CORN RESPONSES TO HIGH LEVELS OF COPPER APPLICATION

AS COPPER RICH PIG MANURE

by

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(ABSTRACT)

The use of copper (Cu) in pig 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 in agricultural land. Results of field experiments conducted on three diverse Virginia soils indicated that the long-term application of Cu as either copper sulfate or Cu rich pig manure, even at high application rates (average application rate for 9 years was 108 metric tons of manure ha^{-1} $year^{-1}$ or 32 kg Cu ha^{-1} $year^{-1}$), had no adverse effects on corn yields. Copper levels in corn grain and leaf tissue were not increased by the nine annual applications of Cu rich manure or $CuSO_4$, except for a slight increase in Cu concentration in corn grain from $CuSO_4$ application on only one soil. Copper concentrations in all plant tissues were well within normal ranges for all treatments at all sites. Corn grain yields were not decreased by Cu application on any of the three soils.

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Chapter I

INTRODUCTION

Copper (Cu) is the most reactive of the agronomically important trace elements in its ability to chemically interact with soil mineral and organic components as well as to form precipitates with sulfides, carbonates, hydroxides, and other anions. It also is an essential element required in small quantities for the normal development and growth of both animals and plants. But, like other heavy metals, at high concentrations Cu can be toxic to animals and plants.

Research has shown that pigs eating Cu rich feed grow faster and use the feed more efficiently. The practice of adding Cu as $CuSO_4$ to pig feed has been used in many countries for a number of years. High Cu levels occur in manure from pigs fed Cu rich diets. The potential environmental hazard associated with Cu accumulation in soils from Cu rich pig manure application is increasingly becoming a subject of concern. The U.S. Environmental Protection Agency has threatened to disallow the use of high Cu in pig feed due to the concern about the potential environmental hazard associated with repeated application of the Cu rich manure to agriculture land.

Research has not been completed to determine the amount of Cu as Cu rich pig manure that can be safely added to soils for corn production. This research was conducted to determine amounts of Cu as Cu rich pig manure that could be applied to three soils with diverse properties. The research was initiated in 1978 and continued through 1986. Research discussed herein is from the ninth year of the long-term field experiments, i.e., from the 1986 growing season. Specific objectives of the research were as follows:

1. To determine effects of long-term applications of Cu rich pig manure and $CuSO_4$ on the elemental composition of corn plants, and
2. To evaluate effects of long-term applications of Cu rich pig manure and $CuSO_4$ on corn grain yield.

Chapter II

LITERATURE REVIEW

Copper has been used by humans since about 8000 B.C. when it was used to make crude hammered articrafts (Kevin et al., 1981). In modern life, Cu has played a versatile role due to its chemical qualities, ductility, malleability, and conductive properties. During the past 50 years, Cu has been established as an essential element for plants and animals (Graham, 1981).

Geochemistry of Soil Copper

Copper distribution varies widely both geographically and geologically. It is distributed in the rocks of the earth's crust typically as chalcophile. The stable Cu minerals are sulfides rather than silicates or oxides. 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} (Krauskopf, 1972). The concentration of Cu in soils generally reflects the Cu content of the soil forming minerals and has a relatively uniform distribution through the soil profile (Oertel, 1961). Minor redistribution of soil Cu can occur through weathering and nutrient cycling by plants (Hodgson, 1963). Copper exhibits greatest mobility in reducing environments. Consequently, the movement of Cu within the soil profile occurs to a greater extent in highly acidic conditions than in oxidizing environments of higher pH levels (Hodgson, 1963; Oertel, 1961).

Mafic rocks contain high concentrations of Cu, up to 100 mg kg^{-1}, as the dark colored ferromagnesium silicates and as the sulfide minerals. The form of Cu in these minerals is unknown but may involve either substitution of Cu^{2+} for Fe^{2+}, Mn^{2+}, and Mg^{2+} in the crystal lattice or inclusion of micro-crystals of CuS due to the similarity in the ionic radii of Fe^{2+}, Mn^{2+}, and Mg^{2+} and due to the strong covalent bonds that Cu ions form with S (Krauskopf, 1972). Copper ions released by weathering processes exist largely as ions adsorbed on fine-grained particles in sedimentary rocks. Copper rarely occurs in carbonate rocks due to the inability of Cu to substitute for Ca and Mg in the carbonate structure.

The most common primary minerals of Cu are insoluble simple and complex sulfides [covelline (CuS), chalcosite (Cu_2S), and chalcopyrite ($CuFeS_2$)] in which strong covalent bonds are formed between reduced Cu (Cu^+) and sulfide (S^{2-}) anions (McBride, 1981). The moderately soluble forms of Cu like hydroxycarbonates [malachite ($Cu_2(OH)_2CO_3$) and azurite ($Cu_3(OH)_2(CO_3)_2$)], silicates (chrysocolla, $CuSiO_3$), and oxides (tenorite, CuO) are considered as secondary minerals. At low pH and under strong leaching conditions at the earth's surface, most of the moderately soluble forms would not persist (McBride, 1981).

Chemistry of Soil Copper

Soil receives Cu as natural as well as artificial sources in various forms in any given environment. For example, Cu released from native soil minerals is considered a natural source. The artificial sources are from fertilizers when applied to soils (Mullins et al.,

1982) and from the direct disposal of wastes such as animal manures (Barker, 1974; Davis, 1974), sewage sludge (Kelling et al., 1977; Ritter et al., 1981), and industrial emissions (Tiller et al., 1981).

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 (McBride, 1981). 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, cation exchange capacity (CEC), redox potential (Eh), and concentration of sesquioxides as well as the concentration of Cu present (Moore, 1972; Baker, 1974; Kiekens and Cottenie, 1980).

Most soluble Cu in surface soils is complexed due to strong specific adsorption of Cu by organic matter (Stevenson and Fitch, 1981), clay minerals (Follett and Lindsay, 1971; Kishk and Harson, 1973), and Al, Fe and Mn oxides (Jenne, 1977). Small quantities of Cu hydroxy and carbonate complexes commonly exist in soil solution. Hodgson et al. (1966) has shown that more than 98 percent of Cu in soil solution exists in an organic complexed form and that only very small amounts of free Cu^{2+} are available for adsorption reactions. The adsorption of Cu by soil increases with an increase in pH and CEC of organic matter. The 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 (Kiekens and Cottenie, 1980).

Adsorption isotherms have been used to describe the adsorption of Cu by soil. An adsorption isotherm study by McLaren and Crawford (1974) showed that clay minerals had a low affinity for Cu compared with amorphous oxides and organic matter. The ability of soil components to adsorb Cu depended on the concentration and nature of functional groups and on the available surface area of the various constituents (McLaren and Crawford, 1974).

The adsorption of Cu by sesquioxides and clay minerals has been shown to be pH dependent (Frost and Griffin, 1977; Kishk and Harson, 1973; McLaren and Crawford, 1973; Sims and Patrick, 1978). McLaren and Crawford (1973) found increased adsorption of Cu by soils and individual soil compounds from a solution containing 5 mg Cu L^{-1} as pH was increased. Frost and Griffin (1977) showed that, at pH > 5.5, retention of Cu increased considerably due to increased adsorption of Cu hydroxy complexes and formation of $Cu(OH)_2$. According to Farrah and Pickering (1976), clay surfaces showed an apparent preference for the Cu hydroxy ion, $Cu(OH)^+$, resulting in a strong pH dependent selectivity.

Many aspects of the chemistry of Cu in soils are related to the formation of complexes with organic matter. In most soils, organically bound Cu represents the major reserve of potentially available Cu. The importance of organic matter in retention and in affecting the behavior of Cu in soils and sediments has been known for many years (McLaren and Crawford, 1973; Petruzzelli et al., 1978; Viets, 1962). Clay, like organic matter, is a major soil component involved in Cu retention. The amount of Cu retention by both clay and organic matter increases with an increase in soil pH. Organic matter forms highly stable complexes with Cu by direct bonding of Cu^{2+} ions to two or more functional groups, i.e., primarily with carboxylic and phenolic groups (Stevenson and Fitch, 1981).

Since Cu has high affinity for soil humic substances, a major portion of Cu in soil solution exists as a soil organic complex (Kerven et al., 1984; Stevenson and Fitch, 1981). According to Stevenson and Fitch (1981), the availability of Cu in these Cu organic complexes depends on the molecular weight of the complex and the concentration of Cu. Metal complexes with fulvic acids generally show a greater availability and mobility compared with humic acid because of their lower molecular weight and greater concentration of acidic functional groups which adsorb Cu.

In summary, labile soil Cu is mainly present as adsorbed Cu^{2+} and as complexed Cu in soil solutions. Due to the strong adsorption of Cu in various complexes, a low concentration of Cu exists in soil solution. The highest amounts of soil solution Cu occur under low pH conditions. In Cu amended soils where large amounts of Cu are present, complexation may reduce the concentration of Cu^{2+} to nontoxic levels. Natural complexing agents may be involved in the mobility and transport of Cu through the soil profile (Stevenson and Fitch, 1981).

Copper in Plants

Copper is an essential element required in a small quantity for the normal growth of all plants. So it has been considered as one of the seven essential micronutrients. The presence of Cu in plants was demonstrated in the early 1800's and the essentiality was clearly established in the 1930's by nutrient solution research (Sommer, 1931). Concentrations of Cu from 5 to 20 mg kg^{-1} in plant tissues are considered in the normal range for growth of most plants, while concentrations of less than 4 mg kg^{-1} are considered as deficient and those above 20 mg kg^{-1} are generally thought to be toxic (Jones and Eck, 1973; Walker and Webb, 1981). A critical Cu concentration of 5 mg kg^{-1} was reported for corn ear leaves at the early silk stage of growth, and a sufficiency range of 1 to 5 mg Cu kg^{-1} was indicated for corn grain at plant maturity (Jones and Eck, 1973).

The Cu in plant tissue is directly involved in the metabolism of plants. Copper plays a vital role in many important enzymes and proteins in plants. The activities of Cu enzymes in plants affects a multitude 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 (Bussler, 1981).

Since Cu was established as an essential element for higher plants, the absorption of Cu by plant roots has received attention from many researchers. Although the exact absorption process is not known, some evidence shows that it is active in nature. In addition, the absorption of Cu into plant cells is among the lowest for the essential elements, because most chelates bind Cu^{2+} strongly in cell walls. Cell walls contain sites for the strong and specific adsorption of Cu which is not easily desorbed (Graham, 1981, Loneragan, 1981). 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 (Graham, 1981; Walker and Webb, 1981). Since there is little correlation between Cu concentrations in plant leaf tissue and extractable soil Cu, it has been assumed that there is only limited translocation of Cu^{2+} complexed by certain amino acids or other soluble protein in xylem and phloem saps from plant roots to shoots (Loneragan, 1981). Once complexed, Cu is able to move freely in the transpiration stream and, thus, the process of excretion of Cu from root cells into the xylem sap may be a key process in the movement of Cu to plant shoots (Loneragan, 1981).

Copper concentration in soil can eventually reach toxic levels following repeated Cu applications. But the amount of applied Cu that will be toxic to plants cannot be accurately predicted due to the lack of general knowledge about the chemistry of applied Cu in soils and due to the inability to estimate plant absorption of Cu from Cu amended soils. The U.S. Environmental Protection Agency bases its guidelines for land application of Cu rich wastes on soil CEC and on maintenance of soil pH at 6.5 or above to minimize solubility of the added Cu (U.S.E.P.A., 1983). These guidelines have yet to be tested adequately with long-term experiments under field conditions.

Copper in Pig Production

Copper is an essential element for man and all animals (e.g., for growth and health), but can be toxic at a high level. The biological importance of this element has been established during the last 50 years (Bories, 1980). In pigs, Cu plays a vital part in major enzymatic systems like hemoglobin synthesis; red blood cell maturation and maintenance; development and integrity of skeletal, cardiovascular, and central nervous systems; pigmentation; reproduction; and function of the immune system (Miller et al., 1979). From 5 to 10 mg Cu kg^{-1} is adequate in the normal fattening diet for pigs to prevent the Cu deficiency (Braude, 1967).

Braude (1945) concluded that pigs on normal rations craved metallic Cu in excess of their minimum dietary requirements after he observed that pigs continuously licked Cu rings located in their pens. Barber et al. (1955a) demonstrated that suckling pigs, which were given free choice of two creep meals, preferred the meal to which $CuSO_4$ had been added (25 g $CuSO_4.5H_2O$, 45.4 kg of diet) and consumed it in preference to the meal without supplementary Cu. It was shown that the addition of $CuSO_4$ at a rate of 250 mg kg^{-1} to normal pig rations caused a positive response in terms of live-weight gain and efficiency of food utilization (Barber et al., 1955a,b). Since then, the response of pigs to Cu-supplemented diets has been studied extensively (Barber et al., 1960; Braude, 1967; Braude et al., 1962; Bunch et al., 1961; DeGoey et al., 1971; Prince et al., 1979; Wallace et al., 1960). In 1967, Braude further reported that the addition of 250 mg Cu kg^{-1} to the diets of growing pigs caused average increases in daily live weight gain of 8.1 percent and in efficiency of food utilization of 5.4 percent. 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 (Braude, 1967; DeGoey et al., 1971; Hanrahan et al., 1968; Hedges et al., 1973).

Prior to the introduction of Cu-supplemented diets in pig production, antibiotics were widely used as feed additives to improve pig performance. But, later many researchers found that growth rate and efficiency of food utilization were as high for Cu rich diets as for antibiotic fortified diets (Braude, 1967; Braude et al., 1962, Bunch et al., 1961). In addition, research has shown that, under some conditions, there may be an additive effect when $CuSO_4$ and antibiotics are supplied together (Braude, 1967; Lucas et al., 1957).

A number of mechanisms have been proposed to explain the growth stimulatory effects of antimicrobial agents such as antibiotics and Cu. Almost all are based upon the concept that the growth response is due to actions on the microbial flora in the gastro-intestinal tract. Some mechanisms frequently proposed are: i) Cu might regulate the microbial flora in the small intestine and thus reduce the level of infectious disease, ii) microbial production of growth depressing toxins might be reduced, iii) microbial destruction of essential nutrients in the gastro-intestinal tract might be reduced, and iv) there might be an enhanced efficiency of absorption and utilization of protein or energy (Ludvigsen, 1981).

Land Treatment of Copper Rich Pig Manure

The current practice of feeding pigs Cu rich diets originated as an economical alternative to use of antibiotics as growth stimulants and as a disease control measure. Use of high levels of Cu in pig production has received considerable opposition due to the concern about the potential environmental hazards associated with repeated application of the resulting Cu rich manure to agricultural land (Baker, 1974; David, 1974). In order to prevent a build-up of excessive levels of Cu in soil, many European countries now limit the amount of Cu which can be added to the pig feed.

Since as much as 95% of the dietary Cu is subsequently excreted in the wastes, the Cu concentration in the manure collected from pigs fed Cu rich diets can be as high as that in sewage sludge (Corcak et al., 1978; Williams et al., 1984). Much of Cu in the manure is in readily soluble forms, which is different from most sewage sludge (Miller et al., 1986). The concen- trations of Cu in manures from pigs fed Cu rich diets are in the range of 600 to 2370 mg kg^{-1} on a dry weight basis.

Few researchers have conducted long-term field experiments to determine the effect of Cu rich pig manure application on plant growth and nutrient composition. Three annual applications of Cu rich pig manure at the rate of 12.3 kg Cu ha^{-1} to a perennial rye grass (*Lolium perenne*) and white clover (*Trifolicum repens*) pasture increased the Cu concentration in the herbage, but not to a level that would be hazard- ous to grazing animals (Batey et al., 1972). Much of the increase in Cu concentration was attributed to direct foliar contamination during application of the manure. They pointed out that Cu rich pig manure should be applied shortly after cutting pastures to avoid risks to grazing animals from ingestion of manure-contaminated herbage. Yield decreases occurred in their studies where plants were smothered by manure application.

There was neither a decrease in corn grain yield nor an increase in Cu concentrations in plant tissue from four annual applications of 15.3 kg Cu ha^{-1} as Cu rich pig manure to a Crosby silt loam (Aquic Hapludalt) (Sutton et al., 1983). Application of 102.9 kg Cu ha^{-1} as Cu rich pig manure over a 3-year period to a Groseclose silt loam resulted in a doubling of the Cu levels in corn roots and in a slight increase in the ear leaves but did not influence grain yield or Cu concentration (Kornegay et al., 1976). Martens et al. (1974) reported that six-annual applications of up to 8.4 kg Cu ha^{-1} as $CuSO_4$ did not affect either soybean seed yield or Cu concentration in soybean foliage. A small increase in Cu concentration in seeds from the $CuSO_4$ applications did not preclude their use as animal feed. Mullins et al. (1982a) conducted experiments on 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 from application of 138 kg Cu ha^{-1} as Cu rich pig manure. A number of investigators attributed decreases in plant growth from Cu rich manure application to smothering of plants when the manure was applied on growing plants and to build up of excessive levels of N, P, and soluble salts in soil, rather than to elevated levels of Cu from the applied pig manure (Batey et al., 1972; Kuo, 1981; Sutton et al., 1983).

The fractions of Cu, both plant available and unavailable, were studied before and after application of Cu, as either $CuSO_4$ or Cu rich pig manure (Mullins et al., 1982a). They found that both plant available and unavailable Cu forms were increased from the Cu additions. Further studies of transformation of Cu in soils following the manure application agreed with these results and with the conclusion that a portion of applied Cu reverted to plant unavailable forms with time, probably due to the conversion to insoluble hydroxides and oxide forms (Kornegay et al., 1976; Kelling et al., 1977).

Evidence from analyses of subsoil samples from the sites where Cu rich manure was incorporated into surface soil showed that there is little downward movement of the applied Cu through the soil profile over a considerable period of time (Batey et al., 1972; Mullins et al., 1982; Sutton et al., 1983). The movement of added Cu within the soil profile is usually restricted because it is bound by various soil components including clay minerals, Fe and Mn hydrous oxides, and organic matter (James et al., 1981; Kickens et al., 1980; McLaren and Crawford, 1973; Stevenson and Fitch, 1981). Due to the rapid immobilization of the applied Cu in the soil, Cu contamination of ground water is generally not considered a major threat in most soils.

The Cu content in soils amended with either organic or inorganic Cu sources have been evaluated with a number of analytical procedures. The extracting procedures included complexing agents such as EDTA (Batey et al., 1972) and DTPA solutions (Follett et al., 1971; Mullins et al., 1982a; Williams et al., 1974); Mehlich-1 solution (Korcak and Fanning, 1978); 1 $\underline{M}$ HCl (Sutton et al., 1983); and 4 $\underline{M}$ HNO_3 (Williams et al., 1984). Results from these analyses have frequently indicated linear increases in extractable Cu with rates of Cu application. Copper extracted from soils to which large amounts of Cu have been added usually did not correlate with Cu concentrations in plant tissue. Dragun et al. (1976) and Kornegay et al. (1976) speculate that the lack of correlation may reflect Cu accumulation in plant roots rather than Cu translocation to plant shoots.

In summary, safe Cu loading capacities of soil cannot currently be predicted due to the lack of scientific knowledge in the area of Cu chemistry in soils (Baker, 1974). The mechanisms of absorption and translocation of Cu by plant tissue also are unclear (Graham, 1981). The behavior and effects of Cu added to soil in pig manure are similar to that applied in sewage sludge (Unwine, 1980). Therefore, the general guidelines proposed by the U.S. Environmental Protection Agency for sewage sludge are also appropriate for application of Cu rich pig manure. These guidelines are based on soil CEC and maintenance of soil pH at 6.5 or above to minimize solubility of the added Cu (U.S.E.P.A., 1983).

CHAPTER III

MATERIALS AND METHODS

Field and laboratory research was conducted to determine the plant availability of Cu from high levels of Cu rich pig manure and $CuSO_4$ applications. Field experiments were initiated during the 1978 growing season and were continued for the 9-year period from 1978 through 1986. Three soils selected for the field studies were a Bertie fine sandy loam (fine-loamy, mixed, thermic, Aquic Hapludult), a Guernsey silt loam (fine, mixed, mesic, Aquic Hapludalf), and a Starr-Dyke Complex (clayey, mixed, mesic, Typic Rhodudult) located in Coastal Plain, Ridge and Valley, and Piedmont physiographic regions of Virginia, respectively. The Ap horizon of each soil was slightly acid when the field experiments were initiated in 1978 and had CEC values ranging from 5.0 to 12.3 cmol(+) kg^{-1} (Table 1). Based on a soil pH of $\geq$ 6.5 and on the soil CEC values, all three soils would have a maximum safe Cu loading rate of 280 kg ha^{-1} (U.S.E.P.A. 1983).

Field Experimentation

The field experiments for this research had five treatments replicated four times in a randomized complete block design on each of the three soils (Table 2). The treatments imposed at each experimental site were as follows: Treatment 1 was a control and did not receive Cu application. Treatment 2 received 33.6 mt ha^{-1} of Cu rich pig manure annually from 1978 through 1982, no Cu application in 1983, 232 kg Cu ha^{-1} applied as $CuSO_4$ in 1984 and 56 kg Cu ha^{-1} as $CuSO_4$ in

Table 1. Properties of soils in 1978 prior to initiation of long-term field experiments to determine the plant availability of copper in pig manure.

Field Experiment Location	Soil Series	Soil Texture	Soil pH	Organic Matter	Cation Exchange Capacity
				%	$cmol(+)kg^{-1}$
Blacksburg - Appalachian Region	Guernsey	silt loam	5.7	1.8	10.4
Holland - Coastal Plain Region	Bertie	fine sandy loam	6.2	1.7	5.0
Orange - Piedmont Region	Starr-Dyke Complex	clay loam	5.9	1.4	12.3

1985 and 1986. Treatment 3 received $CuSO_4$ applications each year to equal the Cu in the manure and $CuSO_4$ applied to treatment 2. For treatment 4, 67.2 mt ha^{-1} of wet Cu rich pig manure was applied annually from 1978 through 1980, 134.4 mt ha^{-1} was applied in 1981 and 1982, 168 mt ha^{-1} was applied in 1983 and 1984, and 84 mt ha^{-1} was applied in 1985 and 1986. Copper as $CuSO_4$ was added to treatment 5 annually from 1978 through 1986 at levels equivalent to that in the Cu rich pig manure for treatment 4.

Cumulative amounts of B, K, Mn, N, P, and Zn, applied to the field experiments from 1978 through 1986 are shown in Table 2. The amounts of K, N, and P applied to the field plots that received pig manure were based on the estimated amounts of these elements supplied by the manure (Donohue and McCart, 1977). 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 approximately 6.5 or above by applications of dolomitic limestone. All amendments were incorporated into the soil by disking and plowing prior to planting.

"Pioneer 3192" corn was grown at each of the three experimental sites. The corn populations were 60,270 plants ha^{-1} on the clay loam soil at Orange, 55,920 plants ha^{-1} on the fine sandy loam soil at Holland, and 57,400 plants ha^{-1} on the silt loam soil at Blacksburg. Weeds were controlled in these experiments by use of herbicides. Corn grain yields were determined for each plot of the field experiments at plant maturity. These yields were corrected to 155 g moisture kg^{-1}.

Table 2. Cumulative amounts of amendments applied as inorganic salts from 1978 through 1986 for the five treatments of the field experiments designed to determine the plant availability of copper in pig manure.†

Treatment No.	Treatment Description	Macronutrients K	Macronutrients N	Macronutrients P	Micronutrients B	Micronutrients Cu	Micronutrients Mn	Micronutrients Zn	Pig Manure
		kg ha^{-1}							mt ha^{-1}
	Field Experiment - Blacksburg								
1	Control	1337	1932	302	6.6	-----	28	107	-----
2	Manure + $CuSO_4$	954	1382	184	6.6	343.7	28	107	168.0
3	High Cu Loading Rate	1337	1932	302	6.6	392.0	28	107	-----
4	High Manure Rate	93	----	110	6.6	-----	28	107	974.4
5	Cu Equivalent to Treatment 4	1337	1932	302	6.6	294.4	28	107	-----
	Field Experiment - Holland								
1	Control	1324	1764	257	5.5	-----	168	107	-----
2	Manure + $CuSO_4$	901	1339	135	5.5	342.3	168	107	168.0
3	High Cu Loading Rate	1324	1764	257	5.5	392.0	168	107	-----
4	High Manure Rate	----	34	---	5.5	-----	168	107	974.4
5	Cu Equivalent to Treatment 4	1324	1764	257	5.5	290.7	168	107	-----
	Field Experiment - Orange								
1	Control	1602	1960	464	5.5	-----	28	90	-----
2	Manure + $CuSO_4$	1097	1408	209	5.5	344.0	28	90	168.0
3	High Cu Loading Rate	1602	1960	464	5.5	392.0	28	90	-----
4	High Manure Rate	----	293	---	5.5	-----	28	90	974.4
5	Cu Equivalent to Treatment 4	1602	1960	464	5.5	285.7	28	90	-----

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

Production and Analysis of Pig Manure

The manure used in this experiment 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 three days after the pigs were given Cu rich rations. The manure was collected daily and placed in plastic cans for transport to the experimental sites. After the collection of manure, samples were taken from the manure to be used at each location. The Cu rich feed used to produce the manure was also sampled.

Triplicate subsamples of manure and feed samples were dried at $105^{o}C$ for 24 hours to determine percent dry matter. The dried samples were ground to pass a 0.84 mm sieve using a stainless steel Wiley mill in preparation for Ca, Cu, Fe, K, Mg, Mn, and Zn analyses. One gram subsamples were ashed at $450^{o}C$ for two hours, the ash was dissolved in 25 mL of 0.5 M HCl, and the mixture was filtered through Whatman number 42 paper. Calcium, Cu, Fe, K, Mg, Mn, and Zn, concentrations in the filtered solution was determined by atomic absorption spectrophotometry. Nitrogen concentration in the feed and manure was determined by a micro-Kjeldhal procedure (McKenzie and Wallace, 1954). Phosphorous was analyzed by a molybdivanado-phosphoric acid procedure (Kitson and Mellon, 1944), and B by the Azomethene-H procedure of Parker and Gardner (1981).

Soil Sampling and Analysis

Soil samples were collected prior to application of treatments and fertilizers from the Ap horizon at each location in the spring of

986. The samples were air-dried and crushed to pass a 2 mm sieve prior to being analyzed. Soil pH of samples was determined in a 1:1 (v/v) soil-to-water mixture after a 1-hr equilibration period. Soil organic matter was determined by a chromic acid oxidation procedure (Donohue and Gettier, 1979). Diethylenetriaminepentacetic acid (DTPA) extractable Cu and Zn were measured by procedures described by Baker and Amacher (1982). The soil samples were first leached with Na acetate (pH 5.0) to remove $CaCO_3$ in preparation for the cation exchange capacity measurement. For the cation exchange capacity determination, samples were saturated with 0.5 M Ca acetate (pH 7.0), and Ca^{2+} was displaced with 0.5 M Mg acetate (pH 7.0). This procedure was chosen because neutral saturation and displacement solutions are used for cation exchange capacity determinations for evaluation of levels of municipal sludge application to soils (Anonymous, 1978; Sommers and Nelson, 1978).

Soil samples were obtained from each plot of the three experiments in the springs of 1982, 1984, 1985, and 1986 for extractable P analyses. The soil samples were air-dried and crushed to pass a 2 mm sieve. Bray P_1 extractable P in the sieved samples was determined by the Murphy-Riley single solution method (Murphy and Riley, 1962).

Plant Tissue Sampling and Analysis

Ten corn ear leaves were sampled at the early silk stage of growth from the two inside rows in each of the four row plots. Corn grain was harvested from 6.1 m of the two inside rows at plant maturity. Ear leaf and grain samples of corn plants were prepared for chemical analysis by the aforementioned drying and grinding procedure for pig manure and feed. Boron, Ca, Cu, Fe, Mg, Mn, P, K, N, and Zn concentrations in the ear leaf samples were obtained by the procedures used for these determinations in pig feed. The corn grain samples were digested in a HNO_3-$HClO_4$ mixture, and the Cu and Zn in the digests were determined by atomic absorption spectrophotometry.

Statistical Analysis

Effects of treatments on corn yield and nutrient concentrations and on soils data were evaluated by analyses of variance and by Duncan's Multiple Range Tests (Duncan, 1955). Relationships between Bray P_1 in soil and rate of manure application were determined by linear correlation and simple linear regression analyses. Mean difference are claimed herein at the 0.05 level of significance.

Chapter IV

RESULTS AND DISCUSSION

Field experiments were continued for a ninth year on the three soils to study corn response to nine consecutive annual applications of Cu rich pig manure. Soils with diverse chemical and physical properties were selected for this research (Table 1). This choice of soils ensured wide applic- ability of the experimental results.

Manure used in the field experiments was produced by pigs fed a ration supplemented with a growth promoting level of Cu as $CuSO_4$. Pig feed used for manure production for the field experiments in 1986 contained 232.5 mg Cu kg^{-1} and averaged 248.9 mg Cu kg^{-1} over the nine years of this investigation from 1978 through 1986 (Table 3). The concentrations of nutrients in the pig feed in 1986 were relatively close to the average nutrient composition for the 8-year period from 1978 through 1985 (Table 3).

Manure Composition

The average macronutrient and micronutrient composition of manure used in this investigation is shown in Tables 4 and 5. Manure produc- ed by pigs on rations with normal Cu levels contained less than 100 mg Cu kg^{-1} (Kornegay et al., 1976, 1977). As expected, much higher Cu levels were present in manure from pigs fed growth stimulating levels of Cu during each of the nine years of this investigation (Table 5). The pig manure used in the field experiments contained a considerable amount of other plant nutrients (Table 4 and 5).

Table 3. Nutrient concentrations in pig feed used for manure production for field experiments during nine growing seasons.

Element	Average Nutrient Concentration 1978 - 1985	1986
	%	
Ca	0.94	0.91
K	0.83	0.68
Mg	0.18	0.15
N	2.68	2.50
Na	0.16	0.14
P	0.56	0.53
	$mg\ kg^{-1}$	
B	10.1	9.3
Cu	250.9	232.5
Fe	173.0	111.9
Mn	37.2	21.1
Zn	65.2	28.7

Table 4. Average macronutrient concentrations and percent solids in pig manure used for three field experiments during nine growing seasons.

Growing Seasons	Macronutrient Ca	K	Mg	Na	P	Solids
	--------	--------	%	--------	--------	--%---
			Field Experiment - Blacksburg			
1978-85	4.1+	1.7	0.80	0.48	2.1	22.1
1986	5.0	1.8	0.89	0.39	2.5	22.2
			Field Experiment - Holland			
1978-85	3.7	1.7	0.79	0.47	1.9	22.7
1986	5.0	1.2	0.83	0.38	2.5	23.0
			Field Experiment - Orange			
1978-85	3.8	1.8	0.77	0.49	2.0	22.2
1986	4.7	1.5	0.89	0.33	2.5	24.7

+Each value represents the average of triplicate determinations for two subsamples for each year.

Table 5. Average micronutrient concentration in pig manure used for three field experiments during nine growing seasons.

Growing Seasons	Micronutrients B	Cu	Fe	Mn	Zn
	mg kg^{-1}				
	Field Experiment - Blacksburg				
1978-85	20.0^{+}	1310	1259	242	319
1986	24.6	1632	838	167	206
	Field Experiment - Holland				
1978-85	18.6	1291	1138	214	293
1986	19.7	1853	847	220	227
	Field Experiment - Orange				
1978-85	19.6	1268	1072	233	318
1986	21.8	1579	1162	193	214

$^{+}$Each value represents the average of triplicate determinations for two subsamples for each year.

Percent solids in three batches of Cu rich pig manure produced for the field experiments in 1986 ranged from 22.2 to 24.7 (Table 4). Raw pig manure, defined as fresh feces and urine, generally has a solid content of 18 percent or less (Meek et al., 1975; Midwest Plan Service, 1975; Donohue and McCart, 1977). The amounts of solids in batches of manure produced for the field experiments from 1978 through 1986 were greater than 18 percent. The relatively high amount of solids in the manure used in our research from 1978 through 1986 reflects drying during fecal matter collection (Mullins et al., 1982b).

Nutrient in Plant Tissue

Corn ear leaves were sampled at early silk growth stage from all treatments on the three soils (Table 6). Ear leaf elemental composition, was not related to Cu application. Concentrations of P, Mn, and Zn were affected by manure application on one or more soil. Manure application increased leaf Mn and P on the Groseclose soil and increased leaf Mn on the Davidson. Leaf Zn was decreased by manure application on the Bertie and Groseclose soils. Sutton et al. (1984) reported elevated P levels in corn leaves following application of pig waste, 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 increase in Mn concentrations on the Guernsey and Dyke soils (Table 6) was probably due to the Mn applied in the feed and to the reduction of insoluble soil Mn to plant available forms. The lower levels of Zn in corn leaves from soils

Table 6: Effect of nine-annual applications of copper as either copper sulfate or pig manure on nutrients in corn ear leaves on three soils.

Copper Treatment		Macronutrients					Micronutrients			
Amount	Source	Ca	K	Mg	N	P	B	Cu	Mn	Zn
kg Cu ha^{-1}		%					mg kg^{-1}			
		Field Experiment - Blacksburg								
0.0	Control	0.41b[†]	2.0a	0.16a	2.8b	0.31a	22.8a	7.9a	48b	37a
392.0	Pig Manure + $CuSO_4$	0.43ab	2.0a	0.14a	2.9b	0.29a	23.8a	8.3a	49b	33ab
392.0	$CuSO_4$	0.39b	2.0a	0.13a	2.9b	0.29a	23.0a	7.9a	43b	32ab
294.4	Pig Manure	0.46a	2.0a	0.14a	3.2a	0.32a	25.1a	9.0a	64a	29b
294.4	$CuSO_4$	0.41b	1.9a	0.16a	2.9b	0.32a	22.4a	8.7a	51b	34ab
		Field Experiment - Holland								
0.0	Control	0.29b	1.5ab	0.14b	2.9a	0.26b	14.5a	8.6a	30b	42ab
392.0	Pig Manure + $CuSO_4$	0.32a	1.6ab	0.15ab	2.9a	0.24b	14.3a	8.1a	30b	38bc
392.0	$CuSO_4$	0.34a	1.4b	0.17a	2.9a	0.24b	15.6a	8.6a	39a	43ab
285.7	Pig Manure	0.34a	1.7a	0.15ab	3.0a	0.31a	16.1a	8.0a	14c	38c
285.7	$CuSO_4$	0.32ab	1.5ab	0.16ab	3.1a	0.26b	14.8a	8.9a	38a	45a
		Field Experiment - Orange								
0.0	Control	0.44a	1.3ab	0.16a	2.9a	0.20a	9.5a	7.8a	46b	22a
392.0	Pig Manure + $CuSO_4$	0.45a	1.2b	0.17a	3.0a	0.21a	8.0a	8.7a	49b	24a
392.0	$CuSO_4$	0.43a	1.3ab	0.16a	2.9a	0.20a	9.1a	7.7a	46b	24a
290.7	Pig Manure	0.46a	1.4a	0.15a	3.1a	0.23a	9.2a	8.0a	58a	20a
290.7	$CuSO_4$	0.42a	1.4a	0.14a	2.9a	0.20a	9.0a	7.7a	48b	24a

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

that received manure applications indicate a greater adsorption of Zn by the manure-treated soils.

The Cu in corn ear leaves under study ranged from 7.7 to 9.0 mg kg^{-1}. The high levels of Cu application as either Cu rich pig manure or $CuSO_4$ did not increase Cu concentrations in the corn ear leaves. Copper concentrations in corn ear leaves from all treatments on the three soils were within the normal range of 6 to 20 mg kg^{-1} (Jones and Eck, 1973).

Application of 285.7 to 294.4 kg Cu ha^{-1} as Cu rich pig manure did not increase Cu concentrations in grain for corn plants grown on the three soils (Table 7). An increase in Cu concentration of a small magnitude, 0.7 mg kg^{-1} or less, occurred in corn grain where a cumulative total of 392 kg Cu ha^{-1} was applied either as $CuSO_4$ or as a combination of Cu rich pig manure and $CuSO_4$ to the silt loam soil over the 9-year period. Application of the 392 kg Cu ha^{-1} either as $CuSO_4$ or as a combination of Cu rich pig manure and $CuSO_4$ did not increase Cu concentration in grain from plants grown on the clay loam and fine sandy loam soils.

The normal range for the Cu concentrations in corn grain is from 1 to 5 mg kg^{-1} (Jones and Eck, 1973). Copper concentrations in corn grain from all treatments of the field experiments on three soils were within this range. 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

Table 7. Effect of nine-annual applications of copper as either copper sulfate or pig manure on copper and zinc concentrations in corn grain and on corn grain yields on three soils.

Copper Treatment		Grain Micronutrient Concentration		Grain
Amount	Source	Cu	Zn	Yield
kg Cu ha^{-1}		-------mg kg^{-1}-------		kg ha^{-1}
	Field Experiment - Blacksburg			
0.0	Control	1.1b+	19.3a	3110a
392.0	Pig Manure + $CuSO_4$	1.8a	25.2a	3420a
392.0	$CuSO_4$	1.5ab	21.2a	3950a
294.4	Pig Manure	1.6ab	22.8a	5250a
294.4	$CuSO_4$	1.3ab	18.5a	2930a
	Field Experiment - Holland			
0.0	Control	2.7ab	43.4a	7130a
392.0	Pig Manure + $CuSO_4$	2.9a	43.8a	7070a
392.0	$CuSO_4$	3.1a	43.9a	6780a
290.7	Pig Manure	2.3b	38.0b	6460a
290.7	$CuSO_4$	3.0a	44.6a	7760a
	Field Experiment - Orange			
0.0	Control	1.6a	30.5a	8430a
392.0	Pig Manure + $CuSO_4$	1.1a	21.6a	8870a
392.0	$CuSO_4$	2.1a	31.1a	8700a
285.7	Pig Manure	1.4a	23.7a	9180a
285.7	$CuSO_4$	1.2a	22.2a	8290a

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

following Cu additions as Cu rich pig manure (Kornegay et al., 1976), sewage sludge (Kelling et al., 1977; Soon et al., 1980), and $CuSO_4$ (Mullins et al., 1982a). Manure application did not increase Zn levels in the grain samples from the three soils.

Grain Yield

Copper concentration in corn grain was not increased by any Cu treatment on any of the three soils. (Table 7). Based on the U.S.E.P.A. (1983) guidelines, the safe Cu loading rate for the three soils is 280 kg ha^{-1}. A higher amount of Cu application as Cu rich pig manure, 285.7 to 294.4 kg Cu ha^{-1}, and as $CuSO_4$, 392 kg Cu ha^{-1}, did not affect corn grain yields on the three soils (Table 7). The relatively low corn grain yields at each experimental site during the 1986 growing season (Table 7) are due to a lack of rainfall.

Soil pH and Organic Matter Content

Soil samples were obtained from each plot of three field experiments in the spring of 1986 prior to treatment application to determine the effect of 8-annual applications of Cu rich pig manure on soil pH and soil organic matter content. As expected, the 8-annual applications of Cu rich pig manure increased the organic matter content in the three soils (Table 8). Soil pH values were at the level of $\geq$ 6.5 desired for Cu disposal sites in the Davidson and Groseclose soils and below this level in the Bertie soil (Tables 1 and 8).

Table 8. Effect of eight-annual applications of copper as either copper sulfate or pig manure on pH and amounts of organic matter in three soils.+

Copper Treatment Amount	Copper Treatment Source	Soil pH	Soil Organic Matter
kg Cu ha^{-1}			%
	Field Experiment - Blacksburg		
0.0	Control	6.7a*	2.1b
336.0	Pig Manure + $CuSO_4$	6.7a	2.4ab
336.0	$CuSO_4$	6.7a	2.1b
264.0	Pig Manure	6.6a	2.6a
264.0	$CuSO_4$	6.7a	2.3ab
	Field Experiment - Holland		
0.0	Control	6.2a	2.1b
336.0	Pig Manure + $CuSO_4$	6.3a	2.1b
336.0	$CuSO_4$	6.3a	2.1b
255.5	Pig Manure	6.4a	2.4a
255.5	$CuSO_4$	6.2a	2.1b
	Field Experiment - Orange		
0.0	Control	6.9a	1.9b
336.0	Pig Manure + $CuSO_4$	7.0a	2.0b
336.0	$CuSO_4$	6.9a	1.9b
253.0	Pig Manure	6.9a	2.8a
253.0	$CuSO_4$	7.1a	2.0b

+Soils were sampled in the spring of 1986.

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

Extractable Soil Copper and Zinc

The effect of 8-annual applications of Cu as either Cu rich pig manure or $CuSO_4$ on DTPA extractable soil Cu and Zn is shown in Table 9. As expected, treatments which received high levels of Cu application as either source contained much higher levels of DTPA extractable Cu than the control treatments on the three soils. Equivalent increases in DTPA extractable Cu were obtained from an equal rate of Cu application as either Cu rich pig manure or $CuSO_4$ at the Blacksburg and Orange sites, but not at the Holland site. This research will be continued to determine future effects of the Cu sources on amounts of DTPA extractable Cu in the three soils. Overall, the data indicate that Cu was not phytotoxic to corn plants (Table 7) where high levels of DTPA extractable Cu occurred in the three soils from the Cu rich pig manure and $CuSO_4$ applications. Soils that received the high rate of manure also showed higher levels of extractable Zn (Table 9).

Extractable Soil Phosphorus

Research was completed to investigate levels of extractable P from 9- annual applications of Cu rich pig manure. For this research soil samples were obtained from each plot of the three field experiments in the springs of 1982, 1984, 1985, and 1986 prior to treatment applications. Greater than 20 mg P kg^{-1} is considered high based on calibration of the NH_4F extractable P procedure (Olsen et al., 1982). Considerably higher amounts of NH_4F extractable P occurred in the three soils from the pig manure application as compared with the control and $CuSO_4$ treatments (Tables 10, 11, and 12).

Table 9. Effect of eight-annual applications of copper as either copper sulfate or pig manure on DTPA extractable copper and zinc in the Ap horizon of three soils sampled in the spring of 1986.

Cu Treatment		DTPA Extractable	
Amount	Source	Cu	Zn
kg Cu ha^{-1}		-------mg kg^{-1}-----------	
	Field Experiment - Blacksburg		
0.0	Control	1.3c+	6.0b
336.0	Pig Manure + $CuSO_4$	55.4a	9.3ab
336.0	$CuSO_4$	50.3a	8.8b
264.0	Pig Manure	28.7b	12.2a
264.0	$CuSO_4$	29.6b	8.4b
	Field Experiment - Holland		
0.0	Control	1.4d	7.0bc
336.0	Pig Manure + $CuSO_4$	43.3a	9.0b
336.0	$CuSO_4$	37.5b	7.4bc
255.5	Pig Manure	17.4c	13.8a
255.5	$CuSO_4$	18.8c	5.7c
	Field Experiment - Orange		
0.0	Control	2.9c	6.8c
336.0	Pig Manure + $CuSO_4$	49.6a	10.5b
336.0	$CuSO_4$	49.5a	8.4bc
253.0	Pig Manure	49.7a	21.3a
253.0	$CuSO_4$	31.2b	9.0bc

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

Table 10. Effect of copper rich pig manure application on ammonium fluoride extractable phosphorous in Bertie fine sandy loam.

Copper Treatment			
Amount	Source	Year	NH_4F extractable P
kg Cu ha^{-1}			mg kg^{-1}
0	Control	1982	152.7a+
133.2	470.4 mt manure ha^{-1}	1982	196.7a
133.2	$CuSO_4$	1982	182.1a
0	Control	1984	213.9b
234.0	86.4 mt manure ha^{-1}	1984	301.0a
234.0	$CuSO_4$	1984	185.7b
0	Control	1985	185.7b
255.5	890.0 mt manure ha^{-1}	1985	587.7a
255.5	$CuSO_4$	1985	188.1b
0	Control	1986	128.8b
290.7	974.4 mt manure ha^{-1}	1986	491.2a
290.7	$CuSO_4$	1986	130.6b

+Column means for each year followed by different letters are significantly different at 0.05 probability level.

Table 11. Effect of copper rich pig manure application on ammonium fluride extactable phosphurous in Guernsey silt loam.

Copper Treatment			
Amount	Source	Year	NH_4F extractable
kg Cu ha^{-1}			mg kg^{-1}
0	Control	1982	143.0b+
137.5	470.4 mt manure ha^{-1}	1982	291.7a
137.5	$CuSO_4$	1982	157.3b
0	Control	1984	141.8b
245.0	806.4 mt manure ha^{-1}	1984	429.7a
245.0	$CuSO_4$	1984	162.7b
0	Control	1985	135.7b
260.0	890.0 mt manure ha^{-1}	1985	547.4a
260.0	$CuSO_4$	1985	143.0b
0	Control	1986	89.3b
294.4	974.4 mt manure ha^{-1}	1986	560.0a
294.4	$CuSO_4$	1986	93.7b

+Column means for each year followed by different letters are significantly different at 0.05 probability level.

Table 12. Effect of copper rich pig manure application on ammonium flouride extractable phosphorous in Dyke clay loam.

Copper Treatment Amount	Source	Year	NH_4F extractable P
kg Cu ha^{-1}			mg kg^{-1}
0	Control	1982	22.4b[+]
132.4	470.4 mt manure ha^{-1}	1982	116.1a
132.4	$CuSO_4$	1982	22.1b
0	Control	1984	26.6b
234.8	806.4 mt manure ha^{-1}	1984	208.4a
234.8	$CuSO_4$	1984	28.1b
0	Control	1985	33.2b
253.0	890.0 mt manure ha^{-1}	1985	327.5a
253.0	$CuSO_4$	1985	23.5b
0	Control	1986	31.7b
285.7	974.4 mt manure ha^{-1}	1986	386.2a
285.7	$CuSO_4$	1986	44.3b

[+]Column means for each year followed by different letters are significantly different at 0.05 probability level.

The concentration of NH_4F extractable P in the three soils exhibited linear relationships with amounts of manure application (Tables 10, 11, and 12). Correlation coefficients between concentration of NH_4F extractable P and amount of manure application were 0.82, 0.84, and 0.79 ($p=0.01$) for the Bertie, Davidson and Guernsey soils, respectively. Overall the NH_4F extractable P data indicate an accumulation of plant available P in the soils from the Cu rich pig manure application. These data suggest that caution should be exercised in future years of this research to ensure that high P availability does not limit corn yields.

CHAPTER V

SUMMARY AND CONCLUSION

Since as much as 95 percent of the dietary Cu is subsequently excreted in the wastes, the Cu concentration in the manure collected from pigs fed Cu rich diets can be as high as that in sewage sludge. The use of high Cu in pig production has received considerable opposition due to the concern about the potential environmental hazards associated with repeated application of the resulting Cu rich manure to agricultural land. Field experiments were conducted on three soils with diverse chemical and physical properties to evaluate the environmental aspects of long-term use of Cu rich pig manure in corn (Zea mays L.) production, and more specifically to determine the effects of nine-annual applications of Cu rich pig manure on Cu concentraton and yield of corn. A search of the literature revealed that these are the only ongoing long term field experiments in the world which are designed to determine safe Cu loading rates from Cu rich pig manure application and which have appropriate control treatments and replecations.

The soils selected for this research were a Bertie fine sandy loam (Aquic Hapludult), a Guernsey silt loam (Aquic Hapludalf), and a Starr-Dyke clay loam complex (Typic Rhodudult), from the Coastal plain, Ridge and Valley, and Piedmont physiographic regions of Virginia, respectively. The U.S.E.P.A. guidelines indicate that the maximum safe Cu loading rate for the three soils is 280 kg Cu ha^{-1} based on their CEC values and on pH levels of 6.5 or above.

Effects of Cu rich manure application on concentrations of various nutrients in soil samples, plant tissue samples, and on corn yields were determined. These parameters were evaluated by statistical analyses. Conclusions drawn during the course of the investigation are outlined below:

1. The concentration of Cu in the pig feed used for manure production for the field experiments in 1986 was 232.5 mg kg^{-1}. The Cu concentrations on a dry weight basis in batches of manure from pigs fed the Cu rich diets ranged from 1579 to 1853 mg kg^{-1}. These Cu concentrations are within the range of those found in sewage sludge.
2. The plant availability of Cu applied as Cu rich pig manure and $CuSO_4$ was evaluated in long-term field experiments on three soils. During the 9-year period from 1978 through 1986, the manure applied at the cumulative rate of 974.4 mt ha^{-1} supplied from 285.7 to 294.4 kg Cu ha^{-1} to the soils. Injury to corn plants did not occur from application of these high Cu levels from the manure or from equivalent levels of Cu as $CuSO_4$.
3. The ninth annual Cu application as either Cu rich pig manure or $CuSO_4$ increased the total amount of applied Cu above the maximum safe loading rate of 280 kg ha^{-1} based on U.S.E.P.A. guidelines for sewage sludge disposal in the three soils. The corn grain yields were not decreased on the three soils with diverse properties from the high level of Cu application. These data indicate that the U.S.E.P.A. guidelines for sewage sludge disposal are applicable for Cu rich pig manure application on corn land.

4. Applications of up to 294 kg Cu ha^{-1} as either Cu rich pig manure or $CuSO_4$ did not increase Cu concentrations in ear leaves from corn plants grown on the three soils. Copper concentrations in grain from corn plants grown on three soils were not increased by applications of up to 294 kg Cu ha^{-1} as either Cu rich pig manure or $CuSO_4$. A small increase in Cu concentration occurred in corn grain where a cumulative total of 392 kg Cu ha^{-1} was applied either as Cu rich pig manure or $CuSO_4$ to the silt loam soil over the 9-year period. Application of the 392 kg Cu ha^{-1} as $CuSO_4$ did not increase Cu concentrations in grain from plants grown on the clay loam and fine sandy loam soils. In all cases, the Cu concentrations were within the normal range for both corn grains and ear leaf tissue where the high levels of Cu were applied to the three soils during the 9-year period.
5. Eight-annual applications of either Cu rich pig manure or $CuSO_4$ increased DTPA extractable Cu concentrations in the Ap horizon of the three soils. Even though the DTPA extractable Cu levels were high, there was no evidence of toxicity of Cu to corn plant grown on the three soils. The highest DTPA extractable Cu level in the soil of 55.4 mg kg^{-1} occurred on the Guernsey soil from application of 336.0 kg Cu ha^{-1} as manure and $CuSO_4$.
6. High levels of NH_4F extractable P were found where Cu rich pig manure was applied to the soils annually for eight years. The build-up of excessive quantities of P represents one of the hazards associated with repeated application of large amounts of manure. It would be desirable to decrease the rate of Cu rich pig

manure application to ensure that high P availability does not decrease plant growth on the three soils in the future.

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