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Uptake of Native and Applied Copper by Corn and Wheat as
Related to Soil Properties

by

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(ABSTRACT)

Copper and Zn deficiencies of crops have been reported in various parts of the world. To prevent these deficiencies sulfate forms of Cu and Zn are generally used. In addition, Cu and Zn also are contributed to soils by manures, pesticides, and waste materials such as sewage sludge. An insufficient supply of Cu or Zn may reduce crop yield; whereas, excess Cu or Zn may be detrimental to both plant and animals.

The field phase of this research was conducted on a Davidson silty clay to evaluate corn response to high levels of CuSO_4 and ZnSO_4 applications up to 338 kg Cu and 830 kg Zn ha^{-1} . The results of this research indicated that corn grain and stalk yields of corn were not affected ($\alpha = 0.05$) by high levels of CuSO_4 and ZnSO_4 either alone or combined. Copper concentration in neither corn grain nor ear leaves was affected ($\alpha = 0.05$) by the Cu application. Zinc concentrations in both grain ($\alpha = 0.01$) and ear leaves ($\alpha = 0.001$) were increased by ZnSO_4 application. Application of ZnSO_4 increased the Cu in the labile pool, whereas CuSO_4 fertilization did not affect Zn in the labile pool.

A greenhouse experiment was conducted to examine the response of wheat to Cu application on 14 soils. Treatments in this study were a control and 5.35 mg Cu kg⁻¹ as CuSO₄. Dry matter yields were not affected ($\alpha = 0.05$) by Cu fertilization whereas tissue Cu levels were increased ($\alpha = 0.001$). Copper uptake was highly correlated with DTPA-Cu ($r = 0.80$, $\alpha = 0.001$), but weakly correlated with Mehlich III-Cu ($r = 0.28$, $\alpha = 0.05$). The DTPA-Cu showed high correlations with clay content ($r = 0.98$, $\alpha = 0.001$) and SA ($r = 0.93$, $\alpha = 0.001$); whereas, Mehlich III-Cu did not correlate with these variables ($\alpha = 0.05$).

A second greenhouse experiment was conducted with 10 Virginia soils to evaluate the response of corn to Cu fertilization. Treatments were a control and 5.35 mg Cu kg⁻¹ as CuSO₄. Dry weights of corn on the 10 soils were not affected ($\alpha = 0.05$) by Cu application; whereas, tissue Cu levels were increased by the applied Cu ($\alpha = 0.001$). Copper uptake by corn plant correlated with DTPA-Cu ($r = 0.62$, $\alpha = 0.001$) and with soil pH ($r = 0.35$, $\alpha = 0.05$). There was no correlation of organic matter with Cu uptake ($\alpha = 0.05$). Both DTPA-Cu and Mehlich III-Cu were unrelated to either organic matter or soil pH ($\alpha = 0.05$).

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TABLE OF CONTENTS

Introduction	1
Literature Review	4
2.1 Copper in Soil	4
2.2 General Chemistry	4
2.3 Mineral Forms of Copper	6
2.3.1 Copper Containing Minerals in Soil	7
2.4 Anthropogenic Addition of Copper	8
2.5 Forms of Copper in Soil	11
2.5.1 Water Soluble Copper	12
2.5.2 Organically Bound Copper	14
2.5.3 Copper in Oxides and Hydroxides and Clay Minerals	18
2.6 Copper in Plants	22
2.6.1 Root Uptake of Copper	22
2.6.2 Other Factors Involved in Copper Absorption	25
2.7 Genotypic Differences in Copper uptake	26
2.7.1 Copper Concentration in Plants	30
2.7.2 Mobility of Copper within the Plant	32
2.7.3 Interaction of Copper with other Ions	34
2.7.4 Physiology of Copper Deficient Plants	37
Literature cited	41

Response of Corn to Long-term Application of high levels of Copper Sulfate and Zinc Sulfate	53
3.1 Introduction	54
3.2 Materials and Methods	55
3.2.1 Field Experimentation	56
3.2.2 Corn Tissue Analysis	58
3.2.3 Soil Analyses	58
3.2.4 Statistical Analyses	59
3.3 Results and Discussion	59
3.3.1 Soil Properties	60
3.3.2 Yield	60
3.3.3 Tissue Concentration of Copper and Zinc	64
3.3.4 DTPA Extractable Copper and Zinc	68
 Literature cited	 70

Response of Wheat to Copper Sulfate Application in Four- teen Virginia Soils	73
4.1 Introduction	74
4.2 Materials and Methods	75
4.2.1 Wheat Tissue Analysis	77
4.2.2 Soil Analyses	77
4.3 Statistical Analysis	78
4.4 Results and Discussion	79
4.4.1 Soil Properties	79
4.4.2 Dry Matter Yield	82

4.4.3	Tissue Copper Concentration	84
4.4.4	DTPA and Mehlich III Extractable Cu	85
4.4.5	Copper Uptake	87
Literature cited		90
 Response of Corn to Copper Application on Ten Virginia		
	Soils	93
5.1	Introduction	94
5.2	Material and Methods	95
5.2.1	Corn Tissue Analyses	96
5.2.2	Soil Analysis	97
5.2.3	Statistical Analysis	98
5.3	Results and discussion	98
5.3.1	Dry Matter Yield	101
5.3.2	Tissue Copper Concentrations	101
5.3.3	DTPA and Mehlich III extractable Copper	103
5.3.4	Copper Uptake and Percent Copper Uptake	107
Literature cited		111
Summary and Conclusions		113

LIST OF TABLES

Table 1.	Cumulative amounts of Cu and Zn applied to the long-term field experiment on the Davidson soil.	57
Table 2.	Physical and mineralogical properties of the Davidson soil under study.	61
Table 3.	Chemical properties of the Davidson soil under study.	62
Table 4.	Corn grain and stalk yields as affected by 19-annual applications of various levels of Cu and Zn to the Davidson soil.	63
Table 5.	Copper and Zn concentrations in corn ear leaf tissue as affected by 19 annual applications of Cu and Zn to Davidson soil.	65
Table 6.	Copper and Zn concentrations in corn grain as affected by 19-annual applications of Cu and Zn to Davidson soil.	66
Table 7.	DTPA extractable copper and zinc in Davidson soil after 18-annual applications of copper sulfate and zinc sulfate.	69
Table 8.	Properties of soils used to evaluate Cu uptake by wheat plants when fertilized with copper sulfate.	80
Table 9.	Classification of 14 Virginia soils used in the greenhouse experiment.	81
Table 10.	Dry matter yield and tissue Cu concentrations of wheat grown on 14 different soils with or without Cu fertilization.	83
Table 11.	DTPA and Mehlich III-Cu in 14 different soils prior to Cu application.	86
Table 12.	Relationship of Cu uptake by wheat with extractable Cu and soil properties.	88
Table 13.	Properties of soils used to evaluate Cu uptake by corn plants when fertilized with copper sulfate.	99
Table 14.	Classification of 10 Virginia soils used in the greenhouse experiment.	100

Table 15.	Dry matter weights and tissue Cu concentrations of corn grown on 10 different soils with or without Cu fertilization.	102
Table 16.	DTPA and Mehlich III extractable Cu in 10 soils prior to Cu application.	104
Table 17.	Relationship of Cu uptake and extractable Cu with soil properties.	106
Table 18.	Uptake and percent uptake of applied copper by corn on 10 different soils.	109
Table 19.	Relationship of uptake and percent uptake of applied copper with soil properties.	110

CHAPTER 1

INTRODUCTION

Copper (Cu) is one of the seven micronutrients considered to be essential for plant growth. Before the turn of this century Cu was regarded as a plant poison, as indeed it may be when applied in excessive amounts. After the turn of this century, many researchers working independently observed that use of Cu containing fungicides such as the 'Bordeaux' mixture improved vigor and yield of plants. Grosenbacher (1916) in Florida reported that application of the 'Bordeaux' mixture to citrus trees or incorporation of copper sulfate (CuSO_4) into soil controlled 'die back' and improved yield and vigor. Felix (1927) reported a similar response on yield and vigor for lettuce (Lactuca sativa) and onions (Allium cepa) by foliar application or soil incorporation of CuSO_4 . It was believed that Cu was responsible for the increase in yield and plant vigor. Thereafter, in the 1930's Cu was shown to be an essential element for plant growth. Later, crop failures due to Cu deficiency were reported in many parts of the world and a global interest in Cu research was thus developed.

The primary source of the labile Cu supply in soil is from rock weathering. In addition, a considerable amount is contributed by anthropogenic activities such as application of

fertilizers, manures and pesticides. The plant availability of this element from soil is mainly governed by the physical and chemical factors of the soil, which vary among soils.

Since Cu is required by all plants and animals in micro amounts and in a very narrow range, little excess or shortage may cause toxicity or deficiency, respectively. Similar to other cations, Cu is involved in enzyme activities. Deficiency symptoms of Cu have been identified since the 1920's and, since then, have been observed on soils in the northeastern part and sandy eastern coastal plains of the United States (Cunningham, 1972). The deficiency of Cu also has occurred in many parts of the world where Histosols were brought into crop production.

Copper has a high affinity to chemically interact with soil minerals and organic matter (OM) and to form precipitates with sulfides, carbonates and hydroxides, and thus exists in many forms in any given soil environment (McBride, 1981). Due to the abundance of Cu in the form of Cu^{2+} compared with Cu^+ under normal soil conditions, plants absorb Cu in the form of Cu^{2+} (Graham, 1981). The rate of Cu^{2+} uptake by plant roots varies proportionately with the Cu^{2+} activity (or effective concentration) at the root surface (Baker, 1971).

Adequate labile soil Cu is required for successful crop production. Due to the low requirement by plants and the abundance in soils, Cu depletion in soil due to crop removal

is negligible. However, in certain soils even with with abundant levels of total Cu, crops may exhibit severe deficiency symptoms as a result of suppressed Cu availability due to physical and chemical soil properties. Under such situations, Cu supplementation is required to increase crop yields. The objectives for the research reported herein were as follows:

1. To determine if selected Virginia soils supplied sufficient Cu for corn (Zea mays L.) and wheat (Triticum aestivum L.) growth.
2. To relate selected soil properties to Cu uptake by corn and wheat plants, and
3. To determine the response of corn to an excessively high level of Cu application.

CHAPTER 2

LITERATURE REVIEW

2.1 COPPER IN SOIL

Copper deposits have been formed throughout geological time and are widely distributed geologically and geographically. Copper ore bodies are very rare and occupy only a small fraction of known rock formation. The average content of Cu in the earth's crust is about 70 mg kg⁻¹ (Hodgson, 1963). Copper has the chemical properties to interact with soil mineral and organic components and to form precipitates with sulfides, carbonates, hydroxides and other anions (McBride, 1981).

2.2 GENERAL CHEMISTRY

Copper is by definition a transition metal element, for it possesses partially filled d orbitals. Due to its high molecular weight, it is called a heavy metal, and in reference to its scarcity in the surface environment, it is referred to as a trace element. In plant nutrition, Cu is known as a micronutrient, not because it is unimportant, but because it is required in minute amounts.

The electronic structure of the free Cu atom is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4s^1$, which leads to production of two ionic forms; Cu^+ the first oxidized form, loses a $4s^1$ electron to become $3d^{10}$, whereas Cu^{2+} the second oxidized form, loses a $4s^1$ and a $3d^{10}$ electron to attain a $3d^9$ configuration. The first oxidized state (Cu^+) is very rare in the soil environment, but can exist under extremely reduced (electron rich) conditions. The Cu^+ ion is very unstable at ordinary temperatures in concentrations greater than approximately 10^{-7} M; at higher concentrations, it exists in solution mainly in the form of anionic complexes such as $CuCl_2^-$ (Krauskopf, 1972).

In solution, Cu^+ easily oxidizes to Cu^{2+} and thus the most relevant Cu species in soil solution is the aquated Cu^{2+} ion, $Cu(H_2O)_6^{2+}$. Since natural ground water has a pH of 5 to 8 and since $Cu(H_2O)_6^{2+}$ is a moderately strong acid ($pK_a = 6.8$), its conjugated base, $CuOH(H_2O)_5^+$, is common in ground waters (Parker, 1981). If the pH of the ground water is higher than 9 or if solutions contain sufficiently high Cu concentrations, solid species such as CuO and $Cu(OH)_2$ may precipitate from a $Cu(H_2O)_6^{2+}$ solution (Parker, 1981). Due to the high affinity of Cu^{2+} for hydroxyl groups (OH^-), most Cu^{2+} salts contain either water of crystallization or OH^- groups (Parker, 1981).

2.3 MINERAL FORMS OF COPPER

Copper released to the geological cycle upon weathering may accumulate to some extent in sedimentary rocks, being either adsorbed on clay surfaces or precipitated as sulfides, and does not easily substitute into the carbonate structure. Therefore, carbonate rocks are generally low in Cu (McBride, 1981). Copper is more abundant in basaltic than granitic rocks (Krauskopf, 1972). The higher amount of Cu in basaltic rocks than in granitic rocks is due to the greater occurrence of sulfides and ferromagnesium silicates in the basaltic rocks (McBride, 1981). While the predominant minerals of Cu in the earth's crust are sulfides (CuS, Covellite; Cu_2S , chalcocite; CuFeS_2 , calcopyrite), the metallic form of Cu also is common in reducing environments (McBride, 1981) and was found in masses weighing several tonnes in the lake Superior region of the United States (Parker, 1981). Upon exposure to aerobic conditions at the earth's surface, Cu^+ and metal Cu are oxidized to the 2+ oxidation state. On the other hand, highly reducing conditions such as flooding of soils may cause Cu^+ or even Cu metal to be thermodynamically more stable than Cu^{2+} (Lindsay, 1979).

2.3.1 Copper Containing Minerals in Soil

The minerals that contain Cu as a major constituent are mainly the sulfides (CuS, covellite; Cu₂S, chalcocite; CuFeS₂, calcopyrite) and the hydroxy carbonate (Cu₂(OH)₂CO₃). A number of secondary minerals of Cu, including oxides, carbonates, silicates, sulfates and chlorides can form, although all are relatively soluble. However, under low pH and leaching conditions at the earth's surface, most of these minerals would not persist. In addition, it has been demonstrated that these Cu minerals, which are expected to be the least soluble under the conditions at the earth's surface, are much too soluble to control the very low activity of Cu²⁺ in soil solution (Lindsay, 1979; Cavallaro and McBride, 1980).

Other Cu minerals include chrysocolla (CuSiO₃), which forms under acid environments in the presence of high amounts of soluble SiO₃, and tenorite (CuO), a rare mineral in the earth's crust, which forms only in the near absence of other anions (Krauskopf, 1972). The hydroxide of Cu [Cu(OH)₂], which is a poorly ordered initial neutralization product of Cu solutions, readily recrystallizes to oxides or hydroxycarbonates with aging (Cotton and Williamson, 1966).

The concentration of Cu in the earth's crust is estimated to vary from 24 to 55 mg kg⁻¹ (Cox, 1979), while the total content of the element in soil is reported to range from 1

to 80 mg kg⁻¹ (Krauskopf, 1972). This large range in total Cu accumulation in soils is due to two main factors, parent material and soil formation processes, which govern the initial amount of Cu in soils (Kabata-Pendias and Pendias, 1985). Soil Cu concentrations generally reflect the Cu contents of the soil forming materials and are not subject to severe depletion by weathering and leaching processes. The distribution of Cu in the soil profile shows little variation other than an accumulation in the A1 horizon due to plant cycling and a decrease in the A2 horizon due to eluviation (Hodgson, 1963). Since Cu is highly absorbed by soil oxide minerals and organic matter, leaching of Cu is unlikely to be an important process in soils (McLaren et al., 1981).

2.4 ANTHROPOGENIC ADDITION OF COPPER

Mining and processing of metallic Cu, along with the disposal of water containing Cu, has in some areas caused major increases in soil Cu and the availability of Cu to plants and other organisms. In addition, the contribution of Cu by aerial depositions of particulates near smelters (Costescu and Hutchinson, 1972) and urban areas (Parker et al., 1978) has increased both soil and plant Cu concentrations. These metal deposition patterns, though depending to a considerable extent on climatic conditions such as wind and rainfall distributions (Cartwright et al., 1977), generally decline

exponentially with distance from the smelter (Kuo et al., 1983).

High concentrations of Cu in soils retard root development and possibly inhibit the Cu uptake mechanism and, as a result, dramatic increases in plant shoot Cu levels seldom occur, which is in contrast to Zn and Cd accumulation in plant shoots (Bennett, 1977). Copper phytotoxicity has occurred in orchards and vine yards where the 'Bordeaux' mixture has been used as a fungicide over many years (Chaney and Giordano, 1977).

Much recent research has dealt with application of heavy metal containing domestic and industrial sludges to agricultural land. Sewage sludge contains from 250 to 1700 mg Cu kg⁻¹ and a variety of other heavy metals (Allaway, 1977). Sewage sludge application as an effort to recycle micronutrients can have adverse effects on crop production (Allaway, 1977). The susceptibility of the crops to phytotoxicity from long-term application of sludge metals including Cu varies with the type of crop. Vegetable crops are much more susceptible to injury from long-term application of sludge metals such as Cu, Cd, Zn and Ni at lower levels of soil pH (Chaney and Giordano, 1977). An increase in soil pH from limestone application overcomes Cu phytotoxicity in corn (Hinesly et al., 1979). Forages showed some tolerance to elevated levels of Cu application, but did not appear to accumulate Cu in above ground tissues (Allaway, 1977).

Farm yard manure, such as swine and poultry manures, also is applied as a soil amendment to agricultural land. Copper salts are included in the diet of monogastric animals like swine, to enhance the growth rate and, as a result, manures from these animals contain high levels of Cu (Davis, 1974). Swine feed containing 250 mg kg⁻¹ of Cu can result in 600 to 900 mg kg⁻¹ of Cu in manure. Such manure has been applied to agricultural lands in Europe over long periods of time without any adverse effect. However, there is a concern in the United States regarding accumulation of Cu in soils from long-term application of high Cu manure (Baker, 1971).

Field studies with Cu 'enriched' manure indicated minimal Cu uptake by forage crops (Batey et al., 1972) and by corn (Kornegay et al., 1976). In fact continued application over three years on several Virginia soils have resulted in no increases in Cu concentrations in corn ear leaf or grain (Martens et al., 1981). Similar results were observed by application of Cu as CuSO₄ (Martens et al., 1981) indicating that relatively high Cu levels in the soil have a limited availability to the plants. This supports the suggestion by Elsokkary and Lag (1978) that Cu availability to the plants may be governed by a dynamic equilibria between metal fractions, rather than by total Cu content, and that this low Cu availability mainly is due to complex formation of Cu with soil hydrous oxides and organic matter.

2.5 FORMS OF COPPER IN SOIL

Forms of Cu present in soil can be categorized based on solubility, exchange reactions and chemical forms as follows: a) water soluble; b) exchangeable; c) adsorbed, chelated or complexed; d) in secondary clay minerals and insoluble metal oxides; and e) in primary minerals (Viets, 1962). McLaren and Crawford (1973a) suggested a similar Cu suite in soils and developed a fractionation procedure to separate exchangeable Cu, specifically bound Cu, occluded Cu in soil oxides and residual Cu mainly in the clay lattice structure. The respective fractions were extracted in sequence with the following reagents: 0.05 M CaCl_2 , 2.5% acetate acid, 0.1 M potassium pyrophosphate ($\text{K}_4\text{P}_2\text{O}_7$), Tamm's reagent and hydrofluoric acid (HF). Exchangeable, water soluble, specifically adsorbed and organically bound Cu are in dynamic equilibrium (Lindsay, 1972). The Cu present in the water soluble, exchangeable and adsorbed forms are considered as readily available to plants, whereas, Cu associated with primary and secondary soil minerals is relatively unavailable to plants and has very little importance in plant nutrition (Viets, 1962).

Total Cu in the soil is no more than an inventory for it does not indicate the amount of Cu available to plants growing on that soil (Gilbert, 1952). Therefore, it is quite

possible for a soil to be high in total Cu and yet to be low in Cu availability.

2.5.1 Water Soluble Copper

Water-soluble Cu is readily available to plants and is replenished by other fractions as it is depleted by plant uptake (Viets, 1962). Many workers use 1:2 or 1:10 soil-to-water ratios to remove water soluble Cu (Viets, 1962). Amounts of water-soluble Cu extracted from soils vary with these ratios, extraction times used, organic matter contents (Deramer and Smith, 1964) and redox potentials (Sims and Patrick, 1978). The association and hydrolysis constants of Cu^{2+} indicate that formation of significant concentrations of soluble nitrate, sulfate, chloride and phosphate complexes are unlikely in most soil solutions (Sanders and Bloomfield, 1980). Only Cu hydroxyl and carbonate complexes are expected to exist in significant amounts in soil solutions (Baes and Mesmer, 1976)

Measurement of water soluble Cu is difficult due to its low concentrations, which is in $\mu\text{g kg}^{-1}$ range (Kabata-Pendias and Pendias, 1985). Research indicates, that at higher pH levels, Cu in soil solution does not exist as inorganic complexes, but exists mainly as organic complexes (Hodgson et al., 1965, 1966). The total amount of complexed Cu in soil solution generally increases above pH 7 due to the greater

solubility of organics (McBride and Blasiak, 1979), while the amount of "free" Cu^{2+} at higher pH levels is much lower and commonly is in the range of 10^{-8} to 10^{-9} M (McBride and Blasiak, 1979; Hodgson et al., 1965). The amount of water soluble Cu extracted from the A horizons of three New York soils varied from 5.0 to 18.0 $\mu\text{gCu kg}^{-1}$ and the fraction organically complexed in solution was 79% at soil pH 4.5 and 99.2% at soil pH of 9.0 (Hodgson et al., 1965). The amount of Cu in soil solution in a calcareous Colorado soil was 3.5 $\mu\text{gCu kg}^{-1}$ and that present as an organic complex varied from 96.0% at a soil pH of 7.6 to 99.8% at a soil pH of 7.9 (Hodgson et al., 1966).

Water soluble Cu is susceptible to changes induced by drying soil samples prior to extraction. When the soil is dried to about 100 °C there is considerable breakup of humus materials, which will eventually change the concentration of water soluble Cu as compared with the undried soil (Bartlett and James, 1980). Dehydration apparently increases the reduction of Mn in soil oxides (Leeper, 1970), which increases the amounts of soluble Mn and associated Cu in soil solution (Schuman, 1980). The amount of Cu released from the Mn oxides, which is present as free Cu^{2+} , depends on soil pH, on amount of soluble organic matter in soil solution, and on the competition for functional groups of the dissolved organic matter.

2.5.2 Organically Bound Copper

Organic matter in soil has a high affinity for Cu^{2+} and this reaction is of importance in plant nutrition, because the plant available soil Cu includes organically-bound Cu form as well as adsorbed and water soluble Cu (Viets, 1962). In soil systems, extractable Cu levels appeared to be more strongly bonded to organic matter than to phyllosilicates (Lombin, 1983). For decades, soil scientists have recognized the high capacity of organic matter to complex Cu even at low pH levels (Lees, 1950). As Kabata-Pendias and Pendias (1985) cited, humic acid of a soil containing 40 g kg^{-1} of organic matter may complex $1,517 \text{ kg Cu}$, $17,927 \text{ kg Fe}$, $1,015 \text{ kg Zn}$, 913 kg Mn and $4,500 \text{ kg Pb ha}^{-1}$. The more widely used schemes for fractionating soil Cu is that of McLaren and Crawford (1973). The organically bound form is extracted in this sequential fractionation procedure with 0.1 M potassium pyrophosphate. From about 20 to 50 g kg^{-1} of the total Cu in 24 soils with diverse properties was accounted for in the organically-bound form (McLaren and Crawford, 1973).

Organic constituents form both soluble and insoluble complexes with Cu (McBride, 1981). The wide variety of compounds are involved in Cu complexation reactions include, aliphatic acids, phenolic acids, peptides and proteins, polysaccharides, and humic and fulvic acids (Stevenson and Ardakani, 1972). Thus far, greater attention has been di-

rected toward bonding of Cu in soil with humic and fulvic acids than with other organic compounds.

Soil organic matter components are broadly defined by dissolution methodology. Humic acid is a soil organic material which is soluble in alkali and insoluble in acid and which possesses a high molecular weight ($\cong 300,000$ average) and a total acidity of 500 to 800 cmol (+) kg^{-1} . Fulvic acid, which is soluble in both alkali and acid, has a much lower molecular weight ($\cong 2000$ average) and a higher acidity of 900 to 1400 cmol (+) kg^{-1} (Stevenson and Ardakani, 1972). The major functional groups in fulvic and humic acids are the carboxylic (-COOH), phenolic (-OH) and amino (-NH₂) groups, which together constitute a larger percentage of the total acidity in fulvic acid than in humic acid. As a result, fulvic acid complexes more Cu than humic acid. In contrast to the amount of complexation, humic acids bond more tightly ($\log K = 9.1$) with Cu (Rosell et al., 1977) than fulvic acids ($\log K = 4.0$) (Schnitzer and Hansen, 1970).

The increase in stability constants of Cu humate complexes with an increase in degree of humification has implications regarding the mechanism whereby Cu is maintained in a soluble forms in soils (Preston et al., 1981). A sequence is envisioned in which Cu present in low concentrations is immobilized by complexation with humic acids. However, as the stronger binding sites become saturated, an increasing amount of the Cu will be solubilized through the action of

fulvic acids and simple organic compounds (Stevenson and Fitch, 1981). Therefore, the plant available portion of Cu comprises much more Cu associated with the smaller molecules of fulvic acids than with the larger molecules of humic acids (Preston et al., 1981).

The mechanism of metal retention by organic Cu complexing materials has been elucidated by a variety of methods. Selective blockage of carboxyl, amide and phenolic groups by methylation and esterification resulted in a drastic reduction in Cu exchange capacity (Broadbent and Bradford, 1952) and, later, similar results were obtained for Zn (Hims and Barber, 1957). Blockage of carboxyl and phenolic groups, either separately or simultaneously, reduced metal complexation, but the greatest reduction occurred when both groups were blocked (Schnitzer and Skinner, 1965). Blockage of alcoholic or N containing groups did not affect metal complexation (Schnitzer and Skinner, 1965). Gamble and Schnitzer (1974) concluded that adjacent carboxyl and phenolic groups were largely responsible for metal retention by forming multiple bonds with the metal. There is evidence that some types of bonding sites in organic materials are purely electrostatic in nature (Mortenson, 1963). McBride (1981) suggested that Cu is only marginally limited in its rotation by the weak humic ligand field, and for this reason, he did not find any chelation or covalent bonding of Cu humic acid by electron spin resonance (ESR) analysis.

The importance of metal-organic interactions in soil sediment environments is widely appreciated by both environmental and soil scientists. Organic matter destruction in soil has been shown to decrease Cu retention (Hims and Barber, 1957), and alkali extraction of humic acid removed nearly 100% of the total Cu from lake sediments (Nriagu and Coker, 1980). Scholkovitz and Copland (1981) suggested that solubility of humic acid trace metal complexes, including Cu complexes are a reverse of these predicted from inorganic solubility considerations. The complexing of these ions with humic substances led to solubilization at high pH (3 to 9.5) and precipitation at low pH (3 to 1). Schnitzer and Kerndorff (1981) established the order of the affinity of metal ions to form water insoluble complexes with fulvic acid (this order depends on pH of the medium) as follows:

Fe = Cr = Al > Pb = Cu > Hg > Zn = Ni = Co = Cd = Mn

The solubility of fulvic acid complexes is strongly controlled by the fulvic acid-metal ratio. Formation of water insoluble complexes are favored when the ratio is lower than two.

Soluble fulvic acid molecules are able to increase soluble metal levels, to desorb metals from sorption sites, and to dissolve precipitates (Jackson and Skippen, 1978). At higher pH levels (as in calcareous soils and reduced sediments),

fulvic acids tends to be more soluble and to possess a high complexing ability: this relationship was shown by organic complexation of Cu in displaced soil solutions of 900 and 600 mg kg⁻¹ in calcareous and acid soils, respectively (Hodgson et al., 1966). Copper-fulvic complexes with low stability constants are readily soluble and release Cu into soil solution for plant sorption (Kabata-Pendias and Pendias, 1985). Despite the high affinity for Cu and the ubiquitous occurrence of organic materials, Jenne (1968) has argued that the importance of organics is over-emphasized in soil sediment Cu reactions, and makes a convincing case that hydrous oxides of Fe and Mn may be more active in Cu adsorption than organic matter. In reduced soils and sediments, organic matter may play a dominant role; whereas, in oxidized environments, hydrous oxides may dominate Cu adsorption reactions.

2.5.3 Copper in Oxides and Hydroxides and Clay Minerals

Several oxides and hydroxides of Al, Fe, Mn, Si and Ti occur in soils. Specific adsorption of Cu by these hydrous oxides surfaces has important implication for the mobility, retention and plant availability of Cu (McKenzie, 1980). Iron and Mn oxides and Al hydroxides are of major importance as trace elements cation adsorbers in soils.

Oxides and hydroxides of Fe and Mn are relatively common constituents in soils and have pigments (mainly Fe oxides)

which determine the color of the soil. These oxides and hydroxides are present in numerous mineral forms and in microcrystalline and amorphous oxides and hydroxides (Kabata-Pendias and Pendias, 1985). In addition, these oxides and hydroxides occur as coating on soil particles, as fillings in cracks and veins, and as concretions and nodules. By electron probe procedures, it was found that many trace elements in soil including Cu are concentrated along the deposited oxides in soil materials (Norrish, 1975). The oxides have a higher sorption capacity for trace elements, of which large amounts can be accumulated in nodules (Norrish, 1975). Mechanisms of sorption involve isomorphous substitution of a divalent or trivalent cation for Al, Fe and Mn ions, cation exchange reactions, and oxidation effects at the surface of the oxide precipitate. The amount of a particular ion such as Cu, that is adsorbed depends mainly on the pH of the equilibrium solution. The maximum adsorption values for various ions on Fe oxides occur between pH 4 and 5 (Schwermann and Taylor, 1977).

Unlike many other metallic ions, Cu^{2+} can be "specifically" adsorbed by layer silicate clays; by Al, Fe and Mn hydrous oxides; and by organic matter. The amount of adsorption on these materials increases with an increase in soil pH (Murray, 1975). Adsorption studies with synthetic Fe and Mn oxides have clearly demonstrated the high affinities of these surfaces for metal cations. Cobalt, Cu and Ni were much more

strongly adsorbed to these surfaces than the alkali or alkali earth cations. The heavy metals were sorbed at pH levels below the zero point of charge of the oxide surfaces, and the amount of adsorption increased with an increase in pH up to the point of hydroxide precipitation (Murray et al., 1968). Oxides of Mn, which had a higher affinity for trace metals than Fe compounds, adsorbed metals at $\text{pH} < 3$ (McKenzie, 1980).

Iron and Mn oxides commonly occur as coating on clay size particles in soils and sediments and, thus, exhibit a high surface area (SA). Since both Fe^{3+} and Mn^{4+} in oxide structures are reducible under poorly aerated conditions (low Eh) to the more soluble Fe^{2+} and Mn^{2+} , fluctuations in Eh result in cyclic dissolution and precipitation phases. These fluctuations tend to retard crystallization of the oxides, leaving them in a more disordered state and result in high SA materials (Jenne, 1977). Crystalline forms of these materials are difficult to detect in soil by X-ray diffraction analysis (Schwertmann and Taylor, 1977) and, therefore, some researchers indicated that most of the Mn materials had an amorphous structure (Ross et al., 1976).

Copper adsorption on layer silicates is depressed if the pH is maintained at relatively low values (< 5). Thus, Cu^{2+} and Ca^{2+} have approximately equal preference for exchange sites of montmorillonite at low pH (El-Sayed et al., 1970). Electron spin resonance (ESR) investigations of

adsorbed Cu^{2+} on fully wetted smectites and kaolinites have shown that the metal tumbles freely as the hexaquo $\text{Cu}(\text{H}_2\text{O})_6^{2+}$ and that the hydrated Cu^{2+} is held tightly in the interlayers of vermiculite. Adsorbed heavy metal cations on hydrous oxides and clay mineral surfaces are readily exchanged by other cations. Decreases in pH as heavy metal cations were adsorbed by hydrous oxides and clay minerals indicated a release of protons from the adsorbate surface (Farrah and Pickering 1976, 1977; Forbs et al., 1976).

When heavy metal cations, such as Cu^{2+} , are specifically adsorbed on Al, Fe or Mn hydrous oxide surfaces, one to two moles of protons are released per mole of sorbed heavy metal cation (Padmanabhan, 1983). The desorption of heavy metals from adsorbed surfaces is not the reverse of adsorption, because hysteresis occurs (Hodgson et al., 1965). The apparent hysteresis between adsorption and desorption was attributed to the existence of two types of adsorption sites with different bonding constants, which were described as "readily desorbed" and "less readily desorbed" fractions. The gradual interchange of some "readily desorbed" Cu^{2+} into the "less readily desorbed" category was attributed to a time dependent reaction involving isomorphous substitution of lattice Fe^{3+} by Cu^{2+} (Padmanabhan, 1983).

2.6 COPPER IN PLANTS

One of the important factors that determines the biological availability of a micronutrient is its adsorption by soil constituents. In general, plants readily take up the species of trace elements that are dissolved in soil solution either in an ionic or a complexed form (Kabata-Pendias and Pendias, 1985). Metals in soil solution often associate with organic ligands to form relatively stable complexes (Stewart, 1963). The stability of complexes and the proportion of metal to be found in the complexed form varies from metal to metal. Copper usually is considered to have a greater propensity towards complex formation than other biologically significant metal ions. Little information is available relative to uptake of complexed metals, the exceptions to this being the uptakes of Fe and Zn supplied as synthetic chelates (De Kock and Mitchell, 1957) and as natural organic metal complexes (Marquenie-vander Werff and Out, 1981).

2.6.1 Root Uptake of Copper

The absorption of micronutrients by roots can be either passive (nonmetabolic) or active (metabolic). Numerous studies indicated that the mechanism of Cu absorption by plant roots is by the active process; but, passive absorption

also is likely to occur, especially in the toxic range of Cu in soil solution (Kabata-Pendias and Pendias, 1985).

Since the amount of Cu required by plants is small, i. e., 5 to 20 mg Cu kg⁻¹ (Jones, 1972), Loneragen (1975) favored the view that absorption of Cu is under metabolic control. Graham (1979) suggested that, if the absorbed species is the Cu²⁺ cation, energy may be required only to maintain the membrane potential for the electrochemical gradient to enable absorption of Cu²⁺ from external concentrations as low as 10⁻¹³ M. Studies which show that addition of chelates to the growth medium decreases the rate of Cu absorption support the conclusion that Cu²⁺ is the commonly absorbed species by plants (De Kock and Mitchell, 1957; Dragen et al., 1976). Dragen et al. (1976) concluded that Cu deficiency in corn was associated with a Cu²⁺ concentration of less than 10⁻¹³ M.

A considerable number of highly specific adsorption sites for Cu occur in root cell walls (Larsen, 1966; Harrison et al., 1979). These specific adsorption sites may play an important role in Cu absorption by plants. In root tissues, Cu is almost entirely in complexed forms, whereas, it most likely enters root cells in a dissociated form.

Graham (1981) compared the results of different studies carried out on the rates of Cu absorption by higher plant roots and indicated that these rates are among the lowest of the essential micronutrients. These rates varied from pico to micro-mole of Cu hr⁻¹g⁻¹ (DW) of roots in the physiological

concentrations range. Several reports indicated that the concentrations of Cu in roots were proportional to those in the soil or solution medium over a wide range of levels of Cu supply, while the concentration in the shoots was not a function of rate of supply (Dragen et al., 1976; Graham, 1979; Graham et al., 1981).

Of primary importance in studies on absorption of Cu is to recognize that most chelates bind Cu^{2+} strongly as a result of a high ligand field stabilization energy (Parker, 1981). Due to strong preference of Cu^{2+} for tetragonal coordination geometry and due to its affinity for peptides, N provides a potential basis for selectivity in Cu absorption, firstly in distinguishing Cu^{2+} from Cu^+ and secondly in selecting Cu^{2+} from Zn^{2+} (tetragonal geometry) and Mn^{2+} which have low affinities for such chelates (Graham, 1981). It is also important to recognize the high affinity of Cu^{2+} for carbonyl, thiol, hydroxyl, carboxylic and phenolic groups since these are present in cell walls. These groups provide cell walls sites for strong and specific adsorption of Cu which is not easily desorbed (Harrison et al., 1979).

Even though the mechanisms of Cu absorption by roots are far from clear, some evidence in both soil and solution environments showed that Cu is entirely complexed either by root exudates or by organic ligands of soil origin (Geering and Hodgson, 1969; Graham, 1979). There is considerable evidence that Cu is not absorbed by roots as a chelate whether

the ligand is EDTA or soil humic material. For example, copper is absorbed more rapidly from Cu^{2+} solutions from inorganic compounds than from Cu-EDTA or Cu-DTPA (Dragen et al., 1976). High molecular weight humic materials extracted from soil also decreased the rate of absorption of Cu (Petruzzelli and Guidi, 1976). Although, Cu exists in the root environment mainly as chelates, it appears that the ligand normally dissociates from Cu prior to Cu absorption.

Mineral elements do not readily leak out of the plasma lemma of root cells once they are absorbed. Yet, for absorption, ions must penetrate this fairly impermeable membrane. This idea led to the carrier hypothesis. According to the carrier hypothesis, Cu moves with a carrier molecule through the membrane and desorption of Cu occurs on the inside. Goodman and Linehan (1979) indicated that Cu was bound to amino acids in complexes such as $\text{Cu}(\text{glutamate})_2$ or $\text{Cu}(\text{glycine})_2$ as it moved through the membranes. This carrier could be a Cu specific polypeptide to which bonding would be very strong (Graham, 1981).

2.6.2 Other Factors Involved in Copper Absorption

There are many factors involved in the rate of absorption of Cu by plant roots. While aeration (oxygen) is required for absorption of many macronutrient ions, it is not clear if aeration is required for absorption of Cu. Cathala and

Salsac (1975) stated that aeration with N had no effect on uptake of Cu by excised roots of either sunflower (Helianthus annus M.) or corn (Zea mays L.). But this may not be relevant, since there was no desorption of Cu from the free space (Graham, 1981).

Growth medium pH affects the degree of chelation and determines Cu availability (Cavallaro and McBride, 1980), especially in soil systems. Both high and low pH extremes can damage tissues and impair their absorption capacity (Bowen, 1979). Hydrogen ions may compete with Cu^+ for adsorption and/or absorption sites.

2.7 GENOTYPIC DIFFERENCES IN COPPER UPTAKE

There are marked genotypic differences in Cu nutrition of plants. Of the temperate cereals, sensitivity to Cu deficiency is generally greater in wheat than in oat (Avena sativa L.), and least in rye (Secale cereale L.) (Smilde and Henkens, 1967). There undoubtedly is genetic variation within each species and, in any particular study, the genotypes chosen affect the order of observed sensitivities. Oat plants proved particularly sensitive to Cu deficiency in the studies of Mulqueen et al. (1961) and Nambiar (1976a), which is in contrast to the research of Smilde and Henken (1967). In two studies, tetraploid wheat (Triticum durum) was more sensitive to Cu deficiency than hexaploid wheat (T.

aestivum) (Nambiar, 1976a; Graham and Pearce, 1979). Large differences in susceptibility to Cu deficiency exist within T. aestivum cultivars (Nambiar, 1976a).

Substantial differences exist among cereals in the ability to respond to Cu supplied after the appearance of deficiency symptoms. Nambiar (1976b) observed that genotype differences in reponsiveness were related to the N:Cu ratio in grain from nine genotypes. He hypothesized that the higher the ability of grain to accumulate protein, the more sensitive the genotype would be to Cu deficiency. However, Hill et al. (1978) found one genotype among four wheats which did not fit Nambiar's hypothesis and argued that, perhaps, marked tillering ability rather than high grain N predispose plants to Cu deficiency. It should be pointed out that use of nine genotypes (Nambair, 1976a) is insufficient to confirm a generality and that one exception is insufficient to destroy the usefulness of the hypothesis. Such a relationship would be useful in view of the trend towards higher protein grains, higher use of N fertilizer, and the apparent increase in the incidence of Cu deficiency.

Genotype differences in sensitivity to Cu deficiency have been studied in oat plants by comparing responses of genotypes in soil and solution culture systems. Smilde and Henken (1967) indicated that these differences were due to varying abilities to extract Cu from soil; whereas, Mulder (1956) suggested such differences reflected a differential

ability of the roots to release Cu from the substrate. The ability of rye (Secale cereale L.) to grow satisfactorily in soils too deficient in Cu for other cereals also has been shown by other European and Australian workers (quoted by Smilde and Henken, 1967). As a result of this difference, rye has been described as "Cu efficient" (Graham, 1978), an agronomic description relative only to the other "Cu inefficient" cereals, e.g., wheat, oat and barley. Even though no mechanism is implied in the term, Cu efficiency of rye appears to be associated with its greater ability to accumulate the Cu in root tissues (Smilde and Henken, 1967; Graham and Pearce, 1979). The differences in rates of Cu absorption by roots are attributable to better exploration of soil through greater root length per plant, better contact with soil through longer root hairs, modification of availability of Cu in soil adjacent to roots by roots exudation, acidification or change in redox potential, more efficient transportation from root to shoot, more efficient translocation of Cu from older tissue to growing plants and/or lower tissue requirement for Cu (Graham, 1981).

Further evidence of genetic control of Cu efficiency in plants is explained by the wheat-rye hybrids in relation to their parent types (Graham, 1978; Graham and Pearce, 1979). The resultant triticale hybrids was shown to inherit the Cu efficiency of its rye parentage, and some had a high yield potential like wheat. The triticales absorbed an amount of

Cu from soil which was intermediate between that of wheat and rye. Because the Cu absorption was greater than the critical amounts needed for pollen viability, yields were not intermediate, but were equal to or greater than those of wheat (Graham, 1981).

Absorption of Cu has not been studied extensively at the toxic end of the spectrum. There have been a number of studies on the mechanism of protection from excess Cu in various species tolerant of soils high in Cu such as mine tailings. These include specific adsorption or chelation of Cu in cell walls; binding of Cu within leaf, root and stem cells; exclusion of Cu from the xylem and thereby from the shoot; and alteration of enzyme systems (Antonovics et al., 1971; Wainwright and Woolhouse, 1975).

Generally, both Cu tolerant and nontolerant plants accumulate Cu in roots to levels which are proportional to those in the outside environment. Concentration in shoots are less responsive until the roots are damaged. Then tolerance breaks down and high levels of Cu may enter the shoot and cause a phytotoxicity. Exclusion from the shoot occurs at a higher critical external Cu concentration in tolerant types than in nontolerant plants, whether Cu is bound in the root cell walls or bound to proteins within the root cells (Reilly, 1969; and Turner, 1970).

2.7.1 Copper Concentration in Plants

Concentrations of Cu in the shoot of cereal plants including barley, corn, oats, rice, rye, sorghum, and wheat normally range from 1.0 to 12 mg Cu kg⁻¹ and depend upon the amounts of labile soil Cu and plant specie and age (Gupta and Macleod, 1970; Chaudry et al., 1973; Gladstone et al., 1975; King and Alston, 1975). Concentrations of Cu in grain from cereal plants normally range from 0.8 to 6 mg Cu kg⁻¹ (Gladstone et al., 1975; King and Alston, 1975; Nambiar, 1976a).

The differences in tissue Cu concentrations among wheat, oat and barley are very small (Gladstone et al., 1975; Nambiar, 1976), but rye, due to its Cu tolerant characteristics seems to accumulate more Cu under the same growing conditions (Smilde and Henken, 1967; Gladstone et al., 1975). In this respect triticale appears to be intermediate in Cu content between its parents (Graham and Pearce, 1979; Harry and Graham, 1981). Copper concentration in older leaves may be twice as high as in young leaves, whereas stems contain intermediate values (Loneragan et al., 1976; Nambiar et al., 1976b; Loneragan et al., 1980); these differences may disappear with plant maturity. Although there is a lack of information on the relationship between the Cu concentrations in straw and grain, tillers generally have a lower Cu con-

centration than main stems (Nambiar, 1976b; Hill et al., 1979a; Loneragan et al., 1980).

Despite a general relationship between the Cu supplying capacity of a soil and uptake by plants (Martens, 1968), the Cu concentration in plant tissue is not a reliable indicator for diagnosis of Cu deficiencies (Caldwell, 1971; King and Alston, 1975). Furthermore, the Cu content of the mature shoot also does not satisfactorily indicate the Cu status of plants (King and Alston, 1975; Nambiar, 1976b; Loneragan et al., 1980). King and Alston (1975) suggested that a critical concentration in grain of 2.0 to 2.5 mg Cu kg⁻¹ could be used to identify potentially Cu deficient field sites. In contrast to their suggestion, Nambiar (1976a) stated that Cu concentrations in grain from Cu sufficient cereal genotypes ranged from 0.88 to 1.58 mg Cu kg⁻¹. When Cu was supplied, the grain yield of wheat was increased 15 to 40 fold, while the Cu concentration of the grain remained close to 1.0 mg Cu kg⁻¹ for both Cu treated and untreated plants (Riceman et al., 1940). A Cu concentration of 1.0 mg Cu kg⁻¹ generally is accepted as marginal in mature wheat grain in western Australia (Graham and Nambiar, 1981).

Variations in Cu content in different plants, as observed by various authors, were due to genotype differences, parts of plant sampled, plant age at sampling, and growth conditions such as level of macronutrient fertilization. Foliar diagnosis of the Cu status of oat and barley plants was best

accomplished by use of the youngest leaves at the beginning of shooting (Thiel, 1972). After studying the mobility of Cu within Cu-deficient and Cu-sufficient plants, Loneragan (1975) and Loneragan et al. (1976) proposed that the analysis of young leaves for diagnosis of Cu deficiency was more suitable, than use of older leaves or stems. Supporting this viewpoint, Nambiar (1976b) also proposed the use of young leaves in that, although their Cu concentrations were generally lower than in old leaves or stems, the range between deficient and sufficient situations are wider. Moreover, genotypic differences in the critical levels were smaller if young leaves were used. A study of the mobility of Cu in plants led to a diagnostic field technique in which the youngest fully emerged leaf blades were sampled at any time from the seedling stage to senescence. A Cu concentration $< 1.3 \text{ mg Cu kg}^{-1}$ was indicative of a moderate to severe deficiency, 1.3 to $1.6 \text{ mg Cu kg}^{-1}$ was indicative of a mild deficiency, 1.6 to $2.0 \text{ mg Cu kg}^{-1}$ was indicative of a marginal deficiency and $> 2.0 \text{ mg Cu kg}^{-1}$ was indicative of sufficient amounts for healthy plants (Gartrell and Brennan, 1979; Gartrell et al., 1979a, 1979b; Loneragan et al., 1980).

2.7.2 Mobility of Copper within the Plant

The extent of Cu mobility in plants has been a subject of debate. When wheat leaves were supplied with Cu, it moved

rapidly from older leaves to other parts of the plant (Bukovac and Wittwer, 1957). In oat plants with an adequate Cu supply, Cu was efficiently translocated from straw to grain (Williams and Moore, 1952). In contrast, little translocation of Cu was observed in oat plants by early workers (Wood and Womersley, 1946). This low mobility was confirmed by the appearance of deficiency symptom first in young leaves. The Cu concentration is relatively high in young wheat plants (2 to 3 leaf stage), decreases rapidly until flowering and, thereafter, any further decrease is very small (Gladstone et al., 1975; Loneragan, 1975; Graham, 1981). The rate of decline is associated with the initial concentration in the plant, i.e., the higher the initial concentration, the more rapid the rate of decline (Loneragan et al., 1976, 1980). When the Cu supply for wheat plants was adequate, Cu readily moved from older leaves when they senesced (14 to 35 days of age) and, at that time, about 70% of the total Cu was lost. In Cu deficient plants, the same leaf retained all its Cu for 50 to 64 days before it finally senesced and, at that time, about 80% of its Cu was translocated (Hill et al., 1978). During the period of retention, younger leaves suffered from acute Cu deficiency.

Translocation of Cu does not occur when the plant would benefit most from additional Cu in the growing point and young leaves. The immobility of Cu in "Cu deficient" plants occurred in all leaves to the extent that Cu was not trans-

located to the developing inflorescence in sufficient quantity to prevent pollen sterility (Hill et al., 1978). As a result, the grain yield declined.

Many workers showed that the immobility of Cu is tied to the immobility of N in plant tissues, and that N moves largely when senescence occurs (Hill et al., 1978, 1979a, 1979b; Loneragan et al., 1980). Hill et al. (1979a) suggested that Cu was mobilized out of the oldest leaf of "Cu deficient" wheat plants by shading due to premature senescence and that removal of Cu paralleled loss of both N and chlorophyll. Release of this Cu stimulated growth of new leaves. In nonsenescent leaves, Cu exists in a immobile form in phloem; whereas, in senescent leaves, it is highly mobile. Due to remobilization of Cu within the plant, 67% of the Cu in "Cu sufficient" vegetative plant was lost during the grain filling (Loneragen et al., 1980).

2.7.3 Interaction of Copper with other Ions

Like any other nutrient, Cu has interactions with other ions with regard to absorption, metabolism and translocation. Oat plants grown on a moderate "Cu deficient" soil produced some grain unless N fertilizer was added without Cu; then grain production failed completely (De Kock and Cheshire, 1968). They also observed that NH_4^+ -N was more toxic than NO_3^- -N and that NH_4^+ -N did not suppress Cu absorption.

Possibly the NH_4^+ nutrition resulted in more bonding sites for Cu. The interaction between Cu and N in the growth of cereal is one of the most important in plant nutrition. This Cu:N interaction is most serious where Cu deficiency is not recognized due to the absence of visual symptoms on growing plants and due to low overall productivity. In such cases, addition of high rates of N fertilizer induces severe Cu deficiency and, thereby, decreases grain production drastically. On the other hand, application of both Cu and N fertilizer can increase yields (Wapakala, 1973).

Copper deficient barley and wheat plants translocated less Ca to young leaves than did healthy plants, especially when phosphate was high (Brown, 1965; Nambiar, 1976b). Brown (1965) suggested that the 'wither-tip' symptom of Cu deficiency may in fact be Ca deficiency mediated by high phosphate. Decreased transpiration under Cu deficiency may be the cause of the lower Ca content in young leaves. Increasing phosphate fertilizer rates on phosphate fixing soils of Central Georgia, decreased the Cu content in wheat plants from acceptable to marginal levels (Touchton et al., 1980).

Zinc fertilization suppressed Cu adsorption by cereals in soil and solution systems (Widdowson, 1966; Chaudry and Loneragan, 1970; Bowen, 1969, 1981). Suppression of Zn uptake due to Cu addition is very prominent in solution cultures (Chaudry and Loneragen, 1972; Loneragan, 1975). In soil systems, the suppressing effect of Cu addition on Zn

uptake was developed slowly over about 45 days (Chaudry and Loneragan, 1970). The decreased rate of absorption of Cu as a result of Zn addition reflects the fact that Cu and Zn are absorbed by the same mechanism (Bowen, 1969).

Aluminium even at a low concentration such as 1 mg kg⁻¹ markedly reduced Cu uptake (Hiatt et al., 1963; Caldwell, 1971); however, higher levels of Cu overcome this inhibition. Hiatt et al. (1963) suggested that Al and Cu did not compete for the same absorption site in active absorption of Cu, but did compete for common binding sites at or near root surface. The inhibition of Cu uptake by Al could be overcome by an increase in pH. Acidification of soil usually increases Cu availability and results in higher grain yields if Cu is otherwise deficient (Harry and Graham, 1981). The small change in Cu availability with a decrease in pH as compared with Fe, Mn and Zn is due to the strong chelation of Cu (Piper and Beckwith, 1949). Addition of lime to a neutral sandy soil may greatly aggravate Cu deficiency (Harry and Graham, 1981).

Copper-Fe antagonism is indicated as a Cu-induced chlorosis (Kabata-Pendias and Pendias, 1985). A high level of Cu in the plant decreases the Fe content in chloroplasts (Reilly and Reilly, 1973). Ferrous, on the other hand, decreased Cu absorption from soil solution, especially when the soil was high in organic matter (Kabata-Pendias and Pendias, 1985). They also reported that the toxic effect of Cu can

be overcome by addition of Fe and that, at very high levels of Cu, the adverse effect has never been overcome by addition of Fe (Olsen, 1972).

2.7.4 Physiology of Copper Deficient Plants

An inadequate supply of Cu results in Cu deficiency symptoms on plants. Although these symptoms vary among plant species a common symptom of Cu deficiency is wilting. This effect has been described as a "peculiar limpness" (Piper, 1972), as "flagger foliage without turgor" (Pizer et al., 1966) or as "flaccidity" (Gartrell and Brennen, 1979). The leaves of Cu deficient plants become white-tipped and withered with strong tendency to collapse sharply. This abnormality has been attributed to damage to tissue structure caused by retarded lignification and by incomplete formation of vascular structures (Rahimi and Bussler 1973a, 1973b; Pissarek, 1974). Rahimi and Bussler (1973a, 1973b) suggested that "Cu deficient" leaves have smaller and fewer chloroplasts, smaller stomata with narrower apertures and thin-walled guard cells. In addition, oat leaves of plants that are deficient in Cu have ruptured chloroplasts and fibrous materials in collapsed chlorenchyma (Wood and Womersley, 1946; De Kock et al., 1971b). The collapsed poorly lignified xylem vessels cannot withstand the transpiration suction, which results in restricted water

supply in Cu deficient plants (Pissarek, 1974) and, consequently, in wilting symptoms due to water shortage. This water shortage is enhanced by a high shoot to root ratio, which results from decreased root development as compared with shoot growth under conditions of Cu deficiency (Chaudry and Loneragan, 1970; King, 1974).

Even mild to moderate Cu deficiencies may delay anthesis and maturity (Graham, 1976a; Nambiar, 1976a) and cause excessive tillering (Nabiar, 1976a). These growth effects lead to the situation that Cu deficient plants use less water than Cu sufficient plants until heading and more water thereafter (Graham, 1976a; Nambiar, 1976a). The head bending symptom, which is apparent towards maturity of cereals, is further evidence of structural weakness in Cu deficient plants (King, 1974; Graham, 1976a; Gartrell and Brennan, 1979). This symptom occurs where grain is sufficiently filled to make the heads heavy, but lignification is impaired, and thus, the peduncles bend under the weight. This is a useful indicator of marginal Cu deficiency in wheat and barley crops.

In cereal crops, economic yield is determined by the number of filled grains per head. If any inhibition takes place in fertilization or grain filling, there is a marked decline in yield. Lipman and Mackinney (1931) observed that barley plants grown with and without added Cu grew equally well until flowering, later the plants without added Cu had fewer blossoms, and did not produce seed capsule or seed. Wood and

Wormersley (1946) realized that Cu deficiency caused "sudden death of spikelets" at about a 20 mm length in oat. Anthers of Cu deficient wheat plants were smaller and produced fewer pollen grains that were small and dented and non viable (Graham, 1975). This clearly indicates the male sterility in Cu deficient plants.

Although male sterility is the cause of the drastic yield loss in conditions of moderate to mild Cu deficiency, yield loss also occurs due to decreased grain size (Nambiar, 1976a; Gartrell et al., 1979a). The latter reflects low photosynthetic efficiency as a result of poor stomatal function or poor translocation of carbohydrates (Agarwal and Pandey, 1972; Baszinski et al., 1978). Low photosynthetic efficiency results in low levels of soluble carbohydrates (Brown and Clark, 1977; Graham, 1980a). This condition results in poor grain filling in mildly Cu deficient plants. The embryos of Cu deficient grain appear to be normal and most of the endosperm are empty. Such grain is shrivelled and often blackened (Hooper and Davies, 1968). The blackening of endosperm is not due to microbial growth but is due to deposition of melanin, an amino acid precursor (Hooper and Davies, 1968). Many other workers also reported deposition of melanin in field grown cereals under Cu deficient situations (King, 1974; Gartrell et al., 1979a).

The relationship of Cu and plant diseases has a long history through the use of Bordeaux mixtures of which Cu salts

are a key ingredient. However, occasionally benefits of Bordeaux mixtures in addition to disease control led to the discovery of Cu as an essential element for plants (Felix, 1927). At the same time, it was recognized that Cu deficient plants were more susceptible to diseases than Cu sufficient plants; for example, Cu deficient barley, oat and wheat are more susceptible to powdery mildew, root rots and septoria (Schutte and Mathew, 1967; Graham, 1980b).

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CHAPTER 3

RESPONSE OF CORN TO LONG-TERM APPLICATION OF HIGH LEVELS OF COPPER SULFATE AND ZINC SULFATE

Abstract

A long-term field experiment was established in 1967 and continued through 1985 to evaluate the response of corn (Zea mays L.) to yearly application of CuSO_4 and ZnSO_4 on Davidson clay loam soil (clayey, kaolinitic, thermic Rhodic Paleudult). By 1985, the 19 annual applications of Cu and Zn supplied a cumulative total of 338 kg Cu ha⁻¹ and 830 kg Zn ha⁻¹. These Cu and Zn additions either alone or combined did not decrease either grain or stalk yield. Copper concentrations in grain and ear leaf were not affected by the treatments; whereas, application of Zn increased both grain and ear leaf Zn concentrations. Tissue Cu and Zn concentrations on all treatments were within the acceptable range for healthy corn plants.

Additional index words: DTPA extractable metals, Ear leaf Cu, Ear leaf Zn, Grain Cu, Grain Zn, Zea mays L.

3.1 INTRODUCTION

Copper and Zn are essential micronutrients for plant growth. Deficiencies of these elements in crop plants have been reported throughout the world (Murphy and Walsh, 1972; Kabata-Pendias and Pendias, 1985). Deficiency of Cu is common in wheat (Triticum aestivum L.); whereas, deficiency of Zn is frequent in corn (Zea mays L.) (Sparr, 1970). Inorganic forms of Cu and Zn (i.e., sulfates) generally have been used in correction of these micronutrient deficiencies (Murphy and Walsh, 1972). In addition to inorganic fertilizers, use of sewage sludge and manure as soil amendments also contribute Cu and Zn in soil (Allaway, 1977; Davis, 1974). Addition of high levels of these materials is considered to pose a potential hazard to plants and animals due to their high concentration of heavy metals including Cu and Zn (Lexmond and deHaan, 1977; U.S. Environmental Protection Agency, 1979). The availability of Cu and Zn applied to soil, as fertilizer or other amendments, depends on many soil factors such as organic matter (Viets, 1962), hydrous oxides of Al and Fe (McKenzie, 1980), pH (Hodgson et al., 1965) and clay minerals (El-Sayed et al., 1970).

Guidelines have been established for maximum levels of heavy metal applications to soils as either inorganic or organic sources (Sommers and Nelson, 1978). Based on guidelines for municipal sludge utilization (U.S. Environmental

Protection Agency, 1983), the maximum tolerable metal applications for the Davidson soil are 280 kg Cu and 560 kg Zn ha⁻¹. Loading rates for application of heavy metals, including Cu and Zn, to soils have been based on an interpretation of a wide volume of heterogenous literature. Therefore, the rate of Cu and Zn application, especially as sulfates, that will either injure plants or increase Cu and Zn concentrations in edible plant portions to undesirable high levels is still unknown (Winarko, 1985).

The purpose of this long-term research was to evaluate the effects of high levels of Cu and Zn application, as sulfates, on corn production. Rates of Cu and Zn application to the soil under study were in excess of those in guidelines (U.S. Environmental Protection Agency, 1983).

3.2 MATERIALS AND METHODS

A long-term field experiment was conducted for the nineteenth year to evaluate corn (Zea mays L.) response to application of high levels of Cu and Zn sulfates. This field experiment was conducted on a Davidson silty clay (clayey, kaolinitic, thermic Rhodic Paleudult) located in Orange County, Virginia. The experiment was initiated in 1967 and continued through 1985. Corn was grown on the experimental area each year, except in 1972, when soybeans [Glycine max (L.) Merr.] were grown on the site.

3.2.1 Field Experimentation

Five treatments applied to the Davidson soil annually during the 19 year period from 1967 through 1985 consisted of various levels of Cu and Zn sulfates either alone or together and a control (Table 1). The treatments were arranged in a randomized complete block design with four replications and with a plot size of 5.0 x 7.6 m.

Boron, K, Mg, N and P were added as Na borate, KCl, MgO, NH_4NO_3 and triple superphosphate to the experimental area at levels to prevent these nutrient deficiencies. The entire experimental area was supplied with 1.1 kg B, 168 kg Mg, 263 kg N, 39 kg P and 74 kg K ha^{-1} . The Cu and Zn sulfates for the treatments were broadcast on the soil surface on March 26, 1985 and disked into the soil to a depth of approximately 12 cm. Corn 'Pioneer 3192' was grown on the experimental area at a population of 80,670 plants ha^{-1} . Weed control was achieved on the experimental area by herbicide application. A total of 9.8 cm of supplemental water was applied to the experimental area by irrigation. Corn grain and stalk (earless shoot) yields were determined for each plot at plant maturity. The grain yields were reported at 155 g moisture kg^{-1} whereas stalk yields were reported on a dry weight basis.

Table 1. Cumulative amounts of Cu and Zn applied to the long-term field experiment on the Davidson soil.

Treatment number	<u>Cumulative micronutrient application⁺</u>			
	1984		1985	
	Cu	Zn	Cu	Zn
	kg ha ⁻¹			
1	0.0	0.0	0.0	0.0
2	0.0	695.4	0.0	830.7
3	151.2	199.8	159.6	210.7
4	334.0	0.0	338.0	0.0
5	334.0	695.4	338.0	830.0

+ The 18th and 19th years of the long-term field experiment were 1984 and 1985, respectively.

3.2.2 Corn Tissue Analysis

Plant tissue samples consisted of ten ear leaves plot⁻¹ sampled at the early silk growth stage and of grain shelled from 10 ears plot⁻¹ at plant maturity. The ear leaf and grain samples were dried in an oven for 48 h at 70 °C. The dried samples were ground in a stainless steel mill to pass a 20-mesh screen in preparation for Cu and Zn analyses. One-g subsamples of the ear leaf tissue were dry-ashed at 450 °C for two h. Thereafter, the ash was equilibrated with successive 10-, 10- and 5 ml portions of 0.5 M HCl for one hour and filtered through Whatman no. 42 filter paper. Copper and Zn in the combined filtrates were determined by atomic absorption spectrophotometry. One-g subsamples of grain were digested in HNO₃-HClO₄, diluted to 35 ml with water, and filtered through Whatman no. 42 filter paper. Copper and Zn in the filtrate were determined by atomic absorption spectrophotometry.

3.2.3 Soil Analyses

Before the 19th annual application of the treatments, soil samples were collected for DTPA-Cu and Zn (DTPA extractable Cu and Zn) determinations. The surface 0- to 20-cm layer (AP horizon) was sampled from all plots. Ten soil cores plot⁻¹ were collected using a sampling probe with a 1.9-cm diameter.

Soil samples were air dried and ground to pass a 10 mesh sieve. Amounts of DTPA-Cu and Zn in these samples were determined as described by Lindsay and Norvell (1978).

3.2.4 Statistical Analyses

Corn yield and tissue Cu and Zn concentration data were evaluated by analyses of variance (ANOVA). Mean separations were performed by linear contrast when the F-value of the ANOVA was significant at the 0.05 probability level. The statistical analyses were performed on an IBM 3081 computer utilizing SAS programs (SAS Institute, Inc., 1982).

3.3 RESULTS AND DISCUSSION

A long-term field experiment was carried out to evaluate the response of corn to high levels of CuSO_4 and ZnSO_4 application. This experiment was carried out for the past 19 years and, in the nineteenth year, the cumulative amounts of Cu and Zn applied to the soil were 338 and 830 kg ha^{-1} , either together or alone, respectively. The evaluation of corn response to applied Cu and Zn was accomplished by measurement of corn grain and dry stalk yields and of Cu and Zn concentrations in both ear leaf and grain tissues.

3.3.1 Soil Properties

This long-term field experiment was conducted in the Piedmont region of Virginia on a Davidson silty clay (Tables 2 and 3). The Davidson is a highly weathered soil, and the Ap horizon of the soil contained mainly kaolinite and 14-A⁰ intergrade clay minerals. In addition, this soil had a low organic matter content (16 g kg⁻¹), moderate CEC (11.2 cmol (+) kg⁻¹), and very high free Fe content (59 g kg⁻¹). The pH of the soil was 6.6 which is in the acceptable range for disposal of high levels of heavy metals (Sommers and Nelson, 1978).

3.3.2 Yield

Addition of high amounts of Cu and Zn to soil may result in high concentrations of Cu and Zn in plant tissue and yield losses. The nineteenth annual incremental addition of Cu and Zn resulting in cumulative totals of 338 kg Cu ha⁻¹ and 830 kg Zn ha⁻¹, either alone or together, did not cause decreases in grain and stalk yields (Table 4). Similar results were reported by Gettier (1986) and Winarko (1985) for the seventeenth and eighteenth incremental applications of Cu and Zn in this long-term experiment respectively. The corn-grain yield was relatively high as would be expected due to the adequate supply of water and other nutrients.

Table 2. Physical and mineralogical properties of the Davidson soil under study.

Particle size analysis*				Minerology**	
Sand	Silt	Clay	Texture	Silt	Clay
_____ g/100 g _____					
9	41	50	Silty clay	Q(85)#, M(10) F(5)	K(40), I(30) Q(10), M(10) G(5), F(5)

Extracted from Mullins et al. (1982).

* Sampling depth was 0 - 20 cm.

** K = kaolinite
 I = intergrade
 Q = quartz
 M = mica
 F = feldspar
 G = gibbsite

Approximate percentages of each mineral are in parentheses.

Table 3. Chemical properties of the Davidson soil under study.

Soil property	value
Soil pH	6.6
Organic matter, gkg ⁻¹	16
Free iron, gkg ⁻¹	59
Cation exchange capacity, cmol(+) kg ⁻¹	11.2

Table 4. Corn grain and stalk yields as affected by 19-annual applications of various levels of Cu and Zn to the Davidson soil.

Treatment number	Total micronutrient application		Corn yield	
	Cu	Zn	Grain	Stalk
	kg ha ⁻¹		kg ha ⁻¹	
1	0.0	0.0	12712	10534
2	0.0	830.0	12697	9012
3	159.6	210.7	13246	10527
4	338.0	0.0	12611	9434
5	338.0	830.0	13058	10107
Analysis of variance			F value	
			0.73 ns	1.77 ns ⁺

ns⁺ - not significant at the 0.05 level of probability

Higher levels of Cu and Zn application as sewage sludge than applied in this research as sulfates did not decrease corn yield (Jones et al., 1975; Page, 1974). However, these data with sewage sludge are not directly comparable for tolerable Cu and Zn loading rates from soluble inorganic sources, because plants absorb different amounts of Cu and Zn from soluble inorganic sources than from sewage sludge (Cunningham et al., 1975; Mullins et al., 1982).

3.3.3 Tissue Concentration of Copper and Zinc

Nineteen annual application of various levels of Cu and Zn, which provided a cumulative total of up to 338 kg Cu ha⁻¹, did not increase the concentration of Cu in either corn grain or ear leaves (Tables 5 and 6). Similar results were observed for 17-annual Cu applications by Gettier (1986) and for 15-annual applications by Mullins et al. (1982). However for 18 annual Cu applications Winarko (1985) reported no increase in Cu concentrations in grain with added Cu but obtained an increase in Cu concentration in ear leaves from added Cu. The levels of Cu in the grain and ear leaf from all treatments ranged from 3.3 to 3.5 and 9.5 to 10.0 mg Cu kg⁻¹, respectively. These Cu concentrations are within the normal range as suggested by many authors (Gartrell and Brennan, 1979; Gartrell et al., 1979b; Loneragen et al., 1980).

Table 5. Copper and Zn concentrations in corn ear leaf tissue as affected by 19 annual applications of Cu and Zn to Davidson soil.

Treatment number	Total micronutrient application		Cu and Zn concentration in ear leaf	
	Cu	Zn	Cu	Zn
	kg ha ⁻¹		mg kg ⁻¹	
1	0.0	0.0	9.65	19.20
2	0.0	830.0	9.50	60.04
3	159.6	210.7	9.50	27.56
4	338.0	0.0	9.81	20.41
5	338.0	830.0	10.06	52.91
			F value	
Analysis of variance			2.10 ns ⁺	26.36 ***
Linear contrasts of ear leaf Zn:				
Treatment	1, 4 vs 3		-----	2.90 ns
Treatment	3 vs 2, 5		-----	40.24 ***
Treatment	1, 4 vs 2, 5		-----	79.10 ***
Treatment	4 vs 5		-----	1.84 ns

*** Significant at the 0.001 level of probability.

ns⁺ Not significant at the 0.05 level of probability.

Table 6. Copper and Zn concentrations in corn grain as affected by 19-annual applications of Cu and Zn to Davidson soil.

Treatment number	Total micronutrient application		Cu and Zn concentration in corn grain	
	Cu	Zn	Cu	Zn
	kg ha ⁻¹		mg kg ⁻¹	
1	0.0	0.0	3.42	25.40
2	0.0	830.0	3.48	34.97
3	159.6	210.7	3.32	29.17
4	338.0	0.0	3.39	24.40
5	338.0	830.0	3.38	33.37
Analysis of variance			0.04 ns ⁺	4.62 **
Linear contrasts of grain Zn:				
Treatment	1, 4 vs 3		----	2.56 ns
Treatment	3 vs 2, 5		----	3.51 ns
Treatment	1, 4 vs 2, 5		----	18.09 **
Treatment	4 vs 5		----	0.27 ns

** Significant at the 0.01 level of probability.

ns⁺ Not significant at the 0.05 level of probability.

Zinc concentrations in both grain and ear leaves increased ($P \leq 0.001$) with rate of Zn application (Tables 5 and 6). The highest Zn values in grain ($35.0 \text{ mg Zn kg}^{-1}$) and ear leaves ($60.0 \text{ mg Zn ha}^{-1}$) were obtained at the highest cumulative application rate of $830 \text{ kg Zn ha}^{-1}$. These results are similar to Winarko's (1985) findings. No interaction occurred with respect to Cu and Zn in either grain or ear leaves in these experiments.

Overall, these data conform to the conclusion by Chaney (1975) that an increase in soil Cu results in a small increase in Cu in the plant shoot. This relationship is expected since Cu is only partially mobile within the plant and accumulates in the roots (Dragun et al., 1976; Woolhouse and Walker, 1981). In contrast to Cu, an increase in levels of soil Zn led to accumulation of substantial amounts of Zn in the aerial parts of plants (Chaney, 1975).

In summary, yearly addition of Cu and Zn as CuSO_4 and ZnSO_4 to cumulative totals of 338 and 830 kg ha^{-1} , respectively, either alone or combined, were not phytotoxic to corn plants. Copper concentrations in both grain and ear leaf were not affected by addition of Cu; whereas, Zn concentrations of both in grain and ear leaves were increased in response to added Zn. Concentrations of both Cu and Zn in leaf tissues were within an acceptable range for growth of healthy plants.

3.3.4 DTPA Extractable Copper and Zinc

Statistical analyses indicated that the 18 annual application of CuSO_4 at a cumulative total of 338 kg Cu ha⁻¹ increased the DTPA-Cu in the Davidson soil (Table 7). The linear contrast analysis for treatment 4 versus 5 showed that addition of Zn increased the DTPA-Cu, i.e., increased the amount of Cu in the labile pool of the soil. Similar results were reported by Winarko (1985). This could be explained by competition of adsorption site, by the applied Cu and Zn. The linear contrast analysis indicated that applied Cu did not affect Zn concentrations in the labile pool. This finding agrees with that of Winarko (1985), where lower cumulative totals of Cu and Zn had been applied to the soil.

Table 7. DTPA extractable copper and zinc in Davidson soil after 18-annual applications of copper sulfate and zinc sulfate.

Treatment number	Total Micronutrient application		DTPA extractable Cu and Zn concentration	
	Cu	Zn	Cu	Zn
	_____ kg ha _____		_____ mg kg ⁻¹ _____	
1	0.0	0.0	1.62	1.70
2	0.0	830.0	3.03	42.82
3	159.6	210.0	7.40	7.65
4	338.0	0.0	19.68	1.70
5	338.0	830.0	24.00	45.30
Analysis of variance			_____ F value _____	
			76.36 ***	174.49 ***
Linear contrast:				
Treat.	1,2 vs 3	1,4 vs 3	12.80 *	8.31 *
Treat.	3 vs 4,5	3 vs 2,5	103.87 ***	311.07 ***
Treat.	1,2 vs 4,5	1,4 vs 2,5	284.60 ***	631.56 ***
Treat.	4 vs 5	2 vs 5	6.98 *	1.08 ns

*** Significant at the 0.001 level of probability.

* Significant at the 0.05 level of probability.

ns Not significant at the 0.05 level of probability.

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CHAPTER 4

RESPONSE OF WHEAT TO COPPER SULFATE APPLICATION IN FOURTEEN VIRGINIA SOILS

Abstract

A greenhouse experiment was conducted to evaluate the response of wheat (Triticum aestivum L.) to applied CuSO_4 in 14 Virginia soils. The 14 soils selected for this research had pH levels from 5.4 to 7.3 and ranged from 0.06 to 1.56 mg kg^{-1} in DTPA-Cu and from 0.01 to 1.10 mg kg^{-1} in Mehlich III-Cu. Short term nutrient uptake procedures developed by Stanford and DeMent (1957) were used in this research. The test plant wheat (cv. Saluda) was harvested when at 7 weeks growth stage for dry matter calculation and tissue Cu determination. Cu application did not increase dry matter yields on the 14 soils, but did increase Cu concentration. Copper uptake was highly correlated ($r = 0.80$, $\alpha = 0.001$) with DTPA-Cu whereas no correlation occurred between Mehlich III-Cu and Cu uptake ($\alpha = 0.05$). DTPA-Cu showed a high correlation with clay content ($r = 0.98$, $\alpha = 0.001$) and SA ($r = 0.93$, $\alpha = 0.001$); whereas, Mehlich III-Cu showed no correlation with either clay content ($\alpha = 0.26$) or SA ($\alpha = 0.40$).

Additional index words: Cu uptake, DTPA-Cu, Mehlich III-Cu, Triticum aestivum L.

4.1 INTRODUCTION

Copper deficiency has been reported in an ever increasing rate in crops grown in various areas of the world, and the use of Cu fertilizers has increased accordingly. Much progress has been made during the past few decades in diagnosing and correcting Cu deficiencies in plants and animals (Graham and Nambiar, 1981). Cereals, however, deserve special attention since they are the worlds major source of food and, as a group, are particularly sensitive to Cu deficiency (Graham and Nambair, 1981). Among cereals, wheat (Triticum aestivum L.) is more sensitive to Cu deficiency than corn (Zea mays L.) (Sparr, 1970).

Response to Cu fertilizer have been reported in all agricultural environments from the temperate regions (Reith, 1968; Talitinen, 1971) to tropical India (Chaudry et al., 1973); and from the arid border of crop production in western Australia (Gartrell, 1977) to humid monsoonal Indonesia (Green, 1957). Copper deficiencies are common in highly leached sandy-textured soils with high organic matter contents and with pH levels in the acid and alkaline extremes (Graham and Nambiar, 1981). The plant availability of Cu

depends on many soil factors such as as organic matter (Emmerich et al., 1982; McLaren and Crawford, 1973a), clay minerals and hydrous oxides of Al, Fe, and Mn (Baker, 1974), and pH (Kabata-Pendias and Pendias, 1985). Aluminium, Fe, and Mn hydrous oxides and phyllosilicates provide a high surface area on which Cu can be adsorbed by exchange reactions (Jenne, 1977).

Since the labile Cu seems to be predominantly organically bound and adsorbed on hydrous oxide surfaces, extractants containing chelating agents have generally been useful in estimating plant available Cu in soils (Reith, 1968; King and Alston, 1975). Since a meager amount of research has been conducted to evaluate the prevalence of Cu deficiency in Virginia, the extent of Cu deficiency in Virginia is unknown.

The objective of this greenhouse experiment was to evaluate the effect of CuSO_4 on wheat dry matter production and Cu uptake and to determine the influence of soil properties on Cu availability. Soil with diverse properties were selected for this research.

4.2 MATERIALS AND METHODS

A greenhouse experiment was conducted to evaluate wheat (Triticum aestivum L.) response to application of CuSO_4 on 14 different soils. Chemical and physical properties and the soil names of the 14 soils are given in Table 8 and 9. These

soils were obtained from the Coastal Plain, Piedmont, and Ridge and Valley regions of Virginia.

The short term uptake procedure devised by Stanford and DeMent (1957) was used in the greenhouse research. Round plastic containers, 15 cm in diameter with bottom removed were nested in identical containers with bottoms intact. Two treatments in the study were a control and 5.35 mg Cu kg⁻¹ soil. Seven hundred and fifty g of acid washed and neutralized quartz sand were placed in the top container. Two hundred and fifty g of the soil to be tested were placed in the bottom container. A solution of CuSO₄ was atomized at the rate of 1.34 mg Cu per 250 g soil for the Cu treatment. All the pots received the following additional 286 mg N as NH₄NO₃ and (NH₄)₂SO₄, 217.2 mg P as triple superphosphate, 332.8 mg K as KNO₃, 15.6 mg Zn as ZnSO₄·7H₂O, 41 mg Fe as FeSO₄·7H₂O, 25 mg Mn as MnSO₄·H₂O, 1 mg B as H₃BO₃, 18.1 mg Ca as Ca(NO₃)₂, 65.5 mg S as (NH₄)₂SO₄, and 75.8 mg Mg as MgSO₄·7H₂O pot⁻¹ during the entire experiment period. Twenty five wheat (cv. Saluda) seeds were planted at a depth of 2 cm in the quartz sand and were thinned to 20 pot⁻¹ when seedlings were 2 cm in tall.

All pots were maintained at near field capacity (determined by weight) by watering twice daily. The treatments were arranged in completely randomized design with three replications. All of the pots within a block were rotated daily to avoid any effect due to light and temperature. Tops

were harvested 2 cm above the surface when they were about 20 cm tall. The tissue was dried at 70 °C for 48 hours and the dry weights were recorded.

4.2.1 Wheat Tissue Analysis

The whole dried tops were ground in a stainless grinder to pass 20-mesh screen in preparation for Cu analysis. One g ground samples were dry-ashed at 450°C for 2 hours. Thereafter, the ash was equilibrated in successive 10-, 10-, and 5-ml portions of 0.5 M HCl for one hour and filtered through Whatman no. 42 filter paper. Copper concentration in the filtrate was determined by atomic absorption spectrophotometry.

4.2.2 Soil Analyses

DTPA (DTPA-Cu) and Mehlich III (Mehlich III-Cu) extractable Cu were measured in the 14 soils prior to the greenhouse experiment. The DTPA-Cu was determined by the procedure devised by Lindsay and Norvell (1978). Ten grams of each air-dry soil was extracted in duplicate with 20 ml of DTPA extracting solution (0.005M DTPA, 0.1 M triethanolamine, 0.01 M CaCl₂, pH 7.3). The extraction was performed by shaking the suspension for 2 hours on a reciprocating shaker at a rate of 120 cycles min⁻¹. The sus-

pension was centrifuged for 10 min at 2000 rpm and filtered through Whatman no. 42 filter paper. Copper in the filtrate was determined by atomic absorption spectrometry.

Mehlich III-Cu was determined by the procedure developed by Mehlich (1984). A volume of 2.5 cm³ of air-dry soil was measured in duplicate into 100 ml plastic extraction bottles (or tubes) and 25 ml of Mehlich III extracting solution (0.2 N CH₃COOH, 0.25 N NH₄NO₃, 0.015 N NH₄F, 0.013 N HNO₃, and 0.001 M EDTA) was added. The suspension was shaken on a mechanical reciprocating shaker at the rate of 200 cycles per min for 5 min and filtered through a Whatman no. 42 filter paper. The Cu concentration in the filtrate was determined by atomic absorption spectrophotometry.

4.3 STATISTICAL ANALYSIS

Analyses of variance were performed on the dry matter yield and Cu concentration data. Simple correlation analyses were completed to determine relationships among Cu uptake and soil characteristics. All statistical procedures were performed on an IBM 3081 computer utilizing SAS programs (SAS Institute, Inc., 1982).

4.4 RESULTS AND DISCUSSION

A greenhouse experiment was conducted to evaluate the response of wheat to applied Cu as CuSO_4 in 14 soils. Of prime interest in this research was whether Cu application would increase plant growth. Therefore, wheat, which is highly sensitive to Cu deficiency was selected as a test crop for the research. Furthermore, a short term uptake procedure (Stanford and DeMent, 1957) was used to place as much stress as possible on absorption of Cu from the labile pool in the soils.

4.4.1 Soil Properties

The 14 soils selected for this study varied widely in chemical and physical properties (Table 8). Although the pH of the 14 soils ranged 5.4 to 7.3, 11 of the soils had a pH levels from 6.0 to 7.4. These high relatively levels of pH are conducive for development of Cu deficiency. The 14 soils had organic matter contents from 10 to 38 g kg^{-1} , surface areas from 11.2 to 104 m^2g^{-1} and clay contents from 54 to 468 g kg^{-1} .

Table 8. Properties of soils used to evaluate Cu uptake by wheat plants when fertilized with copper sulfate.

Soil no.	Soil series	pH	Organic matter — g kg ⁻¹ —	Clay	SA m ² g ⁻¹	Texture
1	Craven	5.5	38	124	34.3	Sandy loam
2	Rumford	6.6	10	54	12.4	Loamy sand
3	Slagle	6.6	35	87	32.5	Sandy loam
4	Emporia	6.5	20	96	20.3	Loam
5	Kempsville	6.0	10	71	11.2	Sandy loam
6	Rains	6.0	24	155	28.0	Sandy loam
7	Myatt	6.8	11	79	11.9	Sandy loam
8	Dragston	6.9	11	95	15.2	Sandy loam
9	Starr	6.1	22	468	104.0	Clay
10	Dyke	6.9	16	421	85.0	Silty clay
11	Lodi	7.4	26	166	36.5	Silt loam
12	Christian	5.7	19	163	35.1	Silt loam
13	Tarboro	5.4	4	63	18.3	Loamy sand
14	Fauquier	7.3	30	248	92.2	Silt loam

Extracted from Jin (1985).

Table 9. Classification of 14 Virginia soils used in the greenhouse experiment.

Soil no.	Soil series	Soil classification
1	Craven	Aquic Hapludults, clayey, mixed, thermic
2	Rumford	Typic Hapludults, coarse-loamy, siliceous, thermic
3	Slagle	Aquic Hapludults, fine-loamy, siliceous, thermic
4	Emporia	Typic Hapludults, fine-loamy, siliceous, thermic
5	Kempsville	Typic Hapludults, fine-loamy, siliceous, thermic
6	Rains	Typic Hapludults, fine-loamy, siliceous, thermic
7	Myatt	Typic Ochraquults, fine-loamy, siliceous, thermic
8	Dragston	Aeric Ochraquults, coarse-loamy, mixed thermic
9	Starr	Fluventic Dystrochrepts, fine-loamy, mixed, thermic
10	Dyke	Typic Rhodudults, clayey, mixed, mesic
11	Lodi	Typic Hapludults, clayey, kaolinitic, mesic
12	Christian	Typic Hapludults, clayey, kaolinitic, mesic
13	Tarboro	Typic Udipsamments, mixed thermic
14	Fauquier	Ultic Hapludults, fine, mixed, mesic

4.4.2 Dry Matter Yield

Fertilization of these soils with 5.35 mg Cu kg⁻¹ did not increase dry weight of the shoots over the control (Table 9). In addition, no visual symptoms of deficiency were observed during the 5-week growing period. An increase in dry weight from Cu application was expected in this research because some of the soils contained extractable Cu levels below the critical levels for the DTPA and Mehlich III procedures. Whitehouse (1973) suggested a tentative critical soil test value of 0.4 mg kg⁻¹ for DTPA-Cu. As a criterion for separating soils that are likely to respond to Cu application, Caldwell (1971) and Lindsay and Norvell (1978) reported the critical value as 0.2 mg Cu kg⁻¹ of DTPA-Cu. Grundon and Best (1982) suggested that DTPA-Cu concentration in soils of less than 0.4 mg kg⁻¹ would supply inadequate for wheat growth. In the present experiment, the DTPA-Cu was above 0.4 mg kg⁻¹ in soils 6, 9, 10 and 14 (Table 9), i.e., in the heavier-textured soils. Whereas other soils all of the lighter-textured soils had DTPA-Cu levels below 0.4 mg kg⁻¹ (range 0.06 to 0.29 mg kg⁻¹). This implies that heavier-textured soils that are high in clay have more adsorbed Cu in their surface exchange sites than the sandy or sandy loam soils. The relationships between DTPA-Cu and both clay content and SA are shown below:

$$\text{DTPA - Cu} = -18.8 \times 10^{-2} + 3.67 \times 10^{-2} \text{ Clay, } r = 0.98 \text{ ***}$$

Table 10. Dry matter yield and tissue Cu concentrations of wheat grown on 14 different soils with or without Cu fertilization.

Soil no.	Soil series	<u>Dry Matter</u>		<u>Tissue Cu</u>	
		<u>Cu fertilization, mg kg⁻¹</u>			
		0	5.4	0	5.4
		g pot ⁻¹		mg kg ⁻¹	
1	Craven	8.67	8.49	1.50 b ⁺	4.58 a
2	Rumford	9.28	9.92	1.50 b	5.00 a
3	Slagle	8.93	8.42	1.92 b	4.75 a
4	Emporia	8.44	8.09	2.58 b	6.25 a
5	Kempsville	8.14	9.03	2.25 b	7.25 a
6	Rains	7.90	8.57	2.83 b	5.08 a
7	Myatt	7.79	8.59	1.17 b	5.50 a
8	Dragston	8.15	7.18	2.41 b	6.83 a
9	Starr	8.68	8.60	4.92 a	4.83 a
10	Dyke	9.64	10.09	4.75 a	6.50 a
11	Lodi	8.82	8.91	2.92 b	5.92 a
12	Christian	8.84	8.69	5.00 a	6.00 a
13	Tarboro	7.61	8.60	2.17 b	6.00 a
14	Fauquier	9.09	9.12	4.83 a	5.92 a
		F value			
Analysis of variance:		0.44 ns		96.82 ***	

*** Significant at the 0.001 level of probability.

ns Not significant at the 0.05 level of probability.

+ Column means followed by different letters are significantly different at 0.05 level of probability.

$$\text{DTPA - Cu} = -13.5 \times 10^{-2} + 1.42 \times 10^{-2} \text{ SA}, \quad r = 0.93^{***}$$

A dry weight response to Cu application did not occur ($\alpha = 0.05$) on the soils with lighter textures and DTPA-Cu below the critical level as well as on soils with heavier-textured DTPA-Cu above the critical level (Table 10). Similar results were observed by Grundson and Best (1982) in their pot experiments. Makarim and Cox (1983) suggested that the critical level of Mehlich III extractable Cu was 0.37 mg L⁻¹. In this experiment, all soils except soil 8 were below this critical level. The lack of dry weight increases from added Cu, even on soils with DTPA-Cu and Mehlich-Cu below the critical levels, may be due to the short growth period during the experiment. Evidently, the soil Cu levels were sufficient to meet plant requirements through the stage of growth at which plants were harvested. However, to identify response of wheat to added Cu, this experiment probably needed to be carried out up to heading stage, at which time plants require more Cu than at the juvenile stage of growth.

4.4.3 Tissue Copper Concentration

Copper application increased tissue Cu concentration over the control on 10 of the 14 soils (Table 10). Tissue Cu concentration in Cu fertilized plants ranged from 4.58 to 7.25 mg Cu kg⁻¹ (DW) and were above the sufficient range (> 2

mg Cu kg⁻¹) suggested many workers (Gartrell and Brennan, 1979a; Gartrell et al., 1979a, 1979b; Loneragan et al., 1980). However, McAndrew et al. (1984) reported that Cu concentrations in plant shoots of 3.0 to 4.9 mg kg⁻¹ are low. In the present experiment, all the Cu concentrations in plant shoots were within the sufficiency range of 2 mg kg⁻¹ in the Cu fertilized plants. Plants harvested from the control treatment had plant shoot Cu concentrations above 2 mg kg⁻¹, or above the sufficiency limit, on all soils except numbers 1, 2, 3, and 7. Apparently, the plant tissue Cu concentrations on the control treatment were related to soil texture. That is, soils with lighter textures (sandy loam to loam) had an inherently lower ability to supply Cu than the heavier-textured soils.

4.4.4 DTPA and Mehlich III Extractable Cu

The amount of Cu solublized from the untreated soils was different for the DTPA and Mehlich III extracting agents (Table 10). The DTPA extracting solution consists of 0.1 M triethanolamine, 0.005 M DTPA, and 0.01 M CaCl₂, whereas the Mehlich III extracting solution consists of 0.2 N CH₃COOH, 0.25 M NH₄NO₃, 0.015 M NH₄F, 0.013 N HNO₃ and 0.001 M EDTA. The DTPA extracting solution has a higher concentration of chelating agent (0.005 M DTPA) than the Mehlich III extracting solution (0.001 M EDTA). In addition, the DTPA solution

Table 11. DTPA and Mehlich III-Cu in 14 different soils prior to Cu application.

Soil no.	Soil series	mg kg ⁻¹	
		DTPA Cu	Mehlich III-Cu
1	Craven	0.14 h *	0.05 e *
2	Rumford	0.09 i	0.04 e
3	Slagle	0.14 h	0.06 e
4	Emporia	0.18 d	0.05 e
5	Kempsville	0.11 bc	0.04 e
6	Rains	0.46 d	0.17 d
7	Myatt	0.06 j	0.01 e
8	Dragston	0.21 f	1.10 a
9	Starr	1.56 a	0.37 c
10	Dyke	1.35 b	0.48 b
11	Lodi	0.28 e	0.09 e
12	Christian	0.29 e	0.08 e
13	Tarboro	0.08 ji	0.01 e
14	Fauquier	0.80 c	0.27 c
		_____ F value _____	
Analysis of variance		2437 ***	160 ***

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

*** Significant at the 0.001 level of probability.

has a pH of 7.3 whereas the Mehlich III solution has a pH of 2.5. The higher amount of chelating agent, the greater complexing ability at the higher pH, and the longer extraction period account for the higher amount of Cu generally extracted from the soils by the DTPA procedure as compared with the Mehlich III method. Another reason for high DTPA extraction values over the Mehlich III values is the higher affinity of Cu for DTPA than for EDTA (Norvell, 1972).

4.4.5 Copper Uptake

Copper uptake by wheat plant on the untreated soil showed a significant correlation with DTPA-Cu ($r = 0.80$, $\alpha = 0.001$), clay content ($r = 0.82$, $\alpha = 0.001$), and surface area ($r = 0.83$, $\alpha = 0.001$), whereas Mehlich III Cu was unrelated to Cu uptake ($\alpha = 0.05$) (Table 11). Likewise, Cu uptake by plants grown on the control treatment were unrelated to soil pH and organic matter content ($\alpha = 0.05$). Overall, these data show that DTPA-Cu was a better predictor of Cu uptake than Mehlich III-Cu.

DTPA-Cu was highly correlated with clay content and surface area, as shown below:

$$\text{DTPA - Cu} = -18.9 \times 10^{-2} + 36.7 \times 10^{-3} \text{ Clay} \quad r = 0.98 \text{ ***}$$

$$\text{DTPA - Cu} = -13.5 \times 10^{-2} + 14.23 \times 10^{-3} \text{ SA} \quad r = 0.93 \text{ ***}$$

Table 12. Relationship of Cu uptake by wheat with extractable Cu and soil properties.

Independent variable	r	Significance level
DTPA-extractable Cu	0.80	***
Mehlich III extractable Cu	0.28	ns
Organic matter	0.17	ns
Clay	0.82	***
Surface area	0.83	***
pH	0.32	ns

*** Significant at the probability level of 0.001.

ns Not significant at the probability level of 0.05.

This suggested that Cu can be specifically adsorbed to layer silicates and associated hydrous oxides impurities on the layer silicates (Gettier, 1986; McBride, 1981).

In summary, addition of CuSO_4 to the 14 different soils did not increase dry weight of wheat plants; however, tissue Cu concentrations were usually increased by Cu application on the soils. Wheat plants had Cu concentrations below the critical level on the control treatment on some of the soils. It seems possible that a longer growth period should have been used in the greenhouse experiment in order to obtain an increase in dry weight on these soils. The DTPA extractant solubilized more Cu than the Mehlich III extractant in most of the untreated soils and was a better predictor of Cu uptake than the Mehlich III procedure. DTPA-Cu was highly correlated with soil clay content and SA, but was unrelated to soil organic matter content.

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CHAPTER 5

RESPONSE OF CORN TO COPPER APPLICATION ON TEN VIRGINIA SOILS

Abstract

A greenhouse experiment was carried out to investigate the response of corn to CuSO_4 application in ten Virginia soils. Two and half kg of each soil was potted in plastic pots double lined with polyethelene. Two treatments replicated four times were i) a control and ii) Cu application at a rate of $5.35 \text{ mg Cu kg}^{-1}$ as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Corn (cv. Pioneer 3291) was used as the test plant. Shoots were harvested when the plants were 50 cm in height were used for dry matter yield calculation and tissue Cu determination. DTPA-Cu and Mehlich III-Cu extracted from untreated soils were related to dry weights and Cu uptake of corn plants.

Corn tissue dry matter weights were not affected by Cu application ($\alpha = 0.05$), but tissue Cu levels were increased as a response to applied Cu ($\alpha = 0.001$). However, both in the treated and untreated soils, tissue Cu levels were within the sufficiency range. Copper uptake by the corn plants correlated with DTPA-Cu ($r = 0.62$, $\alpha = 0.001$) and with soil pH ($r = 0.35$, $\alpha = 0.05$), whereas organic matter content did

not correlate with Cu uptake ($\alpha = 0.05$). DTPA-Cu did not correlate with either organic matter content or soil pH ($\alpha = 0.05$). Mehlich III-Cu was unrelated ($\alpha = 0.05$) to both organic matter content and soil pH. Uptake of applied Cu varied from the 10 soils was unrelated ($\alpha = 0.05$) to either organic matter content or pH.

Additional index words: Copper uptake, Dry matter yields, DTPA extractable Cu, Mehlich III extractable Cu.

5.1 INTRODUCTION

Corn (Zea mays L.) is used as a staple food after wheat in many developing countries. In developed countries this is one of the main fodder crops used in preparation of silage for ruminants. Deficiencies of Cu are very common in wheat (Triticum aestivum L.) but are less prevalent in corn (Sparr, 1970). Application of Cu as CuSO_4 is used to alleviate Cu deficiencies in many crops (Donald and Prescott, 1975; Loneragan, 1975). Many soils, especially those high in organic matter or highly weathered or leached, require Cu applications to obtain high yields (Donald and Prescott, 1975; Murphy and Walsh, 1972). On the other hand, corn grown in cleared vineyards developed toxic symptoms when 1 M ammonium acetate extractable Cu reached 200 mg kg^{-1} (Aubert and Pinta, 1977).

Reversion reactions of Cu between chemical forms within the soil are not well understood. Research clearly indicated that soil pH and ligand concentration greatly affects Cu transformations (Viets, 1962). Copper is strongly bonded to organic matter (Emmerich et al., 1982; Gerritse et al., 1982; McLaren and Crawford, 1973) and is adsorbed on the surfaces of layer silicates and hydrous oxides of Al, Fe and Mn (Baker, 1974). The affinity of Cu to organic matter and clay is a function of soil pH and the affinity increases as soil pH increases (Kabata-Pendias and Pendias, 1985).

The overall objective of this research was to evaluate the effect of applied CuSO_4 on Cu uptake by corn plants and to evaluate the influence of pH and organic matter on Cu availability. A further goal of this research was to determine by a greenhouse assay procedure if Virginia soils supplied adequate Cu for corn production.

5.2 MATERIAL AND METHODS

A greenhouse experiment was carried out to evaluate corn (Zea mays L.) response to application of CuSO_4 on 10 different soils. The soils selected for the research had diverse properties (Table 12 and 13). All of the soils under study were obtained from the Coastal Plain region, except the Groseclose silt loam. The Groseclose soil was obtained from the Ridge and Valley region.

Two treatments applied in the greenhouse experiment were i) a control and ii) Cu application. Round plastic containers 25 cm in diameter were double lined with polyethelene and partially filled with 2500 g of the soils to be tested. The Cu treatment received 5.35 mg Cu as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ kg^{-1} soil. All the soils were mixed individually with following nutrients: 224 mg N as NH_4NO_3 , 187.5 mg P as $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, 187.7 mg K as KCl, 850 mg Mg as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 11.0 mg B as $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, 37.4 mg Mn as $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 37.5 mg Zn as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ pot^{-1} in plastic a lined soil mixer. Corn (Pioneer 3291) was planted 3 cm deep at the rate of 8 seeds pot^{-1} and seedlings were thinned to 4 plants pot^{-1} when they were 5 cm tall. Pots were maintained at near field capacity (by weight) by watering twice daily. Treatments were arranged in a complete randomized block design with three replicates and pots within a block were rotated daily to prevent effects due to differences in light or temperature. Plants were harvested 2 cm from the surface when they were 50 cm tall, and dried in an oven for 48 hours at 70 C. Thereafter, dry weights were recorded.

5.2.1 Corn Tissue Analyses

The whole dried plants were ground in a stainless steel grinder to pass a 20-mesh screen in preparation for Cu analysis. One-g of ground samples were dry-ashed at 450 C for 2 h. The ash was equilibrated with successive 10-, 10-, and 5

mL portions of 0.5 M HCl for 1 h and filtered through Whatman no. 42 filter paper. Copper concentrations in the filtrates were determined by atomic absorption spectrophotometry.

5.2.2 Soil Analysis

DTPA (DTPA-Cu) and Mehlich (Mehlich III-Cu) extractable Cu in the 10 soils were determined prior to the experiment. The DTPA extractable Cu was determined by the procedure devised by Lindsay and Norvell (1978). Ten g of each air-dried soil was extracted in duplicate with 20 ml of DTPA extracting solution (0.005 M DTPA, 0.1 M triethanolamine, and 0.01 M CaCl₂, pH 7.3). The extraction was performed by shaking the suspension for 2 h on a reciprocating shaker at a rate of 120 cycles min⁻¹. The suspension was centrifuged for 10 min at 2000 rpm and filtered through Whatman no. 42 filter paper. Copper in the filtrate was determined by atomic absorption spectrometry.

Mehlich III extractable Cu was determined by the procedure developed by Mehlich (1984). For this procedure, 2.5 cm³ of air dry soil were measured in duplicate into 100 mL plastic extraction tubes and 25 mL of Mehlich III extracting solution (0.2 N CH₃COOH, 0.25 N NH₄NO₃, 0.015 N NH₄F, 0.013 N HNO₃, and 0.001 M EDTA) was added. The suspension was shaken on a mechanical reciprocating shaker at the rate of 200 cycles min⁻¹ for 5 min and filtered through Whatman no 42 filter paper.

Copper in the filtrate was measured by atomic absorption spectrophotometry.

5.2.3 Statistical Analysis

Treatment effects on corn dry matter yield and Cu concentration data were evaluated by analyses of variance (ANOVA). Correlations between extractable Cu in soils and both Cu uptake by corn and soil properties were performed on an IBM 3081 computer utilizing SAS programs (SAS Institute Inc., 1982)

5.3 RESULTS AND DISCUSSION

A greenhouse experiment was conducted to evaluate the response of corn to applied Cu as CuSO_4 in 10 different soils in Virginia. Treatments on each soil, which consisted of a control and 5.35 mg Cu kg^{-1} as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, were harvested to determine the dry matter yield and tissue Cu concentration when the plants were about 50 cm in height. DTPA and Mehlich III extractable Cu in the soil were related to dry weights and Cu uptake of corn. The ten Virginia soils chosen for the research ranged from 5.7 to 7.1 in pH and from 11 to 30 mg kg^{-1} organic matter content (Table 12).

Table 13. Properties of soils used to evaluate Cu uptake by corn plants when fertilized with copper sulfate.

Soil no.	Soil series	Soil texture	pH	Organic matter g kg ⁻¹
1	Altavista	loamy sand	5.7	13
2	Bojac	loamy sand	6.2	12
3	Munden	loamy sand	6.3	11
4	Tomotley	loamy sand	6.9	22
5	State	sandy loam	7.1	17
6	Tetotum	sandy loam	6.9	17
7	Groseclose	silt loam	7.0	30
8	Bertie	fine sandy loam	5.9	19
9	Craven	sandy loam	6.7	12
10	Bojac	loamy sand	6.4	17

Table 14. Classification of 10 Virginia soils used in the greenhouse experiment.

Soil no.	Soil series	Classification
1	Altavista	Aquic Hapludults, fine-loamy, mixed, thermic
2	Bojac	Typic Hapludults, coarse-loamy, mixed, thermic
3	Munden	Typic Hapludults, fine-sandy, mixed, mesic
4	Tomatley	Typic Hapludults, fine-loamy, mixed, thermic
5	State	Typic Hapludults, fine-loamy, mixed, thermic
6	Tetotum	Aquic Hapludults, fine-loamy, mixed, thermic
7	Groseclose	Typic Hapludults, clayey, mixed, mesic
8	Bertie	Aquic Hapludults, fine-loamy, mixed, thermic
9	Craven	Aquic Hapludults, clayey, mixed, thermic
10	Bojac	Typic Hapludults, coarse-loamy, mixed, thermic

5.3.1 Dry Matter Yield

Dry weights of corn shoots ranged 4.49 to 6.27 pot⁻¹ and 4.44 to 6.15 g pot⁻¹ on the control and Cu treatments on the 10 soils, respectively (Table 13). Copper application did not increase dry matter yield of corn shoots on any of the 10 soils ($\alpha = 0.05$). Lack of dry weight increase from Cu application may be due to the low sensitivity of corn to deficiency. Supporting this nonresponsiveness in corn to Cu application, Brown and McCormic (1971) indicated that there was no grain yield increase of corn to added Cu as CuSO₄ in their field experiments on Missouri soils. Mullins et al. (1982) reported that neither grain nor silage yield of corn was affected by 15 years of application of various levels of Cu as CuSO₄ which provided a cumulative total of 172 kg Cu ha⁻¹. In the same field experiment, but in subsequent years, Gettier (1986) and Winarko (1985) did not obtain an increase in corn yield from application of a cumulative total of 280 kg Cu ha⁻¹ and 334 kg Cu ha⁻¹, respectively. The above mentioned data point out the low responsiveness of corn to Cu application.

5.3.2 Tissue Copper Concentrations

Application of CuSO₄ at the rate of 5.35 mg Cu kg⁻¹ increased ($\alpha = 0.001$) tissue Cu levels in plant shoots on all

Table 15. Dry matter weights and tissue Cu concentrations of corn grown on 10 different soils with or without Cu fertilization.

Soil no.	Soil series	<u>Dry matter</u>		<u>Tissue Cu</u>	
		Control	+Cu	Control	+Cu
		_____ g pot ⁻¹ _____		_____ mg Cu kg ⁻¹ _____	
1	Altavista	6.27	6.07	5.22 c	7.56 ab ⁺
2	Bojac	5.45	6.01	4.64 cd	5.63 cd
3	Munden	6.03	5.94	5.58 c	8.72 a
4	Tomatley	4.49	4.44	2.51 c	4.98 d
5	State	5.62	5.84	7.59 ab	7.45 ab
6	Tetotum	5.97	6.15	6.18 bc	7.00 bc
7	Groseclose	4.41	5.66	3.23 de	5.78 cd
8	Bertie	5.14	5.18	7.78 a	8.12 ab
9	Craven	5.79	5.94	2.51 e	7.74 ab
10	Bojac	6.23	5.94	5.46 c	6.68 bc
		_____ F value _____			
Analysis of variance		2.48 ns		40.45 ***	

*** Significant at the 0.001 level of probability.

ns Not significant at the 0.05 level of probability.

+ Column means followed by different letters are significantly different at 0.05 level of probability.

soils. (Table 12). Similar results were reported for corn ear leaves by Winarko (1985) from application of Cu at a cumulative total of 334 kg Cu ha⁻¹. In contrast, Gettier (1986) reported no difference ($\alpha = 0.05$) in corn tissue Cu concentration from a cumulative total application of 280 kg Cu ha⁻¹. He concluded that there was no correlation between tissue Cu levels and added Cu. But all his tissue Cu levels in all treatments were within the sufficiency range. In this experiment, all the tissue Cu concentrations for the control and copper treatment were above 2 mg Cu kg⁻¹, which is the critical Cu level suggested by many researchers (Gartrell and Brenan, 1979; Gartrell et al., 1979a,b; Loneragan et al., 1980).

5.3.3 DTPA and Mehlich III extractable Copper

There were differences ($\alpha = 0.001$) between the untreated soils in amounts of DTPA-Cu and Mehlich III-Cu (Table 14). DTPA-Cu in the ten soils ranged from 0.24 to 6.42 mg kg⁻¹ which is above 0.2 mg kg⁻¹ critical level for the procedure (Caldwell, 1971). Mehlich III-Cu ranged from 0.14 to 1.30 mg L⁻¹. Makarim and Cox (1983) suggested that the critical level of Mehlich III-Cu was 0.37 mg L⁻¹. Accordingly all the soils were above the critical level for the Mehlich III procedure except soils 3, 4 and 7.

Table 16. DTPA and Mehlich III extractable Cu in 10 soils prior to Cu application.

Soil no.	Soil series	DTPA-Cu mg kg ⁻¹	Mehlich III-Cu mg L ⁻¹
1	Altavista	0.69 d ⁺	1.04 b
2	Bojac	0.52 d	1.21 b
3	Munden	0.44 d	0.18 d
4	Tomatley	0.40 d	0.16 d
5	State	6.42 a	1.30 a
6	Tetotum	5.03 b	1.02 b
7	Groseclose	0.41 d	0.22 d
8	Bertie	2.56 c	1.07 b
9	Craven	0.24 d	0.14 d
10	Bojac	1.57 c	0.64 c
		_____ F value _____	
Analysis of variance		43.39 ***	42.9 ***

+ Column means followed by different letter are significantly different at 0.05 level of probability

*** Significant at the 0.001 level of probability.

Copper uptake by corn plants from the 10 soils correlated with DTPA-Cu ($\alpha = 0.001$), but the coefficient of simple correlation ($r = 0.62$) was rather low (Table 14). The regression equation for the relationship is shown below:

$$\text{Cu - uptake} = 22.28 + 3.44 \text{ DTPA - Cu}, \quad r = 0.62^{***}$$

Mehlich III-Cu was more closely related to Cu uptake than DTPA-Cu. The regression equation for the relationship between Mehlich III-Cu and Cu uptake is shown below:

$$\text{Cu - uptake} = 16.97 + 19.39 \text{ Mehlich III - Cu}, \quad r = 0.75^{***}$$

Organic matter did not correlate with Cu-uptake ($\alpha = 0.05$) whereas soil pH was weakly related to Cu uptake ($r = 0.35$, $\alpha = 0.05$). DTPA-Cu did not correlate with either organic matter or soil pH ($\alpha = 0.05$). The lack of a relationship between DTPA-Cu and soil organic matter may reflect differences in organic matter composition and Cu concentration among soils. Neither DTPA-Cu nor Mehlich III-Cu correlated with organic matter and soil pH either individually or combined as shown below:

$$\text{DTPA - Cu} = -8.98 - 0.75 \text{ OM} + 1.86 \text{ pH}, \quad r = 0.36 \text{ ns}$$

$$\text{Mehlich III - Cu} = 1.76 + 2.43 \times 10^{-2} \text{ OM} - 1.84 \times 10^{-1} \text{ pH}, \quad r = 0.17 \text{ ns}$$

Table 17. Relationship of Cu uptake and extractable Cu with soil properties.

Dependent variable	Independent variable	r	Significance level
Cu uptake	DTPA-Cu	0.62	***
	Mehlich III-Cu	0.75	***
	Organic matter	0.33	ns
	pH	0.35	*
DTPA-Cu	Organic matter	0.01	ns
	pH	0.32	ns
Mehlich III	Organic matter	0.06	ns
	pH	0.18	ns

*** Significant at the 0.001 level of probability.

* Significant at the 0.05 level of probability.

ns Not significant at the 0.05 level of probability.

5.3.4 Copper Uptake and Percent Copper Uptake

Uptake of applied Cu by corn plants grown on the 10 soils varied approximately three-fold (Table 16). The percent of applied Cu absorbed by corn plants ranged from 0.323 to 0.007 and differed among soils ($\alpha = 0.05$). The inverse of the percent absorption of applied Cu indicates a high amount of Cu fixation in these soils. Soil organic matter is considered to be a dominant complexer of Cu, and the Cu complexation by soil organic matter increases with an increase in soil pH (Kabata-Pendias and Pendias, 19885). In these soils with generally low organic matter contents and relatively high pH levels, percent absorption of applied Cu by corn plants was unrelated ($\alpha = 0.05$) to either organic matter content or soil pH. Undoubtedly, layer silicates and/or hydrous oxides of Al, Fe or Mn were the main adsorbers of Cu in these soils

As summary, addition of Cu did not increase dry matter yield of corn grown on 10 soils, but did increase Cu concentrations in corn shoots grown on the soils. Copper concentrations in corn plants from the control and Cu treatments were within the normal range (Gartrell and Brennan, 1979; Gartrell et al., 1979 a,b; Loneragan et al., 1980). A very low percentage of the applied Cu was absorbed by corn plants from the 10 soils. This relationship indicates that a high amount of Cu fixation occurred in the soils. The lack of an

increase in dry weight of corn plants from Cu application may be due to the lack of sensitivity of corn to Cu deficiency.

Table 18. Uptake and percent uptake of applied copper by corn on 10 different soils.

Soil no.	Soil series	Uptake of applied Cu	Proportion absorbed
		mg pot ⁻¹	%
1	Altavista	0.013 b*	0.099 b*
2	Bojac	0.009 b	0.067 b
3	Munden	0.018 ab	0.135 ab
4	Tomatley	0.011 b	0.082 b
5	State	0.009 b	0.007 b
6	Tetotum	0.006 b	0.045 b
7	Groseclose	0.018 ab	0.135 ab
8	Bertie	0.002 d	0.015 b
9	Craven	0.031 a	0.232 a
10	Bojac	0.006 b	0.045 b
		_____ F value _____	
Analysis of variance		2.75 ***	2.75 ***

* Column means followed by different letter are significantly different at 0.05 level of probability.

*** Significant at the 0.001 level of probability.

Table 19. Relationship of uptake and percent uptake of applied copper with soil properties.

Dependent variable	Independent variable	r	Significance level
Uptake of applied Cu	Organic matter	0.004	ns *
	pH	0.013	ns
Percent uptake of applied Cu	Organic matter	0.005	ns
	pH	0.013	ns

* ns Not significant at the 0.05 level of probability.

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CHAPTER 6

SUMMARY AND CONCLUSIONS

Copper and Zn are frequently incorporated in to agricultural soils has fertilizers, pesticides and waste materials. High levels of Cu and Zn application to agricultural soils are considered to pose a potential hazard to both plants and animals. Experimental evidence is needed to evaluate the effects of high levels of applied Cu and Zn on agricultural lands.

A field study was undertaken on a Davidson silty clay (clayey, kaolinite, thermic, Rhodic, Paleudult). The main objective of this study was to determine the corn response to 19 annual application of various levels CuSO_4 and ZnSO_4 , which provided 338 kg Cu and 830 kg Zn ha^{-1} either alone or together.

The 19 annual application of CuSO_4 or ZnSO_4 did not effect either corn grain or corn-stalk yield ($\alpha = 0.05$). Copper concentrations in the corn grain and ear leaves were not affected by the various levels of applied Cu. Zinc concentrations in both grain ($\alpha = 0.01$) and ear leaves ($\alpha = 0.001$) were increased by Zn application, and the highest levels was observed at the highest cumulative Zn level (830 kg Zn ha^{-1}). Copper in the labile pool was increased by ZnSO_4 application, but the reverse did not occur.

A greenhouse experiment was conducted with 14 Virginia soils with diverse soil properties to evaluate the response of wheat to Cu application. Treatments in the greenhouse study were a control and 5.35 mg Cu kg⁻¹ as CuSO₄. Copper application did not increase dry matter yields of wheat, but did increase Cu concentration in leaf tissues ($\alpha = 0.001$). Copper uptake was highly correlated with DTPA-Cu ($r = 0.80$, $\alpha = 0.001$) but no relationship occurred between Cu uptake and Mehlich III-Cu ($\alpha = 0.05$). DTPA-Cu highly correlated with clay content ($r = 0.98$, $\alpha = 0.001$) and SA ($r = 0.93$, $\alpha = 0.001$).

A second greenhouse experiment was conducted with 10 Virginia soils with diverse soil properties to evaluate response of CuSO₄ fertilization on corn. Treatments in the greenhouse experiment were control and 5.35 mg Cu kg⁻¹ as CuSO₄. Copper fertilization did not affect ($\alpha = 0.05$) dry matter yields of corn grown on the soils, but tissue Cu concentrations were increased ($\alpha = 0.001$) in response to the applied Cu. Copper uptake by the corn plant correlated with DTPA-Cu ($r = 0.62$, $\alpha = 0.001$) and soil pH ($r = 0.35$, $\alpha = 0.05$). Both DTPA-Cu and Mehlich III-Cu were unrelated to both organic matter or soil pH ($\alpha = 0.05$).

Application of up to 338 kg Cu ha⁻¹ and 830 kg Zn ha⁻¹ as sulfate to Davidson soil in 19 annual applications, which was above the tolerable levels for the application of these micronutrients as sewage sludge based on US EPA guidelines,

did not adversely affect the growth and yield of corn. It was concluded that non significant response of corn to the 19 annual Cu applications were more attributable to the uptake and translocation mechanism of Cu by corn plants than to the Cu concentrations in potentially plant available forms. Therefore, it is suggested that the long term field experiment be continued and if possible with different crop species.

Fourteen different soils with diverse properties were used in the greenhouse experiment to study the response of wheat to Cu fertilization. Since wheat is a Cu responsive crop, dry weight increases of wheat from Cu application were expected. Nevertheless, Cu application did not increase dry weights on any of the 14 soils. The nonresponsiveness of wheat to applied Cu lead to the conclusion that 14 Virginia soils were able to meet the Cu requirement of wheat without Cu fertilization.

Copper application did not increase dry weights of corn in the second greenhouse experiment with 10 Virginia soils. These data indicated that the 10 soils supplied adequate Cu for corn growth. In these soils, the plant available Cu fractions were more related to clay content than organic matter indicating that most of the Cu was adsorbed on exchange sites of the clay minerals. Overall, data from this research agree with data in the literature suggesting a low responsiveness of corn to Cu fertilization.

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