Zn	
kg ha^{-1}		----mg kg^{-1}----		kg ha^{-1}
		Guernsey silt loam		
0	control	2.2a*	29.3a	10320b
280	manure + $CuSO_4$	2.1a	25.7a	9660b
280	$CuSO_4$	2.2a	26.0a	9320b
245	manure	1.9a	25.3a	11960a
245	$CuSO_4$	2.0a	23.2a	10280b
		Bertie fine sandy loam		
0	control	1.9a	24.8a	8950a
280	manure + $CuSO_4$	1.9a	24.9a	8830a
280	$CuSO_4$	1.9a	24.1a	9180a
234	manure	1.5b	21.5a	8710a
234	$CuSO_4$	1.9a	23.2a	9100a
		Starr-Dyke clay loam		
0	control	1.7a	18.8a	11340b
280	manure + $CuSO_4$	1.5ab	16.0ab	12680a
280	$CuSO_4$	1.6ab	16.1ab	10900b
235	manure	1.3b	13.7b	12440a
235	$CuSO_4$	1.7a	18.3a	11830ab

*Nutrient concentrations reported on a dry weight basis. Column means for each soil followed by different letters are significantly different at the 0.05 probability level.

Table 9. Effect of eight annual applications of Cu as either $CuSO_4$ or Cu-enriched swine manure on concentrations of Cu and Zn in grain and on grain yields of corn grown on three soils.

Cu Treatment		Grain Micronutrient Concentration		Grain Yield
Amount	Source	Cu	Zn	
kg ha^{-1}		----mg kg^{-1}----		kg ha^{-1}
Guernsey silt loam				
0	control	2.8a*	27.5a	10230a
336	manure + $CuSO_4$	2.9a	26.2a	10080a
336	$CuSO_4$	3.2a	23.4a	9760a
264	manure	3.0a	24.7a	10710a
264	$CuSO_4$	3.0a	25.3a	10570a
Bertie fine sandy loam				
0	control	3.2ab	31.2ab	9860ab
336	manure + $CuSO_4$	3.7a	32.2a	8850ab
336	$CuSO_4$	3.7a	28.9ab	8220b
256	manure	3.0b	23.0b	9540ab
256	$CuSO_4$	3.6ab	29.3ab	10150a
Starr-Dyke clay loam				
0	control	3.7a	27.4a	7910a
336	manure + $CuSO_4$	3.7a	24.0a	7600a
336	$CuSO_4$	3.6a	25.0a	7370a
253	manure	3.5a	25.3a	9100a
253	$CuSO_4$	3.6a	26.0a	7980a

*Nutrient concentrations reported on a dry weight basis. Column means for each soil followed by different letters are significantly different at the 0.05 probability level.

additions as Cu-enriched pig manure (24), sewage sludge (20, 37, 38), and $CuSO_4$ (34).

Corn grain yields were not adversely affected in 1984 (Table 8) nor in 1985 (Table 9) by repeated applications of Cu as either $CuSO_4$ or Cu-rich manure. In 1984, grain yields were actually increased on the Guernsey silt loam and Starr-Dyke clay loam soils that had received 890 mt of Cu-enriched manure ha^{-1} since 1978 (Table 8). Yield was also increased on the Starr-Dyke soil treated with a lower amount of manure plus $CuSO_4$. These increases can probably be attributed to improvements in the general tilth and water relations of these soils caused by the manure. Yields were not significantly decreased, as compared with the control, by any Cu-treatment on any of the three soils in 1985 (Table 9). The levels of applied Cu in both 1984 and 1985 were well above the 200 kg Cu ha^{-1}, as $CuSO_4$, shown to decrease corn yields on an European soil (26). The levels of applied Cu were below those found to be toxic to bush beans in a greenhouse pot study (45).

The concentrations of DTPA extractable Cu in the three soils used in the study exhibited a strong linear relationship with amount of added Cu (Tables 10 and 11). Correlation coefficients determined through simple linear regression analyses using both the 1984 and 1985 soil test results and assuming no difference in extractability between the two sources of Cu were 0.93, 0.96, and 0.98 ($\alpha = 0.01$)

Table 10. DTPA extractable Cu and Zn in the Ap horizon of three soils following six annual applications of Cu as either $CuSO_4$ or Cu-enriched swine manure.

Cu Treatment		DTPA Extractable	
Amount	Source	Cu	Zn
kg ha^{-1}		----mg kg^{-1}----	
Guernsey silt loam			
0	control	1.9c*	4.9b
48	manure	9.1bc	6.9b
60	$CuSO_4$	12.0b	5.3b
198	manure	38.4a	13.4a
198	$CuSO_4$	40.7a	5.4b
Bertie fine sandy loam			
0	control	0.7c	3.9b
50	manure	4.2bc	4.5b
62	$CuSO_4$	6.8b	2.5c
193	manure	18.5a	7.7a
193	$CuSO_4$	18.9a	3.2b
Starr-Dyke clay loam			
0	control	2.0d	2.3c
48	manure	8.6c	4.5b
59	$CuSO_4$	9.1c	2.5c
185	manure	34.0a	11.1a
185	$CuSO_4$	26.9b	2.6c

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

Table 11. DTPA extractable Cu from the Ap and B horizons
of soils following seven annual applications of
Cu as either $CuSO_4$ or Cu-enriched swine manure.

Cu Treatment		Extractable Cu	
Amount	Source	Ap horizon	B horizon
kg ha^{-1}		--------mg kg^{-1}-------	
Guernsey silt loam			
0	control	1.6b*	0.5c
280	manure + $CuSO_4$	29.9a	4.8bc
280	$CuSO_4$	35.9a	6.0ab
245	manure	37.5a	11.0a
245	$CuSO_4$	31.2a	8.1ab
Bertie fine sandy loam			
0	control	1.3d	0.3c
280	manure + $CuSO_4$	49.9a	1.9a
280	$CuSO_4$	36.8b	0.7bc
234	manure	28.4bc	0.9b
234	$CuSO_4$	23.8c	1.0b
Starr-Dyke clay loam			
0	control	2.6b	0.7b
280	manure + $CuSO_4$	37.1a	1.9a
280	$CuSO_4$	32.6a	1.6ab
235	manure	37.5a	1.5ab
235	$CuSO_4$	30.8a	1.2ab

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

for the Guernsey, Bertie, and Starr-Dyke soils, respectively. The assumption of equal extractability between sources of Cu is substantiated by the data in Tables 6 and 7. These results are consistent with other reports of linear changes in DTPA extractable Cu following Cu additions (13, 14). Soils that received the high rate of manure also showed higher levels of extractable Zn in 1984 (Table 10) and in 1985 (Table 12). The amounts of DTPA extractable Cu in the three soils were not significantly correlated (α = 0.05) to the Cu concentrations in either corn ear leaves or grain sampled during the previous year. Since the soil samples were taken prior to application of treatments, no attempt was made to correlate the extractable Cu in the soil samples with the Cu concentrations in the plants grown during the same year. Similar results were reported during the early stages of this study with lower levels of applied Cu on these soils (14, 35).

Analysis of the subsoil samples taken in the spring of 1985 showed that Cu applications caused only slight increases in the Cu concentrations in the Bertie and Starr-Dyke subsoils (Table 11). No differences in subsoil Cu concentration were observed between Cu sources. These results indicate little downward movement of the applied Cu. Downward movement appears to be the greatest in the Bertie compared with that in the Starr-Dyke soil. The Bertie soil contains lower concentrations of clay and free Fe and Mn

Table 12. DTPA extractable Zn from the Ap and B horizons of soils following seven annual applications of Cu as either $CuSO_4$ or Cu-enriched swine manure.

Cu Treatment		Extractable Zn	
Amount	Source	Ap horizon	B horizon
$kg\ ha^{-1}$		\-\-\-\-\-\-\-mg kg^{-1}\-\-\-\-\-\-\-	
Guernsey silt loam			
0	control	7.0b*	1.6b
280	manure + $CuSO_4$	9.3b	2.5b
280	$CuSO_4$	7.9b	1.6b
245	manure	17.3a	4.4a
245	$CuSO_4$	5.9b	1.8b
Bertie fine sandy loam			
0	control	6.1b	0.3bc
280	manure + $CuSO_4$	8.1b	0.5a
280	$CuSO_4$	5.6b	0.2c
234	manure	15.2a	0.4ab
234	$CuSO_4$	6.4b	0.3bc
Starr-Dyke clay loam			
0	control	5.1bc	0.4b
280	manure + $CuSO_4$	6.7b	0.6ab
280	$CuSO_4$	5.8bc	0.5ab
234	manure	17.2a	0.8a
234	$CuSO_4$	4.7c	0.4b

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

oxides which results in less adsorption of Cu (18, 30) and therefore greater leaching of the applied Cu.

In view of the minor downward movement of the applied Cu in the coarser textured Bertie soil, it appears likely that the large increases in the levels of extractable Cu and Zn observed for the Guernsey soil do not represent downward movement due to leaching. Instead, the increases in extractable Cu and Zn can probably be attributed to movement through deep plowing at the Blacksburg location.

The soil samples taken from the Ap horizon of the three soils in the spring of 1985 were fractionated to determine the forms of Cu present in the soils following seven annual applications of $CuSO_4$ and Cu-rich manure. Results of the procedure show increases in all fractions caused by Cu additions (Table 13). The magnitude of the changes was dependent on the amount of applied Cu and, to some extent, on the source of Cu. Only minor increases in Ex-Cu were found in any of the three soils. The greatest increase in exchangeable Cu was in the relatively coarse textured Bertie soil as would be expected due to the lack of clay and oxides which are known to bind Cu in soils. Higher amounts of exchangeable Cu were found in the Guernsey and Starr-Dyke soils treated with manure compared with the same soils treated with an equivalent amount of Cu as $CuSO_4$. This may reflect chelation of the Cu by soluble organics in soils treated with manure. The Cu in the acetic acid extractable

Table 13. Distribution of soil Cu fractions in the Ap horizon of three soils following seven annual Cu applications as either $CuSO_4$ or Cu-enriched swine manure.

Cu Treatment		Soil Cu fractions				
Amount	Source	EX-Cu	HOAC-Cu	PYRO-Cu	OX-Cu	RES-Cu
kg ha^{-1}		------------------mg kg^{-1}------------------				
		Guernsey silt loam				
0	control	0.04c*	0.23c	4.2b	5.4c	7.8b
280	manure + $CuSO_4$	0.08b	14.54a	43.4a	32.8a	7.6b
280	$CuSO_4$	0.08b	14.78a	40.6a	34.8a	9.6ab
245	manure	0.11a	11.54b	41.9a	29.3b	11.2a
245	$CuSO_4$	0.07b	10.75b	41.6a	33.0a	13.2a
		Bertie fine sandy loam				
0	control	0.04c	0.33d	2.8d	1.8c	5.4d
280	manure + $CuSO_4$	0.19a	25.71a	39.3a	29.2a	8.6c
280	$CuSO_4$	0.16a	19.09b	27.2b	20.5b	9.5c
234	manure	0.08b	9.88c	27.7b	28.1a	11.6b
234	$CuSO_4$	0.09b	13.22bc	19.2c	20.9b	14.7a
		Starr-Dyke clay loam				
0	control	0.03b	0.87d	6.4e	13.2e	32.5b
280	manure + $CuSO_4$	0.04ab	20.72a	46.7b	50.1b	43.8a
280	$CuSO_4$	0.03b	14.60b	40.0c	62.9a	34.8b
235	manure	0.05a	11.82c	51.3a	41.8d	35.7b
235	$CuSO_4$	0.03b	11.20c	33.8d	46.1c	44.7a

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

fraction tended to follow the level of applied Cu with no consistent difference between Cu sources. The K-pyrophosphate extractable fraction showed the greatest increases due to Cu treatment. A greater amount of Pyro-Cu was found in the manure treated soils compared with the soils treated with $CuSO_4$. The oxalate fraction also showed large increases which followed the level of applied Cu. Changes in the level of residual Cu were also observed. The largest changes were generally found in soils to which the lower level of cumulative Cu had been applied. This may be due to the fact that a larger portion of the Cu had been added during the earlier stages of this study allowing for a longer reaction time.

Viets (44) concluded that the forms of Cu most available to plants were the Ex-Cu, HOAc-Cu, and Pyro-Cu fractions which are in equilibrium with the soil solution. This conclusion is supported by another study conducted by McLaren and Crawford (29) who also indicate that the Ox-Cu and Res-Cu fractions do not generally represent a major source of Cu to plants. Therefore, both plant available and plant unavailable forms of Cu have been increased by the applications of Cu (Table 13). Copper concentrations in neither the corn ear leaves (Table 6) nor grain (Table 8) were related to the observed changes in the potentially available forms of Cu (Table 13), which may reflect Cu

accumulation in plant roots rather than translocation to shoots.

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CHAPTER III

DISTRIBUTION OF COPPER IN THREE SOILS AS INFLUENCED BY SOIL REACTION AND Cu-ENRICHED SWINE MANURE OR $CuSO_4$ ADDITION

ABSTRACT

The fate of heavy metals applied to soils is controlled by a number of factors including soil pH. A laboratory incubation study was conducted to determine the effects of soil pH on the distribution of Cu in three soils during a 256 day period following addition of 50 mg Cu kg^{-1} as either $CuSO_4$ or Cu-enriched swine manure. The soils used were a Bertie fine sandy loam (fine-loamy, mixed, thermic Aquic Hapludult), a Guernsey silt loam (fine, mixed, mesic Aquic Hapludult), and a Dyke clay (clayey, mixed, mesic Typic Rhodudult) from the Coastal Plain, Ridge and Valley, and Piedmont physiographic regions of Virginia, respectively. Results of Cu fractionation procedures performed 8, 32, 64, 128, and 256 days after Cu applications indicate that the amount of Cu in all fractions was increased by Cu additions. Only a minute portion of the total soil Cu was found in the $CaCl_2$ extractable fraction at any time. The concentration of $CaCl_2$ extractable Cu in Cu amended soils decreased with time and increasing pH. At higher pH levels, greater amounts of exchangeable Cu were found in the manure treated

soils compared with those that received an equivalent amount of Cu as $CuSO_4$, but at low pH the opposite was true. The amount of $CaCl_2$ extractable Cu (exchangeable Cu) appeared to stabilize in all soils after reaching concentrations of 1 to 5 µg Cu kg^{-1}. Acetic acid extractable Cu (specifically adsorbed Cu) also decreased with time and increasing pH. Levels of organically bound Cu were greater in the manure treated soils than in the soils receiving $CuSO_4$. No consistent differences in organically adsorbed Cu were observed due to either pH or length of incubation. Concentrations of oxalate extractable Cu (occluded Cu), in both the control and Cu amended soils, paralleled the levels of Fe and Mn in the soils. There was a tendency for the soils that received Cu to contain less oxalate extractable Cu at low pH than at higher pH levels. The concentration of occluded Cu increased with length of incubation in the Bertie and Guernsey soils to which Cu was applied but remained unchanged in the Dyke soil.

Additional index words: Soil Cu fractionation, Cu adsorption, Cu complexation.

INTRODUCTION

The potential environmental hazards associated with the accumulation of heavy metals in soils is increasingly becoming the subject of concern. Copper commonly enters agricultural soils from a variety of sources including industrial emissions (32), sewage sludge (6, 14, 29, 31), pesticides (32), fertilizers (26), and animal manures (2, 3, 9, 27). Once in the soil, Cu can exist in a number of forms: i) ionic and complexed forms in soil solution; ii) on exchange sites; iii) on specific adsorption sites; iv) occluded in Al, Fe, and Mn oxides and hydroxides; v) in biological residues; and vi) in the lattice structure of primary and secondary minerals (18, 21, 33). The distribution and availability of Cu to plants is controlled by the physical and chemical properties of the soil such as type and amount of clay, amount of organic matter, CEC, pH, and concentration of sesquioxides as well as the concentration of Cu present (2, 15).

Only very small quantities of Cu generally exist in the soil solution at any one time due to strong adsorption of Cu by organic matter (15, 28), clay minerals (10, 11, 16), and Al, Fe, and Mn hydroxides and oxides (13, 22). The amount of Cu adsorbed by these soil components is dependent on soil pH (7, 11, 20, 22). McLaren and Crawford (22) found increased adsorption of Cu by soils and individual soil

components from a solution containing 5 µg Cu mL^{-1} as pH was increased. Kishk and Hasson (16) reported similar results and attributed the increases in adsorption to an increase in pH dependent charge and to less competition with Al^{3+} and H^+ ions for adsorption sites at higher pH levels.

Increasing pH results in a greater hydrolysis of the Cu to hydroxy-Cu forms. According to Farrah and Pickering (10) clay surfaces show an apparent preference for Cu-hydroxy, $Cu(OH)^+$, ions resulting in a strong pH dependent selectivity. Other Cu-hydroxy species as well, $Cu_2(OH)_2^{2+}$ (23) and $Cu(OH)_2$ (1), have been proposed as possible preferentially adsorbed species. Electron spin resonance studies of Cu adsorbed on clay minerals performed by McBride (17) and Clementz et al. (8) support the hypothesis of preferential adsorption of Cu hydrolysis species. They showed that, at low pH levels, Cu was adsorbed as the $Cu(H_2O)_6^{2+}$ ion which tumbled freely at interlayer and external surface positions and could therefore be displaced easily by other cations. As the pH is increased, layer silicates apparently promote hydrolysis of Cu^{2+} and the Cu becomes much less exchangeable.

Soil pH can also influence the degree to which Cu in the soil solution is complexed. Several researchers (7, 19, 20) have shown that increasing the pH of soil solution increased the amount of Cu found in soluble organic complexes. Increasing the pH of peat extracts to

approximately 6.0 resulted in almost complete organic
complexation of soluble Cu compared with only 60%
complexation at its natural pH of 3.5. The increased
complexation of Cu in soil solution probably results from
greater solubility of organics at higher pH levels (7, 19)
in addition to increased Cu hydrolysis and decreased
protonation of organic functional groups. Despite the
changes in organic binding of Cu, the total Cu concentration
in soil solution has been indicated to be relatively
insensitive to pH (19).

Sims and Patrick (30) evaluated the forms of Cu present
in a Typic Fluaquent following a 6-day incubation in
solutions with pH levels from 4.5 to 7.5 and redox
potentials varying from -150 to 500 mV. Results of the
fractionation procedure conducted on the soil after the
incubation showed only slight changes in the distribution of
Cu caused by pH adjustments. The largest changes occurred
in the water soluble Cu which increased with increasing pH.
They also observed that larger changes in the distribution
of Cu among soil fractions occurred as redox potential was
varied.

Relatively few studies have been conducted to evaluate
the influence of pH on the distribution of Cu in whole
soils, especially in Cu amended soils. This study was
conducted to determine the changes in the forms of Cu
present in three soils over a 256 day period following

applications of Cu as either $CuSO_4$ or Cu-enriched swine manure as influenced by soil reaction.

MATERIALS AND METHODS

The soils used in this study were the Ap horizons of a Bertie fine sandy loam (fine-loamy, mixed, thermic Aquic Hapludult), a Guernsey silt loam (fine, mixed, mesic Aquic Hapludalf), and a Dyke clay (clayey, mixed, mesic Typic Rhodudult) sampled from the border areas of field experiments evaluating the environmental impacts of long-term Cu-enriched swine manure applications to cropland (27). The initial properties of the three soils are given in Table 14. The Dyke series, from the Piedmont, is a clayey soil high in Fe and Mn; the Bertie series, from the Coastal Plain, is a sandy soil low in Fe and Mn; and the Guernsey series, from the Applachian Ridge and Valley is a soil with intermediate texture and Fe and Mn contents. The field capacity of the soils was determined by the pressure plate method at a tension of 33 kPa, CEC by Ca/Mg exchange at a pH of 7.0, and organic matter concentration by a dichromate oxidation procedure.

The manure used during this investigation was collected as a slurry containing 220 g solids kg^{-1} from pigs fed diets containing approximately 250 mg Cu kg^{-1}. The manure contained 1512.5 mg Cu kg^{-1} dry matter as determined by ashing a 1.0-g sample of the dried manure at 450°C followed by dissolution of the ash in 0.5 $\underline{M}$ HCl and determination of Cu concentration by atomic absorption spectrophotometry.

Table 14. Properties of three soils used in an incubation experiment.

Soil Series	Soil Texture	Organic Matter	Cation Exchange Capacity	H_2O at 33 kPa	Total	
					Fe	Mn
		$g\ kg^{-1}$	$cmol(+)\ kg^{-1}$	$kg\ kg^{-1}$	$-g\ kg^{-1}-$	
Guernsey	silt loam	18	10.4	0.40	19	1.7
Bertie	fine sandy loam	17	5.5	0.20	5	0.1
Dyke	clay	14	12.3	0.45	66	3.8

Incubation Experiment

Four pH levels, 3.5, 5.0, 6.5, and 8.0 as measured in water with a soil:solution ratio of 1:1, and three Cu treatments; a control, 50 mg Cu kg^{-1} as $CuSO_4$, and 50 mg Cu kg^{-1} as Cu-enriched swine manure were imposed in each soil. Soil reaction was adjusted to the appropriate pH level by application of $Ca(OH)_2$ or repeated additions of a small amount of a 0.1 $\underline{M}$ HCl solution followed by partial drying. After the pH treatments were completed the soils were equilibrated for approximately 21 days prior to applying Cu treatments. The appropriate amounts of a 0.04 $\underline{M}$ $CuSO_4$ solution or Cu-enriched swine manure were added to a 250 g subsample of each soil and mixed thoroughly. Samples were then brought to 'field capacity' (33 kPa tension) with deionized water and covered with plastic film. The treatments were arranged in a randomized complete block design with two replications within two incubation chambers. The incubation chambers were styrofoam chests maintained at a temperature of 30 ± 0.5°C using an aquarium heater submersed in a four liter container of water within the chambers. The bottom of the chests were filled with several cm of water to ensure a high humidity environment. The moisture contents of the samples were monitored throughout the incubation period and moisture contents readjusted to field capacity as needed. After 8, 32, 64, 128, and 256 days, three samples were taken from each soil. One sample

was dried at 105°C to determine moisture content. The remaining two samples were used in a fractionation procedure to evaluate the forms of Cu in the soils.

Fractionation Procedure

The fractionation scheme used was a modification of that given by McLaren and Crawford (21). The solution and exchangeable, specifically adsorbed, organically bound, and oxide occluded Cu fractions were extracted by the procedures described below.

1) Solution and exchangeable Cu (Ex-Cu): Five grams of soil were weighed into a 100-mL plastic centrifuge tube to which 50 mL of 0.05 $\underline{M}$ $CaCl_2$ were added. The suspension was then shaken at 180 cycles min^{-1} in a 30°C water bath for 24 h.

2) Specifically adsorbed Cu (HOAc-Cu): The residue from the 0.05 $\underline{M}$ $CaCl_2$ extraction was shaken with 50 mL of 2.5% acetic acid for 24 h in a 30°C water bath (12).

3) Organically bound Cu (Pyro-Cu): Copper adsorbed by soil organic matter was determined by shaking a new 0.5 g subsample with 50 mL of 0.1 $\underline{M}$ $K_4P_2O_7$ for 16 h at 30°C. Pyro-Cu was calculated by subtracting the Ex-Cu and HOAc-Cu fractions from that extracted by the 0.1 $\underline{M}$ $K_4P_2O_7$.

4) Oxide occluded Cu (Ox-Cu): The residue from the potassium pyrophosphate extraction was washed with 15 mL of deionized water. The residue was then transferred into a

50-mL beaker and evaporated to dryness in a 85°C oven. Twenty-five milliliters of a solution containing 0.1 $\underline{M}$ oxalic acid and 0.175 $\underline{M}$ ammonium oxalate (pH = 3.25) were added to the dried residue. After covering with plastic, the samples were placed in an 85°C water bath and exposed to UV light for two hours (24). All extracts from the fractionation procedure were analyzed for Cu by atomic absorption spectrophotometry.

Statistical Analysis

Copper fractionation data were evaluated by analysis of variance and LSD mean separation procedures at a 0.05 level of probability.

RESULTS AND DISCUSSION

Results of the fractionation procedures conducted over the 256 day incubation period indicate that the distribution of Cu in the three soils was influenced by both soil pH and length of incubation. Only very small quantities of Cu were found in the $CaCl_2$ fraction (Ex-Cu) at any sampling time (Table 15). This relationship demonstrates the speed in which the added Cu is adsorbed by various soil components. The amount of Ex-Cu in the Bertie soil was generally greater than that in the Guernsey or Dyke soils that received the same treatment. This reflects the smaller amounts of clay and Fe and Mn oxides in the Bertie soil which leads to less adsorption of the applied Cu. The level of Ex-Cu in all three soils was affected by pH, incubation time, and source of applied Cu (Table 15).

Decreasing pH below 6.0 resulted in an increase in Ex-Cu in Cu amended soils at all sampling times. The control soils were not influenced by pH except for the Dyke soil at pH 3.0. No differences were observed between the two highest pH levels in any soil. The increased levels of Ex-Cu can be attributed to less adsorption of the applied Cu by soil components as pH is decreased and possible dissolution of other forms of Cu at low pH levels. Researchers (1, 10, 16, 17, 23) have shown that the inhibition of Cu adsorption by soils, which is often observed at low pH, is probably

Table 15. Levels of $CaCl_2$ extractable Cu in three soils as influenced by soil pH, Cu addition, and length of incubation.

Treatment		Length of Incubation (days)					Row
Cu	pH	8	32	64	128	256	LSD.05
		----------μg kg^{-1}----------					
Bertie fine sandy loam							
control	3.5	9efg*	2e	2d	2e	1f	5
	5.0	7fg	1e	1d	2e	1f	3
	6.5	3g	2e	2d	1e	2ef	3
	8.0	5fg	1e	3d	2e	1f	3
manure	3.5	54c	25c	29b	19c	8cde	5
	5.0	35cd	18d	15c	15cd	12c	9
	6.5	28de	19cd	14c	11cd	9cd	1
	8.0	23ef	16d	14c	9d	5def	2
CuSO$_4$	3.5	202a	166a	156a	81a	62a	16
	5.0	123b	78b	37b	35b	32b	6
	6.5	9efg	3e	3d	3e	3ef	3
	8.0	8gf	2e	5d	3e	1f	3
Dyke clay							
control	3.5	25c	7c	1c	2c	2cde	4
	5.0	5fg	1d	3c	2c	2cde	3
	6.5	2g	1d	1c	2c	1e	4
	8.0	4g	2d	3c	2c	1e	4
manure	3.5	49b	17b	12b	10b	6bc	18
	5.0	23cd	8c	6c	5c	6bc	13
	6.5	17cde	6c	5c	5c	3cd	3
	8.0	14ef	6c	6c	3c	2cde	6
CuSO$_4$	3.5	195a	148a	68a	41a	18a	15
	5.0	10efg	18b	11b	9b	10b	7
	6.5	3g	1d	5c	3c	1e	4
	8.0	6fg	1d	3c	2c	1e	3
Guernsey silt loam							
control	3.5	18d	4c	8efg	5d	2f	3
	5.0	20d	5c	5g	4d	2f	12
	6.5	20d	1c	6g	4d	3ef	10
	8.0	18d	1c	5g	3d	2f	3
manure	3.5	49b	20bc	20b	17b	9bcd	5
	5.0	45bc	10bc	18bc	15b	11bc	.17
	6.5	41bc	12bc	13de	10c	8cd	1
	8.0	39c	9c	14cd	9c	7de	1
CuSO$_4$	3.5	234a	165a	65a	43a	29a	8
	5.0	44bc	29b	20b	17b	13b	10
	6.5	23d	5c	11def	9c	5def	5
	8.0	23d	5c	8efg	7cd	2f	8

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

due to less hydrolysis of the Cu and greater competition for adsorption sites by Al and H^+ ions.

At high pH levels there were greater amounts of Ex-Cu in soils treated with Cu-enriched swine manure as compared with that in soils that received an equivalent amount of Cu as $CuSO_4$, while at lower pH levels the opposite trend was true (Table 15). Miller et al. (25) found that the addition of Cu-enriched swine manure to these soils resulted in an immediate increase in soil pH which was attributed to the high initial pH of the manure, 7.8, and the consumption of protons during reduction reactions. Similar changes in pH and redox status probably contributed to the differences observed between the manure and $CuSO_4$ treated soils in this study. The differences may also reflect the greater organic binding of the applied Cu in the manure treated soils and the greater solubility of the Cu-organic complexes as the pH was increased (20).

The length of incubation also had a significant influence on the amount of Ex-Cu (Table 15). The levels of Ex-Cu in soils that received Cu decreased with time as would be expected as the adsorption processes continued. The control soils generally showed no further change after the 32 day sampling time. The relatively high amounts of Ca-Cu in the control soils at the 8 day time were probably caused by changes in the redox status of the soils as they were brought to field capacity. The amount of Ex-Cu in all soils

appeared to stabilize after reaching a concentration of 1 to 5 µg Cu kg^{-1}.

The level of acetic acid extractable Cu (HOAc-Cu) in the Cu amended soils was also affected by pH and incubation period (Table 16). The level of HOAc-Cu in the control soils was not influenced by pH and remained relatively constant over the entire 256 day period. With few exceptions, the concentrations of HOAc-Cu in soils that received Cu as either Cu-enriched swine manure or CuSO$_4$ decreased with time at all pH levels but remained much higher than those in the control soils throughout the study. The decrease in HOAc-Cu over time is consistent with reports that Cu applied to field soils reverts to plant unavailable forms with time (26, 27).

Concentration of HOAc-Cu also decreased as a result of increasing pH which was probably due to greater binding of Cu in less soluble forms at higher pH levels (Table 16). At lower soil reactions, soils amended with CuSO$_4$ contained greater quantities of Cu in the HOAc-Cu fraction than did the soils that received Cu as Cu-enriched manure. These differences probably result from the increased pH, decreased redox potential, and greater organic complexation of Cu caused by the manure additions.

Organically bound Cu (Pyro-Cu) increased in all soils by Cu additions as either CuSO$_4$ or Cu-enriched swine manure (Table 17). The manure treated soils tended to have a

Table 16. Levels of acetic acid extractable Cu in three
soils as influenced by soil pH, Cu addition, and
length of incubation.

Treatment		Length of Incubation (days)					Row
Cu	pH	8	32	64	128	256	LSD $_{.05}$
		---------------mg kg^{-1}-------------					
			Bertie fine sandy loam				
control	3.5	0.3e*	0.3e	0.3g	0.3e	0.3e	0.1
	5.0	0.2e	0.2e	0.2g	0.2e	0.2e	0.1
	6.5	0.2e	0.2e	0.2g	0.2e	0.2e	0.2
	8.0	0.2e	0.2e	0.2g	0.2e	0.3e	0.4
manure	3.5	11.7b	14.1a	7.9de	7.1c	6.4c	0.1
	5.0	9.0d	9.9cd	6.2ef	6.0cd	5.6cd	0.1
	6.5	8.7cd	8.2d	6.2ef	6.1cd	6.4c	0.1
	8.0	8.6cd	7.7d	5.5f	5.3d	5.3cd	0.1
CuSO$_4$	3.5	14.7a	13.6a	12.2a	11.8a	10.8a	2.7
	5.0	14.9a	13.4a	11.1ab	9.9b	9.6b	2.4
	6.5	12.6b	11.5bc	9.9bc	9.8b	9.5b	2.3
	8.0	10.7bc	12.0bc	9.0cd	9.0b	8.8b	2.4
			Dyke clay				
control	3.5	0.7e	0.7e	0.7e	0.7d	0.7f	0.1
	5.0	0.5e	0.5e	0.5e	0.5d	0.6f	0.8
	6.5	0.5e	0.5e	0.5e	0.5d	0.5f	0.8
	8.0	0.4e	0.4e	0.4e	0.5d	0.4f	0.5
manure	3.5	14.5a	14.5a	10.6b	9.1b	8.8c	0.1
	5.0	13.4b	13.4b	8.3cd	8.0b	7.3d	0.1
	6.5	11.1c	11.1c	8.1cd	7.9bc	7.7d	0.1
	8.0	10.2c	10.2c	7.8d	7.7bc	7.8d	0.1
CuSO$_4$	3.5	13.9b	13.9b	12.4a	12.0a	11.2a	1.4
	5.0	13.7b	13.7b	9.3bc	9.0b	10.1b	2.0
	6.5	10.9c	10.9c	8.4bc	7.4bc	6.9d	1.9
	8.0	8.8d	8.8d	7.4d	6.8c	5.2e	1.5
			Guernsey silt loam				
control	3.5	0.2e	0.2e	0.1f	0.1d	0.2d	0.2
	5.0	0.1e	0.1e	0.1f	0.1d	0.2d	0.3
	6.5	0.1e	0.1e	0.2f	0.1d	0.2d	0.3
	8.0	0.1e	0.1e	0.2f	0.1d	0.2d	0.4
manure	3.5	15.2ab	15.2ab	10.5bc	8.9b	7.8bc	0.1
	5.0	11.0cd	11.0cd	7.0e	6.8c	6.6c	0.1
	6.5	13.1bc	13.1bc	9.3cde	8.7b	7.7bc	0.1
	8.0	9.4d	9.4d	8.0de	7.6bc	6.5c	0.1
CuSO$_4$	3.5	15.3ab	15.3ab	13.4a	12.8a	11.1a	2.8
	5.0	16.5a	16.5a	11.6ab	11.0ab	10.3a	3.3
	6.5	12.9bc	12.9bc	10.1bcd	9.8b	9.0ab	2.6
	8.0	9.6d	9.6d	8.2cde	7.7bc	10.8a	3.2

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

Table 17. Levels of potassium pyrophosphate extractable Cu
in three soils as influenced by soil pH, Cu
addition, and length of incubation.

Treatment		Length of Incubation (days)					Row
Cu	pH	8	32	64	128	256	LSD$_{.05}$
		--------------mg kg^{-1}--------------					

Bertie fine sandy loam

control	3.5	4.8c*	3.0c	2.5d	2.8d	2.9d	0.6
	5.0	3.8c	2.7c	1.7d	2.0d	2.4d	2.1
	6.5	1.2c	2.8c	1.8d	2.1d	2.2d	3.0
	8.0	1.3c	3.1c	2.3d	2.1d	1.8d	2.1
manure	3.5	38.0a	30.6ab	31.7abc	30.5bc	37.2a	0.6
	5.0	34.8ab	31.6a	35.0ab	35.1ab	37.2a	3.3
	6.5	38.7a	36.0a	31.5abc	32.8bc	35.2a	0.4
	8.0	27.2b	35.0a	38.3a	39.4a	36.5a	1.1
CuSO$_4$	3.5	27.4b	29.6ab	27.9bc	25.1c	26.8c	3.8
	5.0	21.8b	24.4b	27.2c	26.4c	27.9bc	4.4
	6.5	27.8b	34.1a	27.4bc	27.1c	26.7c	6.9
	8.0	33.2ab	36.1a	40.3a	37.8a	32.9ab	6.0

Dyke clay

control	3.5	7.6fg	5.7e	5.3e	5.3e	5.5e	2.6
	5.0	5.4g	5.2e	4.7e	5.3e	5.5e	3.1
	6.5	5.4g	4.5e	4.5e	4.8e	5.0e	2.4
	8.0	3.0g	4.0e	4.7e	4.5e	4.9e	2.8
manure	3.5	53.3a	32.5ab	31.5ab	34.6b	37.0ab	0.7
	5.0	42.8b	29.3bc	33.8a	34.9b	39.9a	2.6
	6.5	42.3c	34.5a	33.8a	32.9bc	32.9bc	0.5
	8.0	34.8d	34.0a	32.8ab	31.8bc	28.6c	0.9
CuSO$_4$	3.5	39.7bc	32.5ab	30.1abc	39.1a	28.6c	3.5
	5.0	24.9e	22.8d	28.3bcd	29.7c	30.4c	0.8
	6.5	19.7e	28.5c	23.9dc	23.4d	30.4c	5.8
	8.0	18.8e	23.4d	26.2cd	25.6d	22.2d	5.5

Guernsey silt loam

control	3.5	2.2d	2.9d	2.4d	2.1d	2.0d	2.6
	5.0	2.9d	2.2d	2.5d	2.3d	2.0d	2.9
	6.5	2.5d	1.8d	2.8d	2.5d	1.8d	3.3
	8.0	2.5d	2.0d	2.6d	2.5d	1.8d	2.8
manure	3.5	39.2a	29.4a	29.1abc	30.0b	36.5a	0.9
	5.0	30.7b	26.8ab	30.7ab	31.9ab	34.5a	0.3
	6.5	29.8b	25.9abc	29.7ab	32.4ab	27.1abc	0.6
	8.0	36.1ab	23.9bc	35.3a	35.4a	30.9ab	1.6
CuSO$_4$	3.5	34.4b	25.9abc	26.6bc	28.9b	24.6abc	6.6
	5.0	29.3bc	24.9abc	27.bc	29.1b	26.7abc	5.0
	6.5	18.8c	26.8abc	24.7bc	23.4c	24.3abc	7.9
	8.0	26.6bc	22.2c	21.8c	20.4c	19.6bc	9.5

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

greater amount of Pyro-Cu compared with the CuSO$_4$ treated soils as could be expected due to the addition of organic matter. Because of large variations in the levels of Pyro-Cu observed between sampling times and pH treatments no consistent effect of either soil pH or incubation time could be discerned. Variations in the data may be due to fluctuations in redox status of the soils during the incubation period. Redox changes would not only influence the rate of organic matter oxidation, but the solubility of other forms of Cu as well. The ability of Cu to form strong inner sphere complexes with organic matter over a wide pH range (4, 5) also may have contributed to the lack of a consistent difference among pH treatments. This is supported by the work of Sims and Patrick (30) who found no difference in K-pyrophosphate extractable Cu in a Typic Fluvaquent over a pH range from 4.5 to 7.5 but showed that organically bound Cu decreased dramatically with increasing redox potential.

The concentration of oxalate extractable Cu (Ox-Cu) in both control and Cu amended soils generally followed the levels of total Fe and Mn present in the soils (Table 14) with the highest concentrations of Ox-Cu being found in the Dyke soil (Table 18). Few significant effects of pH on Ox-Cu occurred in any of the soils. However, at low pH levels, some of the soils contained smaller concentrations of Ox-Cu

Table 18. Levels of oxalate extractable Cu in three soils
as influenced by soil pH, Cu addition, and length
of incubation.

Treatment		Length of Incubation (days)					Row
Cu	pH	8	32	64	128	256	LSD $_{.05}$

----------------mg kg^{-1}-------------

Bertie fine sandy loam

Cu	pH	8	32	64	128	256	Row LSD
control	3.5	2.2c*	0.9c	0.7c	1.0c	1.1b	1.1
	5.0	2.1c	0.6c	0.8c	0.8c	0.8b	1.9
	6.5	1.1c	0.8c	0.5c	0.6c	0.5b	2.8
	8.0	0.9c	0.7c	0.5c	0.5c	0.6b	1.0
manure	3.5	8.2b	6.5b	10.3ab	11.2b	13.5a	1.1
	5.0	8.3b	8.2b	15.1a	10.8b	11.6a	2.1
	6.5	11.8ab	8.7b	12.9ab	13.1ab	14.4a	1.7
	8.0	13.9a	11.5a	14.1ab	15.0a	16.0a	0.4
CuSO$_4$	3.5	7.6b	10.8a	11.1ab	11.0b	10.9a	3.2
	5.0	7.8b	11.3a	10.5ab	10.6b	10.5a	4.8
	6.5	9.2b	8.7b	9.2b	10.7b	14.3a	1.4
	8.0	9.6b	10.5a	10.2ab	12.1a	15.8a	4.5

Dyke clay

Cu	pH	8	32	64	128	256	Row LSD
control	3.5	8.6d	9.0d	8.7d	8.9d	9.4d	6.6
	5.0	10.9d	9.3d	9.6d	9.3d	9.4d	2.8
	6.5	10.8d	9.3d	11.4d	10.9d	10.9d	2.7
	8.0	11.9d	10.3d	11.5d	11.3d	10.9d	4.0
manure	3.5	25.2bc	22.2ab	24.0bc	24.3bc	23.0c	4.0
	5.0	25.5b	19.6ab	28.0a	28.5a	28.0b	2.1
	6.5	26.7b	23.8ab	28.6a	28.3a	27.9b	3.9
	8.0	26.5b	23.4ab	29.8a	29.7a	30.8a	3.8
CuSO$_4$	3.5	21.3c	21.4b	20.8c	21.0c	21.4c	2.0
	5.0	26.9b	17.8c	23.2bc	23.0bc	22.1c	3.6
	6.5	26.9b	22.2ab	26.1ab	26.3ab	27.7b	6.1
	8.0	34.7a	26.5a	28.7a	28.5a	28.5ab	4.9

Guernsey silt loam

Cu	pH	8	32	64	128	256	Row LSD
control	3.5	3.9b	3.0b	3.0c	3.1d	3.3c	2.8
	5.0	4.2b	4.8b	3.2c	3.9d	6.4c	4.0
	6.5	3.3b	3.6b	3.3c	4.5d	6.6c	5.3
	8.0	3.6b	3.1b	3.4c	4.9d	6.8c	5.1
manure	3.5	11.4a	13.3a	12.3b	15.4c	16.7b	1.3
	5.0	11.4a	11.5a	17.7a	19.4a	23.8a	3.2
	6.5	13.4a	13.5a	17.9a	18.1a	18.9ab	0.5
	8.0	12.6a	12.8a	18.7a	19.6a	19.7ab	6.0
CuSO$_4$	3.5	13.8a	12.0a	14.4ab	15.1c	18.8ab	3.2
	5.0	13.8a	14.3a	14.1ab	16.0bc	17.4ab	5.4
	6.5	11.6a	14.3a	15.0ab	16.5bc	17.1ab	5.7
	8.0	13.8a	13.9a	16.6ab	17.3b	18.7ab	4.3

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

than at higher pH levels. This reflects the greater solubility of Fe and Mn oxides at low pH. No consistent differences between Cu sources were observed in any of the soils.

The concentration of Ox-Cu in the Dyke soil remained relatively constant over the incubation period. Both the Bertie and Guernsey soils showed small increases in Ox-Cu with time, however. The difference between the soils may be due to the rapidity in which the applied Cu can be bound and occluded by the high level of Fe and Mn oxides in the Dyke soil compared with the other two soils.

Variations in the level of Ox-Cu may again be due to changes in the redox status of the soils which were possible during the incubation period. Such changes in the redox potential of the soils would affect the solubility of the Fe and Mn oxides which would in turn control the amount of Cu found in this fraction.

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CHAPTER IV

SUMMARY AND CONCLUSIONS

Field experiments were conducted on three Virginia
soils with diverse chemical and physical properties in order
to evaluate the potential environmental hazards associated
with the long-term application of Cu as either $CuSO_4$ or Cu-
enriched swine manure on agricultural land. The soils used
were a Bertie fine sandy loam (Aquic Hapludult), a Guernsey
silt loam (Aquic Hapludalf), and a Starr clay loam
(Fluventic Dystrochrept)-Dyke clay (Typic Rhodudult)
complex, from the Coastal Plain, Ridge and Valley, and
Piedmont physiographic regions of Virginia, respectively.
The U.S.E.P.A. predicts the maximum safe Cu loading rate for
these soils to be 280 kg Cu ha^{-1} based on their CEC values
and pH levels of 6.5 or above. A laboratory incubation
study was performed using the same soils to determine the
changes in Cu distribution in these three soils over a
256-day period following additions of $CuSO_4$ or Cu-enriched
swine manure as influenced by soil pH. Conclusions drawn
from these investigations can be summarized as follows:

1. Application of either 336 kg Cu ha^{-1} as $CuSO_4$ or up to
   264 kg Cu ha^{-1} as Cu-enriched swine manure from 1978 to
   1985 did not adversely affect corn growth or composition.
   Corn grain yields for the 1984 growing season averaged

8950, 10310, and 11838 kg ha^{-1} for the Bertie, Guernsey, and Starr-Dyke soils, respectively, and were increased slightly by manure application on the Guernsey and Starr-Dyke soils. These increases in yield probably reflect improvements in the general tilth and water relations of these soils caused by the manure. In 1985, grain yields averaged 9320, 10270, and 7990 kg ha^{-1} for these soils and were not influenced by $CuSO_4$ or Cu-enriched manure additions. Copper concentrations in corn ear leaf tissue were increased only slightly ($\geq$ 2.1 mg kg^{-1}) by Cu additions with no increase in Cu content on corn grain being observed. Corn grain yields were unaffected or decreased slightly by manure treatments. Copper concentrations in both ear leaves and grain remained within normal ranges on all soils and for all treatments.

2. Concentrations of DTPA extractable Cu in the Ap horizon of the three soils ranged up to 49.9 mg kg^{-1} following seven annual applications of Cu with little difference between Cu sources. DTPA extractable Cu was highly correlated (r $\geq$ 0.93**) to the cumulative amounts of applied Cu, but was not related to Cu concentrations in either corn ear leaves or grain.

3. Analysis of subsoil samples indicated little downward movement of the applied Cu due to leaching. The level of extractable Cu in the Bertie and Starr-Dyke subsoils

taken from the control plots were 0.3 and 0.7 mg kg^{-1}, respectively. Addition of up to 280 kg Cu ha^{-1} over the seven year period from 1978 through 1984 resulted in increases of 1.6 mg kg^{-1} or less in the level of extractable Cu in the Bertie and Starr-Dyke subsoils. The larger increases in the level of extractable Cu in the Guernsey subsoil probably represents movement caused by deep plowing.

4. Eight annual Cu additions to field soils have resulted in increased concentrations of Cu in all soil fractions (exchangeable, specifically adsorbed, organically bound, oxide occluded, and residual Cu) indicating that a portion of the applied Cu is reverting to plant unavailable forms over time.

5. Laboratory incubation of three soils showed that the distribution of applied Cu among exchangeable, specifically adsorbed, organically bound, and oxide occluded soil fractions was dependent on soil pH, length of time following Cu additions, and to some degree the source of applied Cu.

6. Only a minute portion of the total soil Cu was found in the exchangeable Cu (Ex-Cu) fraction at any time. The level of Ex-Cu in Cu amended soils decreased with time

and increasing pH. Concentrations of Ex-Cu in all soils stabilized after reaching levels of 1 to 5 μg Cu kg^{-1}.

7. At higher pH levels, greater amounts of Ex-Cu were found in the manure treated soils compared to those that received an equivalent amount of Cu as $CuSO_4$, but at low pH the opposite was true. The difference between Cu sources is probably due to increased pH and greater organic complexation of Cu in the manure treated soils.

8. The concentration of specifically adsorbed Cu (HOAc-Cu) also decreased with time and increasing pH.

9. Levels of organically bound Cu (Pyro-Cu) were greater in the manure treated soils than in the soils receiving $CuSO_4$. No consistent effects of either soil pH or incubation time on Pyro-Cu could be discerned.

10. Concentrations of oxalate extractable Cu (Ox-Cu), in both the control and Cu amended soils, paralleled the levels of Fe and Mn in the soils. There was a tendency for the Cu treated soils to contain less Ox-Cu at low pH than at high pH levels. The levels of occluded Cu increased with time in the Bertie and Guernsey soils to which Cu was applied, but remained relatively unchanged in the Dyke soil.

11. In order to determine the point at which applied Cu will become toxic to plants and to gain a better understanding of the factors controlling the availability of the applied Cu in soils, this research needs to be continued.