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LONG TERM EFFECT OF COPPER SULPHATE FERTILIZATION OF ORGANIC SOILS ON YIELD OF BARLEY AND DTPA-EXTRACTABLE SOIL COPPER LEVELS

By

RAYMOND EDWARD DOWBENKO

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment for the Degree
Master of Science

Department of Soil Science

Winnipeg, Manitoba

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ABSTRACT

Field studies were conducted in 1987 and 1988 to assess the residual effectiveness of various rates of $CuSO_4$ application on organic soils. As well, an investigation was undertaken to assess both plant and soil analyses as methods of assessing plant available copper on organic soils.

Copper, applied as $CuSO_4 \cdot 5H_2O$, at rates of 5 and 10 Kg Cu ha^{-1} increased barley grain yield for 3 to 4 years after initial application. Annual increments of $CuSO_4$ at a rate of 5 Kg Cu ha^{-1} further increased grain yields on plots originally treated with 5 or 10 Kg Cu ha^{-1}. Good relationships were obtained between DTPA-extractable copper concentration of soils and yield ($r = 0.64$) and % yield ($r = 0.58$). Relationships (r values) obtained between the copper content of plant tissue at the heading stage and yield, and % yield were 0.46 and 0.60, respectively. Both the copper concentration of plant tissue at the heading stage, and the DTPA-extractable soil copper test were found to be equally adequate for assessing copper fertilizer requirements on organic soils. Critical levels for DTPA-extractable copper and concentration of copper in plants at the heading stage were 16 and 5.6 $\mu g\ g^{-1}$, respectively.

Two organic soils utilized in the 1987 and 1988 field studies were obtained for growth chamber study to further investigate the use of plant and soil analyses as methods of assessing plant available soil copper on organic soils. The relationships between % yield and plant tissue copper concentration at the heading stage, and % yield and

DTPA-extractable soil copper concentration were close with r values of 0.75 and 0.64, respectively. Critical levels obtained in the growth chamber study were about 1.5 and 3 times lower for plant tissue copper concentration at the heading stage, and DTPA-extractable soil copper concentration, respectively, than those critical levels obtained in the field study.

The relative effectiveness of copper from Cu-EDTA and $CuSO_4 \cdot 5H_2O$ in increasing barley grain yield was assessed in a growth chamber study. Plant copper content at the heading stage was not significantly increased when 0.25 and 0.50 Kg Cu ha^{-1} was added as Cu-EDTA. Both sources of copper applied at 5 Kg Cu ha^{-1} increased copper content at the heading stage. Grain yields, however, did not differ significantly among rate or copper source applied. However, the results obtained indicated that the quality of grain produced with $CuSO_4$ was superior to that produced with Cu-EDTA.

A field study was conducted in 1988 to evaluate the effects of copper source, band size and placement method on the growth of barley. Placement of $CuSO_4$ in wide bands, as opposed to narrow bands, increased the plant availability of copper from $CuSO_4$. The greater utilization of copper with the wide bands was attributed to a greater plant root-copper fertilizer contact. Placement method did not greatly affect plant copper availability from the more soluble and mobile Cu-EDTA copper source. At equivalent rates of application, Cu-EDTA provided greater plant available copper than $CuSO_4$ early in the growing season. However, at the later stages of crop growth both Cu-EDTA and $CuSO_4$ appeared to be equally effective in increasing plant copper content and grain yield.

A growth chamber study was conducted using two organic soils to investigate the relationships between plant growth, soil pH and DTPA-extractable soil copper concentration. Increases in DTPA-extractable soil copper concentration occurred in response to crop growth. DTPA-extractable copper concentration increased from time of plant emergence to heading, and declined from the heading stage to final harvest closely approaching levels found prior to seeding. Increases in DTPA-extractable soil copper concentrations were likely related to crop rhizosphere development and the concurrent decline in soil pH level. The DTPA-extractability of copper applied as $CuSO_4 \cdot 5H_2O$ and Cu-EDTA was investigated in a growth chamber study. The most significant feature of the data obtained was that much lower amounts of copper were extracted from soils treated with Cu-EDTA than from soils treated with $CuSO_4$. It was postulated that copper from Cu-EDTA may have been more subject to soil organic matter-copper complexation reactions than copper from $CuSO_4$ when competitive ions displaced copper from Cu-EDTA. Due to the greater mobility of copper from Cu-EDTA than from $CuSO_4$, copper from Cu-EDTA, shortly after application, would diffuse and contact a larger amount of soil than copper from $CuSO_4$. Once the copper was displaced from the chelate, it would be subject to fixation reactions.

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I. INTRODUCTION

Previous fertility studies conducted in Manitoba indicated that copper deficiency is a major limitation to small grain production on organic soils (Reid 1982; Tokarchuk 1982). Copper, as copper sulphate ($CuSO_4 \cdot 5H_2O$), is usually applied to alleviate this deficiency. Although yields are increased with $CuSO_4 \cdot 5H_2O$ application, the cost of fertilization may, in instances, exceed the value of the yield increase in the year of application. However, residual effects of copper fertilization on plant tissue copper content and grain yield have been shown to occur for five or more years after the initial application (Pizer et al. 1966; Reith 1968; Reuter 1975). Information regarding the residual effectiveness of $CuSO_4$ in Manitoba was very limited and the economic benefits including the residual effects of $CuSO_4$ could not be accurately ascertained. Also, application of copper fertilizer in excess, due to annual applications over a period of time, may have a deleterious effect on plant growth. Thus, a means of predicting the requirement for copper on previously fertilized and unfertilized soil was needed to ensure sound copper fertilizer practices for organic soils.

Although $CuSO_4$ is often applied to alleviate copper deficiency on organic soils in Manitoba, various chelated forms of copper (eg. Cu-EDTA) are also applied. Copper chelates, due to their greater solubility and mobility, appear to offer increased copper efficiency (Gartrell 1981). Work by Karamanos et al. (1986) however, suggested that a soil applied chelate was beneficial to crop yield only in the year of application and offered no residual value. Method of application greatly affects the relative effectiveness of various copper sources such as $CuSO_4$ and chelated copper. Varvel (1983) showed that the greatest differences in yield between $CuSO_4$ and Cu-HEDTA were obtained when the carriers were banded. Copper sulphate was shown to effectively increase yield when broadcast, but not when banded. Chelated copper effectively increased yield for both methods of application.

Studies were conducted to:

1) assess the residual effects of copper fertilizers on organic soils,
2) develop a soil test for assessing plant available soil copper in organic soils,
3) compare the relative effectiveness of $CuSO_4$ and chelated copper (Cu-EDTA) in enhancing copper concentration of plants and grain yield, and
4) determine the effect of placement of $CuSO_4$ and Cu-EDTA in enhancing copper concentration of plants and grain yield.

The objective of the studies listed above was to obtain information to improve copper fertilization practices for organic soils in Manitoba.

II. LITERATURE REVIEW

2.1 Residual Effectiveness of Copper on Organic Soils

Most of the copper fertilizer applied is not utilized by the crop in the year of application. As such, the residual availability of this fertilizer becomes important to subsequent crops. Lucas and Knezek (1972) suggested that a single soil-application of copper could supply plant requirements for many years. They did not, however, relate application rate and soil type to residual effectiveness of the applied copper. Reith (1968) found that a single application of copper fertilizer under field conditions may provide adequate plant available copper for up to 8 yr. The residual effectiveness of applied copper, however, has been shown to be dependent upon application rate and soil type. Smith et al. (1962) stated that the low mobility of copper in soil was an explanation for its persistence in soil and residual effectiveness.

Although an application of copper usually continues to have an effect on crop growth beyond the year of application, the effect usually decreases with time. Barrow and Campbell (1972) showed that a precise measure of the residual effect is often difficult, as is the detection of small effects. Gartrell (1980) found that the residual effect of copper was equal to or greater than that of a fresh copper application. He further suggested that the residual effect could be explained on a chemical basis, and that all the soil applied copper remained in the top 10 cm of soil 4 yr after application. The processes which enhanced copper availability with time seemingly dominated any soil copper-fixing reactions.

Reductions in residual effectiveness of applied copper with time, in the work of Brennan et al. (1980), were postulated to be a result of slow reactions which converted the applied copper to forms of lower plant availability. Brennan et al. (1986a) showed that the availability of applied copper usually declined with increasing time of contact between soil constituents and copper, and that the soil constituent-applied copper reactions were of major importance in decreasing plant available copper with time (Brennan et al. 1986b). Brennan et al. (1980), had shown, in earlier studies, that reductions in residual effectiveness were usually greater for soils of high organic matter content than for soils of low organic matter content. However, the exact nature of the reaction between applied copper and soil constituents were not elucidated.

Copper adsorption studies have indicated that the reactions involved are either; irreversible, extremely slowly reversible, slowly reversible, require a high activation energy for desorption, or involve a combination of these (McLaren et al. 1983). McLaren and Crawford (1974) suggested that some irreversible, or slowly reversible reaction can take place soon after adsorption of copper by soil components. The limited or poor reversibility of copper adsorption, however, would have serious implications regarding the residual effectiveness of applied copper.

The availability of applied copper decreases with time. The residual effects of such applications have been reported to last from 3 to 12 years. The reduction in residual effectiveness seemingly occurs due to slow reactions occurring in the soil which convert copper into plant unavailable forms. The mechanisms by which these reactions occur are, at present, not clearly understood (James and Barrow 1981). The residual effectiveness of any given application of copper will be affected by such soil factors as: organic matter content, soil pH, soil temperature and, soil water relations. As well, both crop type and crop variety will affect the determination of residual effects.

Residual effects from copper applications are known to occur, and Follet and Lindsay (1971) suggested that the quantities of copper extracted by DTPA reflect residual effectiveness. Although many researchers indicate definite residual effects for copper applications, few, if any, have quantified these effects for organic soils. Graham and Nambiar (1981) have most appropriately stated that an urgent need exists for research which determines when reapplication of copper fertilizer is necessary, and the soil properties which affect the residual value of copper applications.

2.2 Factors Affecting Residual Copper Availability

McBride (1981) stated that copper is likely the most versatile of all trace elements. Due to its ability to chemically interact with soil organic and mineral components, as well as to form precipitates with hydroxide, carbonate, sulphide and other anions, numerous forms of copper are likely to exist in any given soil environment. Specific adsorption by silicate clays, iron, aluminum and manganese oxides, and organic matter is known to occur for copper (McBride 1981).

Copper-organic matter complexation is recognized as an effective mechanism of copper retention in soils. Ardakani and Stevenson (1972) stated that many aspects of copper chemistry and mobility in soils are related to organic matter-copper complexes. Evidence exists that copper is bound in forms of low availability to plants through complexation to soil organic matter (Davies et al. 1969). As such, copper deficiency is most frequently found on organic soils.

Organic soil constituents may form both soluble and insoluble complexes with copper. Compounds such as humic and fulvic acids, aliphatic acids, peptides, proteins, amino acids and polysaccharides are involved (Stevenson and Ardakani 1972). McLaren and Crawford (1973a) have shown that the majority of plant available copper was contained in the organically bound fraction. They further proposed that plant available copper was dictated by an equilibrium involving specifically adsorbed forms. The relation was as follows:

$$\begin{array}{c}\text{Exchangeable and}\\ \text{Soluble Copper}\end{array} \rightleftharpoons \begin{array}{c}\text{Specifically Adsorbed}\\ \text{Copper}\end{array} \rightleftharpoons \begin{array}{c}\text{Organically Bound}\\ \text{Copper}\end{array}$$

Hodgson et al. (1966) found that the organically bound fraction represented greater than 90% of the solution copper in neutral or alkaline soils. McLaren and Crawford (1973b) found that the copper bound to organic matter represented from one-fifth to one-half of the copper in soil. Kline and Rust (1966), in fractionation studies, showed that the largest fraction of soil copper was the fraction associated with organic matter. Nriagu (1979) calculated that, on a world basis, 36% of the total copper content of soils would be associated with organic matter. A variety of reactions can occur between organic matter and copper which lead to stable organic matter-copper complexes (Stevenson and Fitch 1981).

As the stability of these complexes increases, the copper becomes less available for plant uptake (Ennis and Brogan 1966; Goodman and Cheshire 1976). The more weakly held copper can, however, be absorbed by plants (Petruzelli and Guidi 1976). Thus the availability of copper from organic matter would be a function of the method of binding (Brennan et al. 1983). To a great degree this association involves phenolic, hydroxyl and/or carboxyl groups (Davies et al. 1969). While these groups provide relatively weak linkages, Goodman and Cheshire (1976) suggested the more stable, permanent fixation of copper may result from complexing with porphyrin groups. Porphyrin group copper complexes are of exceptionally high stability and it appears that these groups may control the availability and concentration of copper in soil solution. The result of Goodman and Cheshire (1976) suggest that porphyrin groups must approach saturation with copper before copper deficiency is eliminated.

Copper adsorption to organic matter occurs through a variety of bonds; DeMumbrum and Jackson (1965) noted chelation of copper by organic soils through alcoholic hydroxyls, C=O and N=O groups. McBride (1978) suggested copper may be held directly to organic functional groups such as carboxylic, carbonyl and phenolics. McBride (1981) further stated that organic matter held copper in plant available, but thermodynamically stable form.

Copper exists mainly in a complexed form in soil solution (Guy et al. 1975), with humic and fulvic acids playing a significant role in complexation (Schnitzer and Khan 1972). The solubility of humic and fulvic acids depend upon their degree of saturation. Fulvic acids are of higher acidity and lower molecular weight and are generally more soluble than humic acids. Normally, decreased solubility of humic and fulvic acids occurs with increased saturation by copper (Stevenson 1982).

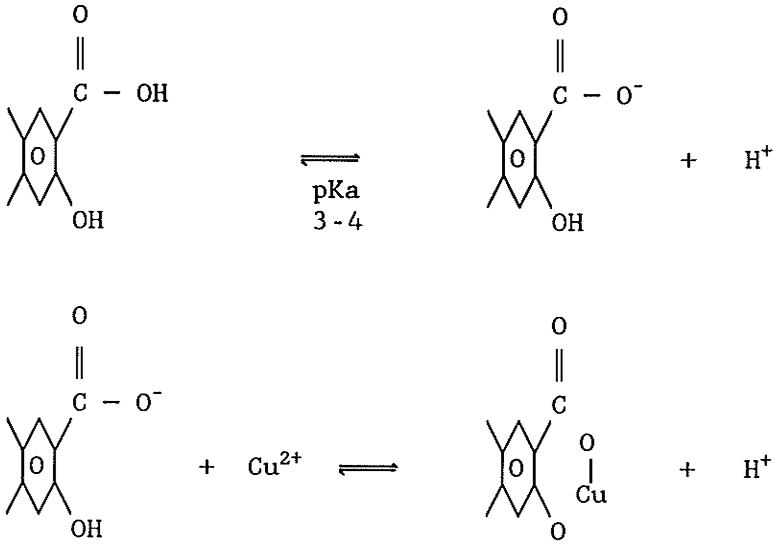
Geering and Hodgson (1969) showed that humic and fulvic acid constituents, and individual biochemicals are involved in Cu^{2+} movement to plants. The fulvic acids are considered to be the most efficient in complexing metals. Content of functional groups such as COOH, phenolic OH, and ketonic C=O are largely responsible for the complexing ability of humic and fulvic acids (Stevenson and Ardakani 1972).

Multiple bonding sites are found on organic matter, the strength of bonding varies with several factors. For low saturation, copper will form covalent bonds, bonds become more ionic as the binding capacity is approached (Preston et al. 1980). Stevenson and Fitch (1981) conclude that bonding sites with a high stability constant would be dependent on the level of saturation of the complexing sites. An increase in strength of binding of copper by humic acid with decreasing amount of copper applied has been noted by Davies et al. (1969) and Goodman and Cheshire (1973; 1976). The organically bound fraction of soil copper adsorbed copper strongly, and also released less copper (Cu^{2+}) to the soil solution as copper levels were lowered (Petruzelli and Guidi 1976). The organic bound fraction was defined as the capacity factor, and the copper specifically adsorbed to inorganic sites was defined as the intensity factor by Graham and Nambiar (1981).

Soil pH is a major factor in determining the degree of complexation occurring between copper and organic matter (Guy et al. 1975; Ghosh and Schnitzer 1981). The species distribution of copper in soil solution will be affected by both organic matter content and soil pH. The soil pH determines the degree of ionization of acidic groups for humic substances and thus the binding sites for copper (Stevenson and Fitch 1981). Sanders and Bloomfield (1980) found that for an organic soil, the degree of complexation of copper increased as pH increased. Jarvis (1981) found increasing sorption with increasing pH and suggested that copper was largely complexed to stable organic matter. Amounts of copper sorbed by surfaces generally increased with increasing pH (James and Barrow 1981; Jarvis 1981; McBride 1981). Thus, it would be expected that copper availability to plants would decrease with increasing pH. However, the literature is contradictory with respect to the effect of soil pH on plant availability of copper. The amounts of copper extracted by chemical extractants vary more with soil pH than does the amount removed by plants. This may be due to the greater efficiency of the plant uptake process at higher pH levels. Jeffery and Uren (1983) and Blevins and Massey (1958) have shown that there is little dependence of copper in soil solution on soil pH. As a result, they concluded that most copper occurs as either moderately labile or as a non-labile species. Kerven et al. (1982) however, showed that increasing pH levels from 3.4 to approximately 6.0 increased the degree of complexation of soluble copper in organic soils to greater than 98%. Computer modelling of soluble organic copper complexes has shown Cu^{2+} to be predominant at pH levels less than 5.5 (99%), whereas above this pH, $CuOH^+$ is formed. Lindsay (1979) suggested that below a pH level of 6.9, Cu^{2+} is predominant, while $Cu(OH)_2$ is a major species at higher pH levels. McBride and Blasiak (1979) have indicated that solution copper complexation starts at about pH 4.0 and increases as pH rises. The general effect of an increase in sorption with increasing pH level likely results from increased pH dependant sites on the colloids, reduced H^+ competition, and changes in the hydrolysis state of copper in soil solution (Jarvis 1981).

Humic component complexes are soluble over a wide pH range, and the increased complexation of copper with rising pH is most likely due to increased solubility of humic substances. Kiekens and Cottenie (1981) stated that the precipitation of complexes at acid pH levels immobilize copper, and as such may lead to the variable results obtained when attempting to correlate copper availability and pH.

Posner (1964) and Gamble (1970) suggested that soil organic matter in the pH range of most soils may be considered a polycarboxylic acid. Carboxylic acid groups bind Cu^{2+} with the release of H^+. As well, the ionization of phenolic hydrogen must also be considered (Stevenson and Ardakani 1972). Bloom and McBride (1979) concluded that the binding of Cu^{2+} may involve exchange of one or two aquoligands by carboxylate oxygens. Kerven et al. (1982), working with an organic soil, showed that once all complexing sites were saturated, further copper additions remain in ionic form. Further, they pointed out that this complexation was of a reversible nature, and suggested the involvement of a weak acid group at the functional site. The degree of ionization was pH dependant, and the ability to complex copper was also pH dependant. Gamble et al. (1970) and Schnitzer and Hansen (1970) suggested the complexation involved a carboxyl group ortho to a phenolic group as shown below.



Boyd et al. (1981), using electron spin resonance spectroscopy, obtained information which confirmed the above mechanism. Boischot and Quillion (1952) and Lucas and Davis (1961) had earlier stated that increasing pH would result in increased complexing of copper by phenolic and carboxylic groups and that the pH at which copper availability was maximal would vary with soil conditions such as organic matter content. A soil will hold copper tightly in pH range 7.0 to 8.0, less securely at pH 6.0, and progressively less with further decreasing pH levels (Stevenson 1982). In this respect, factors influencing soil pH will affect the copper concentration in soil solution and therefore plant available copper.

The plant root is capable of modifying the soil environment by absorbing ions and excreting chemically active substances. Organic acids, amino acids, HCO_3^- and H^+ ions can affect the release of copper from soil colloids (Loneragan et al. 1981). Occurrence of differential uptake of cations and anions results in the plant root altering the pH of its environment through release of H^+ and OH^- and/or HCO_3^- ions (Soon and Miller 1977). Work by Smiley (1974) has shown changes in the rhizosphere soil to be great enough to influence micronutrient concentration and availability for plant uptake. Harry and Graham (1981) stated that acidification usually increases the available copper in soil and may lead to increased grain yield if copper is otherwise deficient. Linehan et al. (1985) showed that a substantial drop in pH occurred during early rhizosphere development. While this has the possibility of mobilizing copper for plant uptake, this low pH level did not continue into the phase of maximum copper mobilization. It was concluded that acidification of the root zone may have contributed to copper mobilization, but was not fully responsible for mobilization of copper due to rhizosphere effects. Rovira and Davey (1974) suggest that a possible mechanism of mobilization is the exudation of low molecular weight organic ligands by the plant roots and/or microorganisms associated with the rhizosphere. Rovatt and Katznelson (1961) recorded a higher proportion of acid-producing organisms in the rhizosphere as compared to non-rhizosphere soil. Louw and Webley (1959) suggested that acid producing organisms are preferentially stimulated in the rhizosphere. These organisms have the effect of both lowering the soil pH and producing natural chelates. Vedy and Bruckert (1979) have shown that organic acids capable of complexing copper occur in soil solution at concentrations reaching 10^{-5} M.

Soluble organic matter and natural chelates such as organic acids are capable of forming copper complexes. Soluble complexes such as these are an important influence on plant available copper. Jarvis (1981) stated that although complexing ligands increase copper mobility and availability, they may also offer competition to the plant roots for copper. Root exudates are considered to regulate uptake of copper from the soil directly (Chaberek and Martell 1959). Sequi et al. (1975) determined apparent stability constants for copper organic matter complexes and found stability constants to be much higher for root secretions than for various soil organic matter fractions. It is however, often reported that biochemicals (eg. aliphatic acids, sugar acids, phenols and amino acids) are of little consequence due to their rapid destruction by microorganisms. Although the total amount of any individual biochemical may be low, the combined total of all potential chelate-formers is sufficient to influence copper availability to plants (Stevenson and Ardakani 1972). Elgawhary et al. (1970) emphasized the importance of natural complexing biochemicals in copper transport to plant roots. Nielson (1976a) has shown that for plants growing in an organic soil, a large increase in organic complexing ligands is associated with an increase in copper concentration of the soil solution. The correlation coefficient was 0.96 during this period. No such increase in soil solution copper occurred when the same experiment was conducted without plant cover.

Clearly, the interaction of organic matter, soil pH, plant root and soil are major factors in the determination of plant available copper.

2.3 Assessing Plant Available Copper in Organic Soils

Both soil and plant tissue analysis are used in the determination of plant and soil nutrient status. The utility of plant and soil analysis are different; soil analysis leads to the indication of a possible copper deficiency, whereas tissue analysis will likely indicate that a deficiency is already present. From a practical point of view, the discovery of the possibility of a deficiency allows action to prevent that deficiency from occurring. Discovery that a deficiency is present allows for only action which may not be beneficial in many instances.

2.3.1 Plant Analysis

Relationships between plant micronutrient levels and crop yield have been established for a large number of crops. Established relationships are often used to compare a particular micronutrient level in terms of deficiency or adequacy. Critical levels are often used to describe the relationship between plant micronutrient concentration and expected crop growth. Provided that no other factors are limiting yield, critical level for a particular nutrient is defined as the plant concentration when yield is 5 to 15% below maximum (Jones 1972).

The determination of the copper status of plants by tissue analysis has often proved to be difficult. Plant tissue copper levels vary with crop species, variety and cultivar (Nambiar 1976a, b; Hill et al. 1978; Graham and Nambiar 1981; Brennan et al. 1986b). As well, plant tissue copper levels decline over the growing season (Gladstones et al. 1975; Reuter 1975). Loneragan et al. (1981) showed that copper behaves as if it were immobile in non-senescent leaves and mobile in senescent leaves. Retention of copper in non-senescent copper deficient leaves resulted in copper levels being greater in deficient plants than in copper sufficient plants. Results such as these lead to an often observed C-shaped Piper-Steenbjerg growth curve (Piper 1942; Steenbjerg 1951) which makes interpretation of tissue analysis difficult. Robson and Reuter (1981) demonstrated that the concentration of copper in plants increases as the degree of deficiency becomes more severe. Bates (1971) indicates that weather patterns affected plant copper concentration. Thus the same crop grown in the same location over a period of years, may in each year have a different critical level. In addition, the interaction between plant copper and other nutrients may further confound interpretation of tissue analysis.

Despite the existence of a general relationship between copper uptake by plants and the capacity of the soil to supply copper, the measure of the copper content of shoots have been unreliable in diagnosing copper deficiency (Caldwell 1971; King and Alston 1975). The work of Loneragan et al. (1980), as well as that of several earlier researchers (Caldwell 1971; King and

Alston 1975; Loneragan 1975; Reuter 1975; Hill et al. 1978), indicates that the copper concentration in mature shoots did not reflect plant copper status at earlier stages, nor when comparison was made between copper-adequate and deficient plants.

As a means of alleviating these problems in tissue analysis interpretation, Loneragan (1975) and Loneragan et al. (1976) suggested analysis of young leaves for diagnosis of copper deficiency. Other work has indicated that diagnosis of copper status of barley should be conducted on the two youngest leaves at the beginning of shooting.

As a result of the aforementioned problems, a measure of the copper concentration of plant samples is not always a useful diagnostic technique. As such, the literature often contains conflicting information regarding critical copper levels in plant tissue.

The usual range of copper concentration in plant tissue is between 5 to 20 $\mu g\ g^{-1}$ Cu (dry matter basis). Generally, copper concentrations in plants below 4 $\mu g\ g^{-1}$ indicate a deficiency is likely to occur (Jones 1972). King and Alston (1975) suggested that a critical copper concentration in cereal grain of 2.0 to 2.5 $\mu g\ g^{-1}$ may be used in identification of potentially deficient soils. A glasshouse study by Gupta and MacLeod (1970) suggested a critical level of 4.8 $\mu g\ g^{-1}$ for barley at the boot stage. Akinyede (1977), using a glasshouse study, suggested that barley at the boot stage containing less than 5.2 $\mu g\ g^{-1}$ Cu would be deficient. Ward et al. (1973) suggested that at less than

5.0 $\mu g\ g^{-1}$ Cu barley would be deficient. Reuther and Labanauskas (1966) stated that levels between 6.2 to 11.9 $\mu g\ g^{-1}$ were low for barley at harvest. McAndrew (1979), in a glasshouse study, indicated that 2.3 to 3.7 $\mu g\ g^{-1}$ Cu was the critical concentration in barley tissue at the boot stage.

The various critical levels for barley exemplify the range found for most cereal crops at each respective growth stage. The variation in values are likely a result of varying varieties, sampling times, type of plant tissue sampled and growth environment. As a result, the practical utility of tissue analysis is often limited due to these factors. The critical levels arrived at through controlled environment studies are often suspect. Soil moisture, temperature, rooting density, light intensity and subsoil fertility greatly affect copper uptake. Critical levels determined in glasshouse studies are unlikely to be the same as those obtained for field studies.

2.3.2 Soil Analysis

Cox and Kamprath (1972) stated that a most effective method of determining nutrient limitations is through the use of soil analysis ("soil tests"). Soil tests for micronutrients are most commonly used in determining soil nutrient status and whether an application of the nutrient of interest will result in increased yield. Caldwell (1971) has shown that copper deficiency is usually more prevalent on muck and organic soils than on mineral soils. The use of a soil test would be helpful in determining the levels at which this deficiency occurs. Viets and Lindsay (1973) however, have shown that difficulties in interpretation of soil tests for micronutrients are compounded by the low micronutrient status of some soils. Despite difficulties, soil analysis, if properly correlated with plant growth studies, is one means by which plant available copper may be assessed, and a large number and variety of extractants and methods have been developed.

The use of micronutrient soil tests allows the separation of deficient and non-deficient soils. As well, determination of a soil critical level with respect to a particular trace element can also be established. A soil critical level would be indicative as to whether or not a soil is capable of supplying adequate micronutrients for maximum crop yield.

As previously discussed, copper mobility in soils is affected by a number of soil properties. Through extraction techniques, attempts are made to relate mobile copper (a form that could be available for plant uptake) to plant copper content (Loneragan et al. 1981). Most micronutrient soil tests would involve correlating plant uptake of the trace element in question, and yield, with the amount of nutrient extracted. Calibration or standardization of the soil test to fit local conditions aids in correlation and use of the test for a specific trace element.

A great variety of micronutrient soil tests has been developed, each test possessing specific qualities that either render it suitable or unsuitable for a specific soil type, trace element, or environmental condition. Most tests developed are extraction procedures which attempt to measure the concentration or amount of plant available form, and the soils capability of resupplying available forms of the trace element for plant uptake.

Calibration and determination of the appropriateness of the test is usually conducted under controlled environment conditions. However, the same reasons which make controlled environment studies (glasshouse) inappropriate for determination of plant tissue critical levels apply as well to soil analysis.

According to Bray (1948), an appropriate micronutrient test should be such that the extractant will be able to extract the plant available form or forms of the trace element, the amount extracted should be correlated with crop growth response, and the test should be rapid and reproducible.

Water, neutral salts, dilute acids and various chelating agents are extractants which have been widely used to assess plant available copper (Gupta 1979). Mortvedt (1977) suggested that none of these tests is particularly useful over a range of soils. Caldwell (1976), however, indicated that identification of some soils which are likely to lead to copper deficiency in plants is possible using some of the above mentioned soil tests. Cox and Kamprath (1972) generally suggested that correlations of extractable copper with yield response or uptake are usually better with weaker extractants than extractants such as the acids. Several researchers have found better correlations between extractable copper and yield with boiling water extractions than with neutral salts or acidic extractants. Extractions using dilute HNO_3 have been previously used on organic soils to assess available copper. Critical levels were found to vary between 3 to 4 $\mu g\ g^{-1}$ Cu. Nelson et al. (1956) reported poor correlations between copper extracted with dilute acid extractants and copper utilized by plants. Dilute acid extractants have generally provided little or no useful information on the copper status of soils. Whitney (1975) proposed the use of 1.0 M HCl for organic soils. A one to sixteen soil to solution ratio was used in the extraction procedure. His research led to the following copper fertilizer recommendations. It was suggested that 7, 3, and 0 Kg Cu ha^{-1} should be applied to soils testing < 9, 10 to 20, and > 20 $\mu g\ g^{-1}$ Cu, respectively. Whitney (1975) further advised that different extractants should be recommended for the determination of plant available copper in organic and mineral soils. Lucas and Knezek (1972) suggested that for copper responsive crops grown on organic soils, total copper content of the soil should exceed 20 to 30 $\mu g\ g^{-1}$.

More recently, chelation extractants such as EDTA, EDDHA and DTPA have been employed to evaluate the copper status of soils. The marked ability of copper to form complexes with naturally present organic ligands in solid and solution phases of soil, and those added in solution as extractants, formed the basis for their use (Lindsay and Norvell 1978). Copper brought into solution would be a function of its activity in soil and the soils capacity to maintain this activity. Dolar and Keeney (1971) stated that chelating agents often provide good predictions of plant available copper as opposed to other extractants. The ability of chelating agents to act as good extractants for plant available soil copper on organic soils is evidenced by their increased usage as soil tests and generally good relationship between extractable copper and crop response to copper fertilizer (Gartrell 1980; Varvel 1983, 1984; Kruger et al. 1985; Karamanos et al. 1986).

One of the more commonly used chelating agents is EDTA. Reith (1968) used EDTA as an extractant and was able to classify mineral soils as: responsive ($< 0.7\ \mu g\ g^{-1}$ Cu), moderately responsive (0.7 to 1.0 $\mu g\ g^{-1}$ Cu) and non-responsive ($> 1.0\ \mu g\ g^{-1}$ Cu). Dolar and Keeney (1971) related that this extractant was able to extract soil solution copper as well as forms of copper associated with organic matter. Most literature reviewed suggested that a reasonably good relationship exists for EDTA extractable soil copper and plant uptake of copper and yield (Reith 1968; Dolar and Keeney 1971; McGregor 1972; Singh and Prasad 1986).

Wallace and Heimadin (1962) and McGregor (1972) employed the use of Na_2EDDHA [ethylenediamine-di(-o-hydroxyphenol acetic acid)disodium salt] to evaluate plant available copper in soils. This chelating agent is, however, not commonly used as an extractant for plant available soil copper. At lower copper concentrations copper does not compete well with Fe^{3+} for chelation, at higher concentrations a stable species of Cu-EDDHA will form. Accordingly, this extractant is not extremely useful for soils having low copper levels.

The use of DTPA as a chelating agent provides results similar to that of EDTA (Norvell and Lindsay 1972). Lindsay and Norvell (1978) suggested that since EDTA and DTPA metal complexes behave similarly, DTPA could be used as an extractant for plant available copper.

Osiname et al. (1973), however, found EDTA to be a better extractant than DTPA for assessing plant available copper on Nigerian soils. Soltanpour et al. (1976; 1979) reviewed factors which affect copper extracted by DTPA and indicated that standardized extraction procedures and conditions were necessary to obtain reproducible and meaningful results. Follet and Lindsay (1971) indicated that DTPA was useful in measuring residual effects of applied copper as well as determining plant available native copper. Haq and Miller (1972), working with Ontario soils, suggested that neither DTPA nor EDTA were suitable extractants for evaluating plant available soil copper. King and Alston (1975) suggested that better relationships between plant available soil copper and extractant - soil copper were likely to occur when soils of similar characteristics were grouped together.

The DTPA soil test for copper has not always proven useful. Lindsay and Norvell (1967) suggested a critical level of 0.2 μg g^{-1} DTPA extractable copper for mineral soils. Most researchers fail to find agreement with this value. Most work involving DTPA extraction for plant available copper has occurred on mineral soils. Critical levels obtained are not applicable to organic soils due to the variable nature of the soil properties involved.

Tokarchuk (1982), in a controlled environment study using several extractants, including DTPA, concluded that none of the extractants used were able to adequately assess plant available copper in unfertilized organic soils. However, it was noted that all extractants were able to extract copper from soil, proportional to the amount of copper applied. Field studies using both fertilized and unfertilized soils indicated that all extractants provided good relationships between extractable soil copper and plant tissue copper levels. It was suggested that DTPA extraction may provide adequate assessment of plant available soil copper.

A measure of soil-plant interaction may not always be provided by a soil test alone. Jones (1972) stated that the elemental nutrient content of a plant reflected the soil nutrient status better than a soil test since the plant micronutrient content at any particular time reflects the plants ability for uptake of that nutrient in that particular environment. Thus, plant tissue analysis has been widely used for diagnosing micronutrient deficiency. Bates (1971) indicates that tissue samples should be taken when deficiencies first appear. Jones (1972) showed that plant analysis must be related to certain growth stage development and specific plant tissues. Both Bates (1971) and Jones (1972) emphasize the utility of plant analysis, but also noted its limitations.

Soil tests may be used to determine a suspected occurrence of deficiency. These tests may be used to delineate between deficient and non-deficient soils. The test selected should be judged on its appropriateness, calibrated against response, and estimate a critical level separating deficiency from adequacy.

The content of a particular nutrient in a plant reflects the availability of the nutrient in the soil. The use of both plant and soil analysis may provide a more definitive assessment of the nutrient status of the soil than either one alone provided that these tests are properly correlated under the appropriate field and environmental conditions.

2.4 Source and Method of Placement of Copper Fertilizer on Organic Soils

During the early stages of growth of cereals (approximately 4 leaf stage), the growing point begins a change from a vegetative to a reproductive phase. It has been suggested that a crop must receive adequate copper before flowering otherwise growth at maturity will be restricted. As such, a copper fertilizer must supply available copper to the crop during the active stages of growth.

Form, solubility and the method of placement are factors which dictate copper fertilizer efficiency. Soluble inorganic forms such as copper sulphate, and organic forms such as various copper chelates are the most widely applied forms of copper. In addition, insoluble inorganic forms such as phosphates, and organic non-chelates such as polyflavinoids are also used. For organic soils, the most widely used carriers include copper sulphate ($CuSO_4 \cdot 5H_2O$) and organic chelate forms (Cu-EDTA). Organic chelates remain more soluble in soil than the sulphate form and, as such, are more mobile in the soil. When copper sulphate is applied to the soil, a large proportion of the Cu^{2+} ions which are brought into solution are initially immobilized by adsorption to exchange sites (Mengel and Kirkby 1979). The nature of a chelated form is such that this strong adsorption is initially prevented.

Uptake of copper usually increases with an increase in root to fertilizer contact (Gartrell and Brennan 1979; Gilkes and Sadleir 1979; Gartrell 1980), and therefore the method of placement will affect this relationship. For equally mobile copper fertilizers, placement method would hold little influence on uptake. However, due to the lack of mobility of $CuSO_4 \cdot 5H_2O$ in soil (Gilkes and Sadleir 1978, 1979; Barber 1980; Varvel 1983), placement method plays an important role regarding plant uptake of copper. The crop roots must contact the applied fertilizer, thus, for copper sources which are relatively non-mobile, the copper must be placed in an active rooting area.

The literature often reports little or no effect of placement on copper uptake for chelated copper sources, citing the mobility of these sources as an explanation. However, for $CuSO_4 \cdot 5H_2O$ which is relatively immobile in soil and forms relatively insoluble reaction products, one would suspect banding to be the most efficient placement method. Banding of the fertilizer minimizes contact between fertilizer and soil, and loss of copper through reaction product formation and adsorption would be minimized. The decrease in soil-copper contact, however, does not seem to be as important as increasing the copper-root area contact (Barber 1980; Gartrell 1980; Varvel 1983). As such, thoroughly mixing $CuSO_4 \cdot 5H_2O$ with the soil has often provided better uptake of copper by the crop in comparison to banding this form of copper fertilizer (Reuter 1975; Gartrell 1980; Varvel 1983). In this respect, Graham and Nambiar (1981) related that copper uptake rate by plants is slow, and that solubility will very rarely limit availability, thereby suggesting positional availability as an important factor.

Varvel (1983) indicated that $CuSO_4 \cdot 5H_2O$ was extremely ineffective in increasing yield when banded, but very effective when broadcast. The work of Gilkes (1981), however, suggested that copper application with the seed would offer maximum fertilizer utilization. He did suggest, however, that mixing of surface applied copper may improve its utilization. Barber (1980) showed that banded $CuSO_4 \cdot 5H_2O$ remained in a small area near the seed. Further, it was evident that over the growing season crop roots grew out of this zone and were unable to effectively utilize the applied copper. Murphy and Walsh (1972) suggested that broadcast applications were less effective than banded applications. In a review by Reuter (1975) it was indicated that banding may cause seed injury, and that broadcasting $CuSO_4 \cdot 5H_2O$ with subsequent incorporation was the most efficient method because of increased plant root-fertilizer contact. Karamanos et al. (1986) showed that incorporation of broadcast $CuSO_4 \cdot 5H_2O$ was essential in obtaining maximum fertilizer efficiency. The work of Varvel (1984) generally indicated that banded $CuSO_4 \cdot 5H_2O$ was only about 23% as effective as broadcast and incorporation methods in increasing yield.

The variable results of broadcast and banded placement methods for $CuSO_4 \cdot 5H_2O$ may in part be explained by poor positioning of the copper fertilizer relative to plant roots. Gartrell and Brennan (1979) suggested that poor positional availability would likely occur when the granule size of the copper source exceeded 3 mm in diameter. Barber (1980), Gilkes (1981), and Varvel (1983) explained inconsistent results regarding method of copper fertilizer application and yield, with soil water relations. They suggested that precipitation may act to move broadcast $CuSO_4 \cdot 5H_2O$ into active rooting areas, and also keep the surface root system actively absorbent thereby making effective use of the thoroughly mixed copper. In drier seasons, roots actively growing into the zone of banded copper may absorb enough copper from this concentrated source for growth (Nambiar 1977), whereas broadcast applications may not be as available. Graham and Nambiar (1981) suggested that banded copper applications may experience a more favorable moisture state than copper placed at the surface of soils. The relative effectiveness of banded and broadcast applications of an immobile copper source such as $CuSO_4 \cdot 5H_2O$ is a function of water relations as well as granule size. Work previously cited has not always considered soil moisture status in explaining yield differences obtained when studies with banded and broadcast copper fertilizer application are undertaken.

Placement method for mobile sources such as chelated copper forms has little influence on fertilizer effectiveness. The literature generally indicates banded and broadcast methods to be equally effective.

Differences in effectiveness due to placement however, are noted at the lower rates of application. Broadcast applications of chelates do not appear to provide a high enough copper concentration for adequate crop growth. McAndrew (1979) indicated that banded applications of chelated copper sources were usually less effective than those which have been thoroughly mixed with the soil. He suggested that interactions with macronutrient fertilizers, such as precipitation of copper with phosphorus to relatively insoluble compounds, were responsible for reduced effectiveness of banded copper chelates. Banded applications of chelated micronutrients are as effective as broadcast applications in many instances. Chelated forms, being mobile, move out into the soil by diffusion and convection thereby allowing a larger root-copper interface (McAndrew 1979). Work by Varvel (1983) indicated that broadcast applications of copper hydroxyethylenediaminetriacetic acid, 5% copper (Cu-HEDTA) were more effective in increasing yield than banded applications. However, more recent studies by Varvel (1984) showed that yield increase and copper uptake was greater with banded application of Cu-HEDTA than with broadcast applications. The contradictory results from the two studies were explained on the basis of water relations. Banded applications of chelates may be more effective in drier years due to greater mobility of copper in the crop rooting zone. In contrast, when adequate precipitation is available throughout the growing season, broadcast and incorporated applications by virtue of the greater fertilizer - root contact are usually more effective than banded applications.

The importance of positional availability was illustrated by the work of Oliver and Barber (1966). Due to the relative immobility of copper, the majority of copper supplied for plant uptake was a result of root interception. The work of Gilkes and Sadleir (1979), as well as Jarvis and Whitehead (1981), also indicated that the uptake of copper was, to a large degree, dependent upon root exploration and interception. As such, methods of application which increase root-copper contact would generally provide the greatest efficacy of soil applied copper fertilizers.

Comparisons of equivalent application methods for copper chelates and $CuSO_4 \cdot 5H_2O$ have seldom been undertaken. As well, comparison at equivalent rates of copper product are equally scarce. The rate of copper chelate application to soil is usually one-fifth that of sulphates (Karamanos et al. 1986), and many workers consider chelated forms to be five times more effective than sulphates. The cost of chelates however, may be two to four times that of sulphates, and at rates normally applied chelates offer no detectable residual value.

Barnes and Cox (1973) found that at 2.8 Kg Cu ha^{-1}, $CuSO_4 \cdot 5H_2O$ and chelated forms broadcast as a spray and disced in showed no significant difference in terms of yield. Younts (1964) indicated that broadcast $CuSO_4 \cdot 5H_2O$ and broadcast Na_2Cu chelate gave comparable results with respect to yield, at equivalent application rates. Varvel (1984) showed that at equivalent rates Cu-HEDTA banded was more efficient than $CuSO_4 \cdot 5H_2O$, either banded or broadcast. It has also been found that banded or broadcast Cu-HEDTA was more effective than $CuSO_4 \cdot 5H_2O$ banded or broadcast, respectively (Varvel 1983). Generally, the literature indicates that at equivalent rates the chelated copper sources are more effective than the sulphate forms. However, at high rates of application the cost of chelate application is prohibitive, and at lower rates no residual benefits are evident. Due to this factor, the application of $CuSO_4 \cdot 5H_2O$, or a low rate of a chelated form, is often implemented.

The literature cited previously showed that application method of either carrier affects plant availability. Inorganic copper fertilizers such as $CuSO_4 \cdot 5H_2O$ are usually applied as a broadcast and incorporated application. Chelate forms are applied as either broadcast or banded, however, at lower rates broadcasted chelate forms may not supply a high enough copper concentration for adequate crop growth. In addition, growing season precipitation often affects the relative effectiveness of various placement methods and ultimately crop yield.

The possibility exists that at equivalent rates of application, chelated forms banded into the soil may supply greater available copper to crops during the seedling stage. Due to low root interception, $CuSO_4$ broadcast and mixed with the soil may not supply plant available copper to the same extent. Additionally, copper from inorganic copper fertilizers such as $CuSO_4$ may bind to organic matter constituents (as previously discussed), effectively reducing plant available copper. Lindsay and Norvell (1969a) indicated that Cu-EDTA at pH 7.3 was stable after 30 days with approximately 40% of the copper still associated with the EDTA molecule. With time, the stability of the molecule decreased, the result being that copper was tied-up with soil organic matter constituents. In this respect, chelated forms may supply available copper early in the growing season, but provide progressively less plant available copper with time. Hill et al. (1978) indicated that copper absorbed early in the season may not be retranslocated in quantities large enough for fertile pollen production. Thus, although available copper may be present during early crop growth, it may not always be enough to ensure adequate yields.

As previously discussed, the first step in complexation of added copper may involve saturation of higher molecular weight soil organic matter constituents. When these sites are exhausted, copper is absorbed by lower molecular weight constituents. It is these sites which may then provide plant available copper.

Kline and Rust (1966) working with copper release curves, have suggested that although most copper ($CuSO_4 \cdot 5H_2O$ source) applied to soils may be in insoluble forms and held to soil organic matter constituents, partial dissociation may occur. It was indicated that these forms could supply plant available copper through the dynamic equilibrium which is established when the insoluble forms come in contact with water. Nielson (1976a) has found that for soils receiving copper applications, available copper levels initially decreased and then reached a relatively constant value after 35 days.

Seemingly, added copper is initially tied-up to soil organic matter constituents satisfying most available copper binding sites, whereupon a dynamic equilibrium is established. It may be envisioned that this equilibrium state occurs in an earlier time frame for $CuSO_4 \cdot 5H_2O$ than for chelated copper applications. Thus, chelated forms will be able to supply plant available copper during the early growth stages of crops whereas the $CuSO_4 \cdot 5H_2O$ form may better be able to supply copper for late vegetative growth and fertile pollen production.

III. GENERAL MATERIALS AND METHODS

Several individual and related studies are reported in this manuscript. For purposes of brevity and clarity, experimental procedures specific to each study are discussed with the results obtained in the appropriate section. Analytical procedures used for plant and soil analysis for all growth chamber and field studies are homologous for each study and are described herein.

3.1 Soil Analysis

Organic matter content of soils was determined by the Walkley-Black method as described by Allison (1965). An automated potentiometer was employed for the titrations. Soil pH was determined using a glass-calomel electrode and was made on a 5:1 water to soil paste. Electrical conductivity was determined on the paste used for pH determination using a standard conductivity meter.

Nitrate-nitrogen was determined by the modified phenoldisulphonic acid method (Harper 1924). Five g of air dried soil were extracted with a solution of 0.1 M $CuSO_4$ and 0.6% Ag_2SO_4. The nitrate concentration was measured colorimetrically using a spectrophotometer set at a wavelength of 415 nm.

Plant available phosphorus was assessed using the method of Olsen et al. (1954). Five g samples were extracted using 100 ml of 0.5 M $NaHCO_3$. The phosphorus extracted was determined colorimetrically using the acid-molybdate-antimony method described by Murphy and Riley (1962).

Exchangeable K^+, Ca^{2+} and Mg^{2+} were determined by displacement of the cations using 1.0 M ammonium acetate adjusted to a pH of 7.0. Five g of soil were shaken with 100 ml CH_3COONH_4 for 30 min, filtered through #1 filter paper and the concentration of K^+, Ca^{2+} and Mg^{2+} determined using atomic absorption spectrophotometry.

Sulphate-S in soils was extracted using a 5 g sample in 50 ml 0.001 M $CaCl_2$, and a 30 min shaking period. One g activated charcoal was added after the 30 min shaking period and the sample further shaken to remove coloration due to dissolved organic matter. The methylthymol blue method (Lazrus et al. 1965) was used to measure SO_4-S concentration in the extracts. The amount of uncomplexed methylthymol blue (sulphate) was measured on a Technicon Autoanalyzer at 460 nm.

Plant available Cu, Fe, Mn and Zn were assessed using the DTPA method of Lindsay and Norvell (1978). A modified procedure was utilized in that a 2 g sample was shaken for 1 h in 20 ml of extracting solution. The suspensions were then centrifuged at 12,000 rpm for 15 min. The supernatant was analyzed using a Perkin-Elmer 760 atomic absorption spectrophotometer.

Bulk density, field capacity moisture content, volumetric water content, and air-filled pore space were determined by filling a plastic cylinder of known volume with a known weight of soil. The soil was then saturated with water, and the saturated weight determined. Cylinders were allowed to drain for two days through #42 filter paper and reweighed. Air-filled porosity at field capacity moisture content was calculated as follows: (weight of saturated soil - weight of soil after drainage)/volume of soil. After 2 d, all soil in each cylinder was oven dried at 105°C. Volumetric water content at field capacity was calculated as follows: (weight of soil after drainage - oven dry weight of soil)/volume of soil.

3.2 Plant Analysis

Phosphorus concentrations were determined using 1 g of finely ground plant material. The sample was digested at 228°C using 5.0 ml concentrated HNO_3 and 2.5 ml 70% $HClO_4$. Following the method of Isaac and Kerber (1971), solutions were digested until clear, and quantitatively transferred to a 25 ml volumetric cylinder and made up to volume using deionized distilled water. The phosphorus concentration of the 25 ml solution after dilution was determined using molybdate-ascorbic acid (Murphy and Riley 1962).

Potassium concentration was determined using the same digestion solution. A 0.25 ml aliquot of the 25 ml solution was diluted with 4.75 ml deionized distilled water. To a 0.5 ml aliquot of this solution was added 1.0 ml 2500 $\mu g\ g^{-1}$ $LiNO_3$ and 8.5 ml deionized distilled water. A portion of this solution was aspirated into a Perkin-Elmer 760 atomic absorption spectrophotometer and concentration of potassium calculated.

Concentrations of copper, iron, manganese and zinc were determined using a portion of the diluted digestion solution. Concentrations were determined using a Perkin-Elmer 760 atomic absorption spectrophotometer.

Total nitrogen was determined using a 0.5 g finely ground sample and the method of Schuman et al. (1973) for automated total N analysis.

IV. EXPERIMENTAL PROCEDURES AND RESULTS

4.1 Residual Effectiveness of Various Rates of $CuSO_4$ and an Investigation of Soil and Plant Analyses as Methods of Assessing Plant Available Copper in Organic Soils - Field Study

The long-term benefit of $CuSO_4$ fertilization of organic soils had not been investigated for Manitoba conditions even though the residual benefit of copper fertilization is of great importance to producers. Also, methods of assessing plant available soil copper in fertilized and unfertilized soils had not yet been evaluated for organic soils in the province. Thus, field studies were conducted to evaluate the residual benefit of $CuSO_4$ applications, and to investigate soil and plant analyses as methods of assessing plant available copper in organic soils.

4.1.1 Experimental Procedures

a) Materials and Methods

Field trials were established in 1985 and 1986 on organic soils in the northern, south eastern, and east central regions of Manitoba. All soils utilized in the field studies were of the Great Group Mesisol and of the subgroup Terric Mesisol. Some of the chemical and physical properties of the field sites are shown in table 1.

Table 1 Physical and Chemical Characteristics of Soils Used in the Field Studies

Site No	1	2	3	4	5	6	7
Legal Location	SW ¼ 33-9-12E	NE ¼ 14-12-11E	NW ¼ 26-12-11E	NW ¼ 14-15-10E	SE ¼ 12-2-9E	NE ¼ 21-1-11E	NE ¼ 36-25-3E
Organic Matter (%)	73.4	69.5	59.5	68.2	70.0	64.3	67.0
pH	6.9	7.5	5.6	6.4	7.6	7.2	6.5
Conductivity (ds m^{-1})	1.3	0.7	0.4	0.7	0.6	0.7	1.2
NO_3-N (Kg ha^{-1})*	36	64	136	96	61	104	124
$NaHCO_3$-extr. P (Kg ha^{-1})	6.1	15.2	7.0	8.6	3.2	7.8	8.3
CH_3COONH_4-extr. K (Kg ha^{-1})	29	151	81	136	68	64	99
SO_4-S (Kg ha^{-1})	12+	12+	12+	12+	12+	12+	12+
CH_3COONH_4-extr. Ca (mg g^{-1})	46.6	37.6	40.2	30.4	37.2	44.6	32.8
CH_3COONH_4-extr. Mg (mg g^{-1})	3.2	3.2	2.9	2.2	2.0	3.7	4.1

* Amount of NO_3-N in the 0 to 60 cm depth of soil. Other physical and chemical characteristics are for the 0 to 15 cm depth.

The field plots consisted of two types. Experiments initiated in 1985 consisted of two copper treatments (0 and 10 Kg Cu ha^{-1}). Experiments initiated in 1986 were composed of three copper treatments (0, 5, and 10 Kg Cu ha^{-1}). The experiments initiated in 1985 and 1986 were non-replicated field strip trials. The fertilized strips were usually 50 m wide and about 150 m long. The residual effects of copper applied in 1985 and 1986 were assessed in 1987 and 1988 using barley as a test crop. At each site, the original treatments were split in 1987, with 0 and 5 Kg Cu ha^{-1} as $CuSO_4$ being applied. The $CuSO_4 \cdot 5H_2O$ applied (25% Cu) was fine granular fertilizer grade. Broadcast applications were attained using a Valmar fertilizer spreader. All plots were then rotovated to a depth of approximately 15 cm and evenly packed using a cylindrical cast iron rolling drum.

Basal treatments of nitrogen, phosphorus, and potassium were applied to each experimental field site in both years according to soil test recommendations to ensure that available macronutrients did not limit yields. Barley (*Hordeum vulgare* var Argyle) was seeded as a test crop at a rate of 93 Kg ha^{-1}. As required, each experimental site was treated with Roundup ten to fourteen days prior to seeding. Early season weed control was accomplished through a tank mix of MCPA and Banvel at recommended rates.

b) Soil Sampling and Soil Analysis

Soil samples were obtained in the spring from the 0-15 cm depth prior to fertilization on all experimental sites and treatments. Samples from nine to eighteen sites on each treatment were taken and composited. Each sample was air-dried, crushed with a wooden roller, and passed through a 2 mm plastic mesh sieve. DTPA-extractable copper on the soil samples was determined using the DTPA-$CaCl_2$ method (0.005 M DTPA, 0.1 M triethanolamine, and 0.01 M $CaCl_2$ adjusted to pH 7.3) of Lindsay and Norvell (1978). Two grams of soil was extracted with 20 ml of extracting solution using a 1 hr shaking period. The suspensions were then centrifuged at 27,000 g for 15 min. The copper concentration in the supernatant was determined by atomic absorption spectrophotometry.

c) Plant Sampling and Analysis

Plant samples were obtained twice during the growing season. Entire barley plants were sampled at time of heading and at maturity. Four 1-m^2 samples were taken from each treatment plot. At maturity plant samples were air-dried, threshed and weight of grain determined. Seeding and harvest dates are shown in tables 1a and 1b, respectively (Appendix A). The chemical composition of barley at the heading stage, as affected by copper fertilization, is shown in tables 1c and 1d (Appendix A).

Plant material was ground in a Wiley mill and a 1-g sample digested using 5.0 ml concentrated HNO_3 and 2.5 ml 70% $HClO_4$. The copper content of the digest was determined using a Perkin-Elmer 760 atomic absorption spectrophotometer.

d) Statistical Analysis of Data

In 1985 and 1986 the experimental sites were designed as demonstration field strips, and altered to completely randomized split plot designs in 1987. Data obtained for yield, plant tissue copper content, and extractable copper content of the soils, were analyzed using Duncan's multiple range test.

Relationships between percent yield for all field sites and copper concentration of plant tissue, and percent yield and DTPA-extractable soil copper were expressed using a modified Langmuir adsorption isotherm equation of the form $y = x/(a + bx + cx^2)$, where y = % yield, x = concentration of tissue or soil copper, a is the intercept and b and c are partial regression coefficients calculated from a quadratic regression analysis of x vs. x/y.

Percent yield (relative yield) was defined as:

$$\frac{\text{yield without copper}}{\substack{\text{yield with copper} \\ \text{(maximum yield)}}} \times 100$$

A plot of % yield versus each of the previously mentioned variables provided information on the percentage of potential yield obtained at a particular concentration of plant tissue copper or DTPA-extractable copper.

Relationships between yield for all field sites and copper concentration of plant tissue, and yield and DTPA-extractable soil copper for the residual treatments were expressed using a quadratic equation of the form $y = a + bx + cx^2$, where y = yield, x = concentration of tissue or soil copper, and a, b and c are defined as noted above.

4.2 Results and Discussion

Yields of barley were increased by $CuSO_4$ applications for periods of at least three to four years after application (tables 2 and 3). Although the application of $CuSO_4$ was shown to have good residual effects, all sites responded to reapplication of copper. Average yields without copper fertilizer were 1668 Kg ha^{-1} in 1987 and 1534 Kg ha^{-1} in 1988. Yields in 1987 and 1988 were 2477 Kg ha^{-1} and 3644 Kg ha^{-1}, respectively, on plots previously treated with 10 Kg Cu ha^{-1}. Copper at 5 Kg ha^{-1} added in 1987 to plots previously treated with 10 Kg Cu ha^{-1} in 1985 or 1986 (sites 1, 2, 3, 5 and 7) increased yields from 2369 Kg ha^{-1} to 2547 Kg ha^{-1} in 1987 and from 3324 Kg ha^{-1} to 3813 Kg ha^{-1} in 1988. Ten or 5 Kg Cu ha^{-1} as copper sulphate were shown to have good residual effects, however, reapplication of copper in addition to that originally added, was required to maximize yield.

Lack of statistically significant differences among treatments at site 7 in 1987 was likely due to variability among treatment samples (table 2). Although not statistically significant, the yield increase obtained as a result of addition of 5 Kg Cu ha^{-1} to previously unfertilized soil at site 7 was sufficient to more than offset the cost of the copper fertilizer.

Table 2 Effect of Previously and Newly Added $CuSO_4$ on Yield of Barley (1987) (Kg ha^{-1})

	Initial Application Rate[*] (Kg Cu ha^{-1})					
	0		5		10	
	New Application Rate (Kg Cu ha^{-1})					
Site No	0	5	0	5	0	5
1	— c[‡]	2715 b	--	--	3123 ab	3545 a
2	1798 d	3590 a	2298 cd	2953 b	2580 bc	2723 bc
3	460 b	1985 a	--	--	1800 a	1625 a
4	3920	3655[§]	3800	3808	3833	4233
5	898 b	1401 a	--	--	370 c	713 bc
6	1510	--	--	--	1660	--
7	3090	3725	3750	3950	3973	4128

[*] Date of Initial Application - Sites 1, 3 and 5: Spring 1985
Sites 2, 4 and 7: Spring 1986
Site 6: Spring 1984.

[‡] Means followed by the same letter do not differ significantly ($P = 0.05$).

-- Treatment not included in experiment.

[§] Application rate in 1987 20 Kg Cu ha^{-1}.

Table 3 Effect of Previously and Newly Added $CuSO_4$ on Yield of Barley (1988) (Kg ha^{-1})

	Initial Application Rate[*] (Kg Cu ha^{-1})					
	0		5		10	
	New Application Rate (Kg Cu ha^{-1})					
Site No	0	5	0	5	0	5
1	730 b[‡]	4140 a	--	--	4295 a	4425 a
2	990 f	2858 e	3095 d	4360 b	3338 c	4525 a
3	148 c	3680 b	--	--	4480 a	4513 a
4	2930 b	4023[§] a	3085 b	4005 a	4170 a	4205 a
5	353 b	490 b	--	--	300 b	635 a
6	3620 b	--	--	--	4716 a	--
7	1965 d	3795 bc	3265 c	3885 bc	4208 b	4965 a

[*] Date of Initial Application - Sites 1, 3 and 5: Spring 1985
Sites 2, 4 and 7: Spring 1986
Site 6: Spring 1984.

[‡] Means followed by the same letter do not differ significantly ($P = 0.05$).

-- Treatment not included in experiment.

[§] Application rate in 1987 20 Kg Cu ha^{-1}.

Poor yields were obtained at sites 3, 5 and 6 in 1987. Severe Canada thistle and sow thistle infestations were responsible for the poor yields on sites 3 and 5. The crop at site 6 was severely lodged in 1987, and lodging of the crop was largely responsible for reduced grain yield. Analysis of plant tissue at the heading stage indicated that plants at sites 5 and 6 may have been deficient in manganese (Appendix A, tables 1c and 1d). Manganese content of plant tissue was one-third to one-half that of the suggested critical level of 24 $\mu g\ g^{-1}$ (Ward et al. 1973).

Grain yields in 1988 generally exceeded those in 1987 (table 3). As noted by Gartrell (1980), copper applications often result in a greater yield increase in the year following application than in the year of application. This is likely due to increased root-fertilizer contact due to soil mixing processes facilitated by field operations. In contrast, yields for treatments without copper fertilizer declined from 1987 to 1988. A notable exception was the 0 Kg Cu ha^{-1} treatment at site 1, for which grain yield increased from 1987 to 1988. Tillage operations conducted by the farmer in the fall of 1987, and movement of soil via wind erosion in the spring of 1988 were suspected of mixing the copper fertilized soil, with non-fertilized soil on the experimental site.

Although copper fertilization increased yield in 1987, and resulted in further yield benefits in 1988, poor yields were obtained at site 5 in both years. The low yields were attributable to the low concentrations of manganese in the plant (Appendix A, tables 1c and 1d), as well as to poor germination and stand establishment.

The plant copper content of barley was increased by the application of $CuSO_4$ both in the year of application and the year following application. The applied $CuSO_4$ provided sufficient copper to maintain plant tissue copper contents at adequate levels for at least three to four years after application (tables 4 and 5). Average plant copper content without copper fertilizer was 3.0 $\mu g\ g^{-1}$ in 1987 and 3.2 $\mu g\ g^{-1}$ in 1988. The increase in copper content from 1987 to 1988 with a decline in yield seems anomalous. However, Robson and Reuter (1981) found plant copper content to increase with increasing copper deficiency of crops. Under copper stress, plants continue to absorb the limited soil supply of available copper with no subsequent increase in yield. Two other points also need be considered, however; 1) the soil supply of plant available copper also increased from 1987 to 1988 on the unfertilized treatments and, 2) the increase in plant copper content was quite small and may have been a result of variability.

Plant copper content in 1987 and 1988 was 5.7 and 5.9 $\mu g\ g^{-1}$, respectively, on plots previously treated with 10 Kg Cu ha^{-1}. It is evident that 10 Kg Cu ha^{-1} supplied reasonably adequate amounts of plant available copper to barley for four years. The addition of copper at 5 Kg ha^{-1} in 1987 to plots previously treated with 10 Kg Cu ha^{-1} in 1985 or 1986 (sites 1, 2, 3, 5 and 7) increased plant tissue copper content

Table 4 Effect of Previously and Newly Added $CuSO_4$ on Copper Concentration in Barley Harvested at the Heading Stage (1987) ($\mu g\ g^{-1}$)

	Initial Application Rate[*] (Kg Cu ha^{-1})					
	0		5		10	
	New Application Rate (Kg Cu ha^{-1})					
Site No	0	5	0	5	0	5
1	— c[‡]	2.6 b	--	--	4.3 a	4.7 a
2	1.8 c	3.1 b	3.6 b	4.6 a	4.9 a	5.3 a
3	2.1 b	3.4 ab	--	--	4.6 a	5.5 a
4	4.2 bc	4.7[§] bc	3.4 c	3.6 c	6.3 ab	7.6 a
5	2.9 c	4.4 b	--	--	4.4 b	5.6 a
6	6.3	--	--	--	8.6	--
7	3.8 b	6.8 a	8.2 a	8.8 a	6.8 a	9.0 a

[*] Date of Initial Application - Sites 1, 3 and 5: Spring 1985
Sites 2, 4 and 7: Spring 1986
Site 6: Spring 1984.

[‡] Means followed by the same letter do not differ significantly (P = 0.05).

-- Treatment not included in experiment.

[§] Application rate in 1987 20 Kg Cu ha^{-1}.

Table 5. Effect of Previously and Newly Added $CuSO_4$ on Copper Concentration in Barley Harvested at the Heading Stage (1988) ($\mu g\ g^{-1}$)

	Initial Application Rate[*] (Kg Cu ha^{-1})					
	0		5		10	
	New Application Rate (Kg Cu ha^{-1})					
Site No	0	5	0	5	0	5
1	3.1 d[‡]	4.1 c	--	--	5.4 b	7.0 a
2	1.6 e	2.1 d	3.0 b	2.6 bc	2.5 cd	4.2 a
3	1.0 c	5.1 a	--	--	4.0 b	5.3 a
4	3.1 c	10.6[§] a	7.9 b	10.1 a	7.9 b	9.7 a
5	3.1 b	6.6 a	--	--	7.4 a	7.1 a
6	8.9	--	--	--	9.2	--
7	1.3 c	4.7 b	4.6 b	6.3 a	5.1 b	5.3 b

[*] Date of Initial Application - Sites 1, 3 and 5: Spring 1985
Sites 2, 4 and 7: Spring 1986
Site 6: Spring 1984.

[‡] Means followed by the same letter do not differ significantly ($P = 0.05$).

-- Treatment not included in experiment.

[§] Application rate in 1987 20 Kg Cu ha^{-1}.

from 5.0 $\mu g\ g^{-1}$ to 6.0 $\mu g\ g^{-1}$ in 1987 and from 4.9 $\mu g\ g^{-1}$ to 5.8 $\mu g\ g^{-1}$ in 1988. Although the original application of copper at 10 or 5 Kg ha^{-1} provided reasonably adequate amounts of plant available copper for four and three years, respectively, reapplication of copper further increased plant tissue copper content.

The copper content of the plant at the heading stage was assessed as a means of predicting plant available soil copper on organic soils. Plant copper content was not closely related to yield (figure 1). The regression equation relating yield and copper concentration in plants harvested at the heading stage had an r value of only 0.46 ($P = .05$). However, plant copper content was reasonably closely related to response to copper fertilization or percent yield (figure 2). The r value for this relationship was 0.60 ($P \leq .01$). The critical plant tissue copper level was determined to be 5.6 $\mu g\ g^{-1}$ for barley grown on organic soils. Treatments which increased plant tissue copper content above this level further increased yields. Yield increase, however, was small and would not account for the cost of copper application.

Plant tissue analyses at the heading stage appears to be useful in assessing the need for copper application on organic soils. Soils could be classified as deficient in plant available copper when plants contained less than about 4.0 μg Cu g^{-1} plant material, marginal in plant available copper when plants contained 4.0 to 5.0 $\mu g\ g^{-1}$, and sufficient in plant available copper when plants contained more than 5 μg Cu g^{-1} plant material.

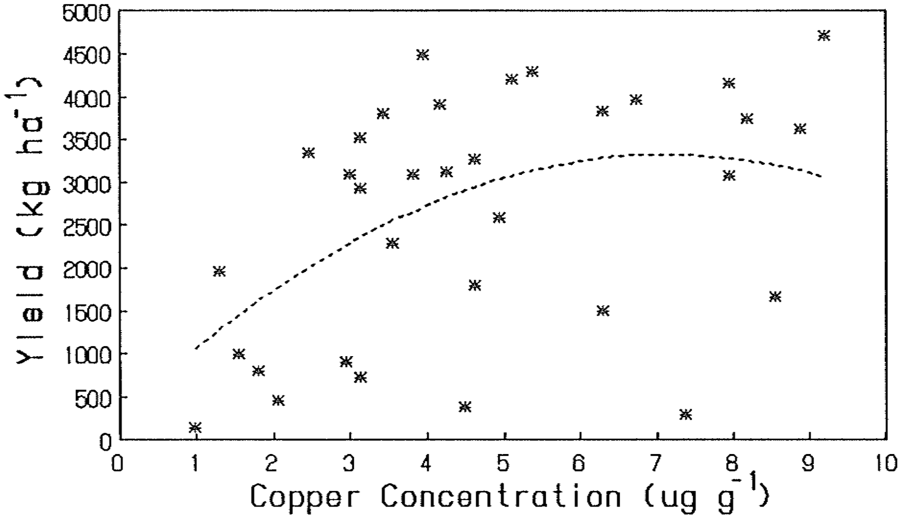


Figure 1. Relationship Between Grain Yield and Concentration of Copper in Barley at the Heading Stage

$y = 269 + 861x - 60.5x^2$

$r = 0.46$

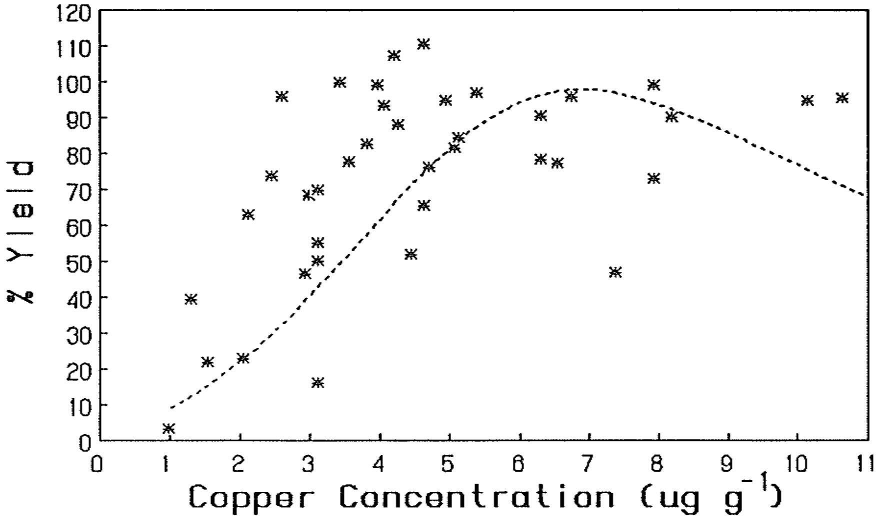


Figure 2. Relationship Between % Grain Yield and Concentration of Copper in Barley at the Heading Stage

$y = x/(0.137 - 0.030x + 0.003x^2)$

$r = 0.60$

Soil copper levels, as assessed by DTPA-extraction, were increased by $CuSO_4$ application for a period of three to four years following application (tables 6 and 7), which was the duration of the study. The data in table 6 reflect soil copper levels prior to copper reapplication in 1987. There was a close agreement of soil copper level with amount of applied copper. Average soil copper levels for treatments without copper fertilizer were 0.8 $\mu g\ g^{-1}$ in 1987 and 1.9 $\mu g\ g^{-1}$ in 1988 (tables 6 and 7). Increased soil copper levels from 1987 to 1988 may have been partly due to mixing of copper fertilized soil with unfertilized soil during tillage operations at site 1, ashes from the burning of a portion of the field at site 2, and error in analysis. Soil copper levels in 1987 and 1988 were 11.4 $\mu g\ g^{-1}$ and 14.2 $\mu g\ g^{-1}$, respectively, for plots previously treated with 10 Kg Cu ha^{-1}. The increase in DTPA-extractable copper from 1987 to 1988 was, in part, likely due to the above-mentioned reasons as well as a soil temperature effect. Soil thawing occurred about 2 wk earlier in 1988 than in 1987 and as a result soil temperatures in spring were about 3 to 5 C warmer in 1988 than in 1987. The higher soil temperatures likely resulted in increased mineralization of soil organic matter and increased microbial activity. Both of these processes would enhance the availability of copper as a result of copper release from soil organic matter-copper complexes (Rovatt and Katznelson 1961; Stevenson and Ardakani 1972; Rovira and Davey 1974).

Table 6. Effect of Previously and Newly Added $CuSO_4$ on Spring DTPA-extractable Copper (1987) ($\mu g\ g^{-1}$)

	Initial Application Rate[*] (Kg Cu ha^{-1})					
	0		5		10	
	New Application Rate (Kg Cu ha^{-1})					
Site No	0	5	0	5	0	5
1	0.4	0.4	--	--	2.0	1.5
2	0.5	0.5	9.2	9.2	10.0	10.0
3	0.5	0.5	--	--	6.4	6.4
4	0.6	0.6[§]	8.6	0.4	20.0	20.0
5	0.2	0.2	--	--	7.3	4.1
6	2.4	--	--	--	3.0	--
7	1.2	1.2	10.0	10.0	31.0	31.0

[*] Date of Initial Application - Sites 1, 3 and 5: Spring 1985
Sites 2, 4 and 7: Spring 1986
Site 6: Spring 1984.

-- Treatment not included in experiment.

[§] Application rate in 1987 20 Kg Cu ha^{-1}.

Table 7. Effect of Previously and Newly Added $CuSO_4$ on Spring DTPA-extractable Copper (1988) ($\mu g\ g^{-1}$)

	Initial Application Rate[*] (Kg Cu ha^{-1})					
	0		5		10	
	New Application Rate (Kg Cu ha^{-1})					
Site No	0	5	0	5	0	5
1	2.2 d[‡]	6.2 c	--	--	13.8 a	10.0 b
2	3.0 d	2.7 d	11.6 c	16.6 a	13.3 bc	15.9 ab
3	1.0 c	9.7 b	--	--	11.4 b	24.2 a
4	1.4 d	28.0[§] c	34.2 b	38.2 a	26.1 c	27.6 c
5	0.9 b	1.5 b	--	--	4.2 a	4.4 a
6	2.4 b	--	--	--	9.9 a	--
7	2.6 d	16.0 b	7.0 c	19.0 b	20.4 ab	23.4 a

[*] Date of Initial Application - Sites 1, 3 and 5: Spring 1985
Sites 2, 4 and 7: Spring 1986
Site 6: Spring 1984.

[‡] Means followed by the same letter do not differ significantly ($P = 0.05$).

-- Treatment not included in experiment.

[§] Application rate in 1987 20 Kg Cu ha^{-1}.

The addition of copper at 5 Kg ha^{-1} in 1987 to plots previously treated with 10 Kg Cu ha^{-1} in 1985 or 1986 (sites 1, 2, 3, 5 and 7) increased soil copper levels from 11.3 $\mu g\ g^{-1}$ in 1987 to 15.6 $\mu g\ g^{-1}$ in 1988 (table 6). The increases in DTPA-extractable copper as a result of newly applied copper were reflected in both increases in yield and plant tissue copper content.

Soil copper level was closely related to yield (figure 3) and response to copper fertilization (figure 4). A better relationship was obtained between yield and DTPA-extractable copper ($r = 0.64$, $P \leq .01$) than between yield and plant tissue copper content ($r = 0.46$, $P = .05$). Thus it would appear that the assessment of plant available copper through DTPA-extraction, would be a better method of determining the need for copper fertilizer on organic soils, than tissue analysis. However, the relationship between copper content of plants at the heading stage and percent yield was slightly better than the relationship between DTPA-extractable copper content of soil and percent yield. The r value obtained for the relationship between plant tissue copper content and percent yield was 0.60 ($P \leq .01$), the r value obtained for the relationship between DTPA-extractable copper and percent yield was 0.58 ($P \leq .01$). Thus the use of either method to determine the need for copper application on organic soils would be equally useful. Soil copper level, as assessed through DTPA extraction, offers more promise as a method of determining the need for copper fertilizer application than plant analysis

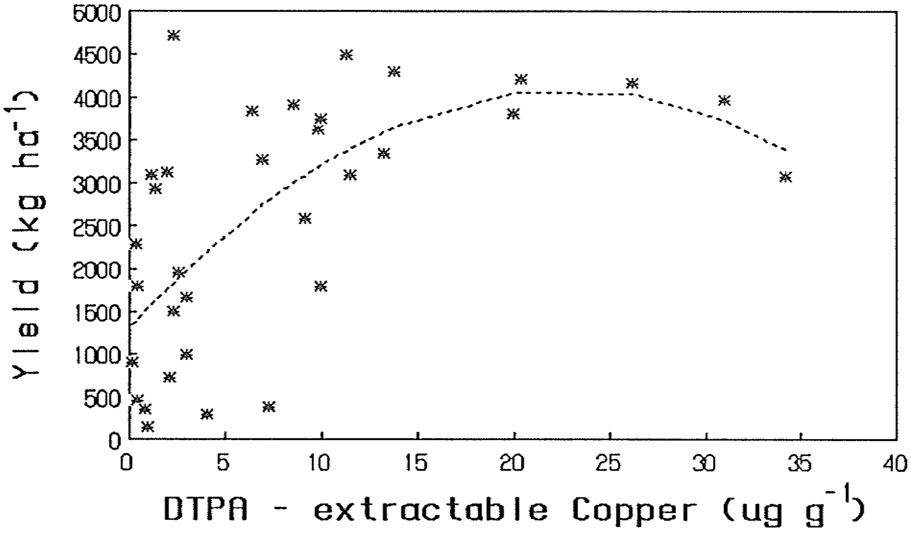


Figure 3. Relationship Between Grain Yield and DTPA-extractable Copper

$y = 1301 + 245x - 5.39x^2$

$r = 0.64$

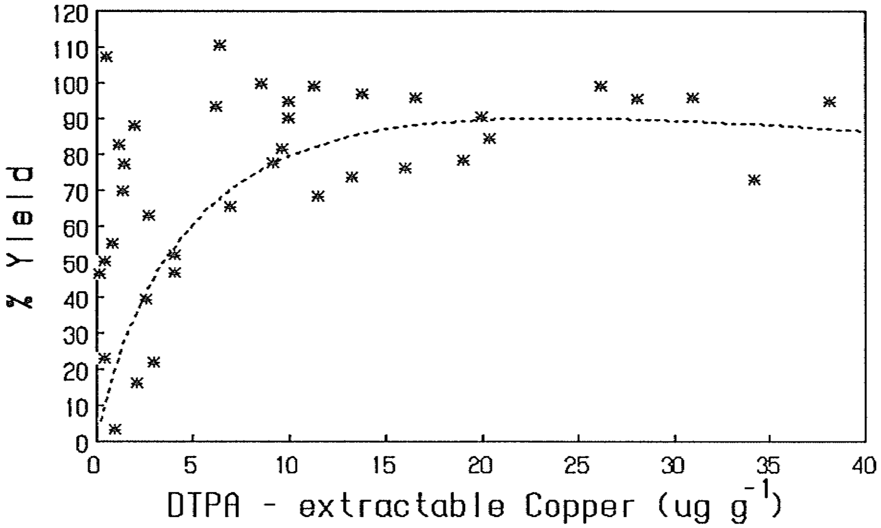


Figure 4. Relationship Between % Grain Yield and DTPA-extractable Copper

$y = x/(4.32 + 0.747x + 0.008x^2)$

$r = 0.58$

since an assessment of Cu requirements can be made prior to planting. Plant analysis has the limitation that remedial action (addition of copper fertilizer) usually cannot be effectively taken to enhance the yield of the crop being grown. The immobility of copper in soils precludes efficient plant uptake of copper from copper fertilizer applied to the surface of soils, unless a very mobile source of copper is applied.

Grain yield was usually low when DTPA-extractable copper levels in the soil were below 2 $\mu g\ g^{-1}$ (figure 3). Yields were usually high when DTPA-extractable copper levels in the soil exceeded a value of about 16 $\mu g\ g^{-1}$. Large increases in yield were usually obtained when the soil contained less than 7 μg Cu g^{-1} soil. Yield increases due to copper fertilization were small when the soil contained high levels of DTPA-extractable copper, i.e. greater than 16 μg Cu g^{-1} soil. Thus it was determined that the critical soil copper level for barley grown on organic soil was 16 $\mu g\ g^{-1}$. Increases in yield occurred when treatment application increased soil copper level above this value. Yield increases, however, were small and did not justify cost of copper application.

4.3 Residual Effectiveness of $CuSO_4$ and an Evaluation of Soil and Plant Analyses as Methods of Assessing Plant Available Copper in Organic Soils - Growth Chamber Study

A growth chamber study was conducted to assess the usefulness of soil analysis (DTPA-extractable copper) and plant tissue analysis at the heading stage as a means of predicting the need for copper fertilization of organic soils. The study was conducted to augment results obtained from the 1987 and 1988 field studies. Studies conducted in the field in 1987 showed that even under adequate fertilizer regimes, widely varied response to added copper occurred. Variations in yield were thought likely to be due to climatic variation among experimental sites, principally soil and air temperature. Two soils exhibiting differential response to copper in the field studies were selected for the growth chamber studies.

4.3.1 Materials and Methods

Soil from the surface 15 cm of two experimental field locations (sites 2 and 7) was obtained in the fall of 1987 for study. Soil from each location was collected by treatment, well mixed to facilitate uniformity, sealed in polyethylene bags and frozen until use. Soil from the following field plot treatments were collected; control, 5 Kg Cu ha^{-1} added in 1986, 5 Kg Cu ha^{-1} added in 1987, 5 Kg Cu ha^{-1} added in 1986 with 5 Kg Cu ha^{-1} reapplied in 1987, 10 Kg Cu ha^{-1} added in 1986, and 10 Kg Cu ha^{-1} added in 1986 with 5 Kg Cu ha^{-1} reapplied in 1987. Some of the chemical and physical properties of the soils selected are shown in table 8. The DTPA-extractable copper levels of the selected treatments are shown in table 9.

Table 8. Physical and Chemical Characteristics of Soils Used in Growth Chamber Studies

Soil No.	2	7
Legal Location	NE ¼ 14-12-11E	NE ¼ 36-25-3E
Soil Type	Terric Mesisol	Terric Mesisol
Organic Matter (%)	64.6	56.3
pH	7.9	7.0
Bulk Density (g cm^{-3})	0.14	0.12
Conductivity (ds m^{-1})	0.52	0.48
NO_3-N (μg g^{-1})	228.9	100.1
$NaHCO_3$-extr. P (μg g^{-1})	16.6	14.6
CH_3COONH_4-extr. K (μg g^{-1})	466.9	436.6
SO_4-S (μg g^{-1})	1792.0	2584.0
CH_3COONH_4-extr. Ca (mg g^{-1})	17.2	12.0
CH_3COONH_4-extr. Mg (mg g^{-1})	3.0	3.0
DTPA-extr. Fe (μg g^{-1})	259.7	53.4
DTPA-extr. Mn (μg g^{-1})	23.2	11.3
DTPA-extr. Zn (μg g^{-1})	2.4	23.1
Field capacity (% H_2O)	399.0	510.0
Volumetric water content at field capacity (ml cm^{-3})	0.35	0.57
Pore space at field capacity (cm^3 g^{-1})	0.03	0.08

Table 9. DTPA-extractable Copper Concentration for Various Experimental Treatments (μg g^{-1})

Previous Treatment and Year of Application (Kg Cu ha^{-1})	Soil 2	Soil 7
0	0.7	1.5
5 (1986)	2.2	2.9
5 (1987)	6.4	23.4
5 (1986) - 5 (1987)	12.5	19.3
10 (1986)	5.9	7.0
10 (1986) - 5 (1987)	9.8	27.3

An additional treatment to those listed above was included to ensure that the experiment contained one treatment or soil with adequate plant available copper for maximum yield. This treatment consisted of augmenting the field soil treated with 10 Kg Cu ha^{-1} in 1986 plus 5 Kg Cu ha^{-1} in 1987 with 5 Kg Cu ha^{-1} just prior to potting of the soil. The newly applied copper was added as $CuSO_4 \cdot 5H_2O$ dissolved in deionized water. The $CuSO_4$ solution was sprayed onto the soil which was thinly spread on a sheet of paper. The soil and copper fertilizer was then thoroughly mixed.

A 2200 g portion of moist organic soil (199 and 246% moisture for sites 2 and 7, respectively,) or 735.8 and 636.7 g portions of oven dry soil for soils 2 and 7, respectively, was placed into 5.1 L plastic pots. Prior to potting, each soil was treated with 100 Kg N ha^{-1} as NH_4NO_3, 40 Kg P ha^{-1} as $NH_4H_2PO_4$, 150 Kg K ha^{-1} as K_2SO_4 and 5 Kg Mn ha^{-1} as $MnSO_4 \cdot H_2O$. Amounts of fertilizer were added on an area basis. All nutrients were added as technical grade reagents and were dissolved in deionized water and sprayed onto 2200 g of moist organic soil. After thorough mixing, the soil was replaced into the pots. All treatments were replicated three times and arranged in a completely randomized design in the growth chamber.

Fifteen barley seeds (*Hordeum vulgare* var Argyle) were placed in each pot at a soil depth of 1.5 cm. The pots were then watered to field capacity moisture content. Plants were thinned to eight seedlings pot^{-1} shortly after emergence. Plants were checked daily for visual symptoms of nutritional deficiency and water content was maintained between 70 and 100% of field capacity moisture content.

Additional nitrogen at 100 Kg N ha^{-1} as NH_4NO_3 was added to each pot 39 d after seeding. A 16 h photoperiod with a light intensity of 500 micro-Einsteins m^{-2} s^{-1}, and a day/night temperature regime of 22/16 C was maintained.

The above-ground portion of four plants from each pot was harvested 52 and 47 d after seeding (heading stage) for soils 2 and 7, respectively. Plants were removed near the soil surface and any adhering soil particles were removed by a deionized water rinse. The plant material was then air-dried, weighed, finely ground and analyzed for nutrient composition. A final harvest of the remaining four plants was conducted at time of maturity (82 d after seeding). The samples were air-dried, threshed by hand and grain and straw weights obtained. Both grain and straw samples were finely ground and analyzed for nutrient composition.

Statistical analysis of the data was conducted as previously described for the field studies.

4.4 Results and Discussion

4.4.1 DTPA-extractable Copper Concentrations

Extractable copper concentration usually increased with increasing amount of copper applied in 1986 (table 9). Extractable copper concentrations were greater for soils treated with copper in 1987 than for soils treated with copper in 1986 when equivalent rates of copper were applied. The decrease in extractable copper concentration with time indicates that copper applied as $CuSO_4$ undergoes soil reactions decreasing plant available copper. Also, adding additional copper in 1987 to plots treated with copper in 1986 resulted in increases in DTPA-extractable copper. Extractable copper concentrations were higher for all treatments for soil 7 than for soil 2, at equivalent rates of application. It is possible that the binding between organic matter constituents and copper occurred to a greater extent in soil 2 than in soil 7. Brennan et al. (1983) stated that the method of binding of copper to soil organic matter largely dictated the availability of copper in soil. Davies et al. (1969), Goodman and Cheshire (1976), and McBride (1978) suggested that a myriad of organic functional groups were present in organic soils, all or some of which, at any one time, may take part in complexing copper. Given the heterogenous nature of organic soils and the large spatial variability of DTPA-extractable copper levels found by Karamanos (1986), it is not unreasonable to postulate that organic soils formed in different geographical locations possess differential ability to complex copper.

4.4.2 Yield and Copper Content of Plants at Heading

For both soils total dry matter yields were greatest for the soils treated with 5 Kg Cu ha^{-1} in 1986 plus 5 Kg Cu ha^{-1} reapplied in 1987 (table 10). It is noteworthy that for both soils the

Table 10. Effect of Copper Fertilization on Yield, Tissue Copper Concentration and Copper Uptake by Barley at Heading

	Soil 2			Soil 7		
Treatment (Cu Kg ha^{-1} yr^{-1})	Yield (g pot^{-1})	Copper Concentration of Plants (μg g^{-1})	Copper Uptake (μg pot^{-1})	Yield (g pot^{-1})	Copper Concentration of Plants (μg g^{-1})	Copper Uptake (μg pot^{-1})
0	15.4	2.4 c‡	37.0 d	19.2 ab	3.8 b	73.0 b
5 (1986)	19.1	6.4 ab	122.2 ab	15.8 cd	6.1 ab	96.4 ab
5 (1987)	18.2	5.5 bc	100.1 bc	15.3 d	7.6 a	116.3 a
5 (1986) - 5 (1987)	20.4	7.4 a	151.0 a	19.9 a	7.0 a	139.3 a
10 (1986)	18.2	6.4 ab	116.5 abc	18.4 abc	6.2 ab	114.1 ab
10 (1986) - 5 (1987)	15.3	5.0 bc	76.5 cd	16.8 bcd	6.4 ab	107.5 ab
10 (1986) - 5 (1987) - 5 (1987)	19.2	7.8 a	149.8 ab	17.0 bcd	8.0 a	136.0 a

‡ Means followed by the same letter do not differ significantly ($P = 0.05$).

treatment with the greatest amount of copper applied [treatment 10 (1986) - 5 (1987) - 5 (1987)] did not result in the greatest yield.

Copper content varied from 2.4 to 7.8 $\mu g\ g^{-1}$ on soil 2 and from 3.8 to 8.0 $\mu g\ g^{-1}$ on soil 7. Copper concentration in plant tissue increased when copper was applied and usually increased with amount added. Augmenting soils previously treated with copper fertilizer with additional copper fertilizer usually increased copper concentration of plant tissues. The highest tissue copper concentration occurred on soils treated with 10 Kg Cu ha^{-1} in 1986 plus 5 Kg Cu ha^{-1} in 1987 augmented with a further 5 Kg Cu ha^{-1} in 1987.

Copper uptake by plants at the heading stage was greatest for the soils treated with 5 Kg Cu ha^{-1} in both 1986 and 1987 for both soils. This was likely a function of increased plant copper concentration for soil 2 and increased dry matter yield for soil 7. Plant uptake of copper usually increased with increasing copper fertilizer applied. Augmenting soils previously treated with copper fertilizer with additional copper fertilizer usually increased plant uptake of copper.

4.4.3 Yield and Copper Content of Plants at Maturity

Yields of barley grain without added copper were low, being 7.8 and 12.6 g pot^{-1} for soils 2 and 7, respectively (table 11). A significant grain yield increase with added copper was observed on soil 2, but grain yield among treatments with added copper were not significantly different. For soil 7, grain yield was

Table 11. Effect of Copper Fertilization on Grain and Straw Yield and Copper Content

Treatment (Cu Kg ha^{-1} yr^{-1})	Soil 2			Soil 7		
	Grain Yield (g pot^{-1})	Copper Concentration of Grain (μg g^{-1})	Straw Yield (g pot^{-1})	Grain Yield (g pot^{-1})	Copper Concentration of Grain (μg g^{-1})	Straw Yield (g pot^{-1})
0	7.8 b‡	3.5 d	17.8 a	12.6 bc	4.5 c	11.4 b
5 (1986)	14.0 a	6.0 abc	13.4 b	13.9 abc	6.0 bc	11.9 b
5 (1987)	12.2 a	4.7 cd	14.0 b	13.3 bc	7.0 abc	12.0 b
5 (1986) - 5 (1987)	14.4 a	6.4 ab	14.1 b	15.0 ab	7.3 ab	11.1 b
10 (1986)	14.7 a	6.2 abc	13.6 b	11.0 c	6.1 bc	11.2 b
10 (1986) - 5 (1987)	13.9 a	5.0 cd	13.7 b	16.6 ab	8.1 ab	13.6 a
10 (1986) - 5 (1987) - 5 (1987)	12.9 a	6.7 a	12.4 c	18.2 a	9.0 a	13.4 a

‡ Means followed by the same letter do not differ significantly ($P = 0.05$).

significantly increased above that without added copper only when soils were fertilized with 10 Kg Cu ha^{-1} in 1986 plus 5 Kg Cu ha^{-1} in 1987 and further augmented with 5 Kg Cu ha^{-1}.

Straw yield without added copper was 17.8 g pot^{-1} on soil 2 and was significantly higher than yield of straw with applied copper. Yield of straw declined when copper was added whereas grain yield tended to increase with copper added on soil 2. This suggests that adequate copper was available for vegetative growth for all treatments but was limiting for retranslocation and subsequent grain production unless additional copper was added (Chaudry and Loneragan 1970; Hill et al. 1978). A negative relationship between straw and grain yield ($r^2 = 0.73$) was obtained for soil 2. In contrast to the data obtained for soil 2, straw yield tended to increase with increasing copper addition for soil 7. Straw yield without added copper was 11.4 g pot^{-1} on soil 7, and was significantly less than the yield of straw for the following treatments; 10 Kg Cu ha^{-1} in 1986 plus 5 Kg Cu ha^{-1} in 1987, and 10 Kg Cu ha^{-1} in 1986 plus 5 Kg Cu ha^{-1} in 1987 augmented with 5 Kg Cu ha^{-1}. Apparently, for soil 7, adequate copper was available for both vegetative growth and normal grain production. A reasonably strong positive relationship between straw and grain yield with increasing copper addition was found when these variables were compared through a linear regression analysis ($r^2 = 0.66$).

Grain copper contents without added copper fertilizer were 3.5 and 4.5 $\mu g\ g^{-1}$, respectively, for soils 2 and 7. Grain copper content usually increased with increasing copper addition for both soils.

4.4.4 Relationship Between % Yield and Tissue Copper Concentration

The relationship between % yield (grain yield without added copper/grain yield with copper x 100) and the copper concentration in plant tissue at the heading stage was investigated. The relationship, shown in figure 5, was relatively close and had an r value of 0.75 (P = .05). The results obtained indicates that the critical concentration of copper in the plant for barley was 3.8 $\mu g\ g^{-1}$. The critical value of 3.8 $\mu g\ g^{-1}$ is reasonably similar to that obtained by McAndrew (1979), who reported a critical range of 2.3 to 3.7 $\mu g\ g^{-1}$ for barley grown in the growth chamber. The critical value obtained in the growth chamber studies reported here is, however, about 1.5 times lower than that obtained in the field study (section 4.2). The reasons for the discrepancy are not clear, but the difference between the results from the field study and growth chamber study suggests that results from growth chamber studies may not be directly applicable to field conditions.

4.4.5 Relationship Between % Yield and DTPA-extractable Copper

The relationship between % yield (yield of grain without added copper/yield of grain with added copper x 100) and DTPA-extractable soil copper level prior to seeding was investigated. The relationship, shown in figure 6, was reasonably close and had an r value of 0.64 ($P = .05$). The results indicate a value of 5.2 $\mu g\ g^{-1}$ as a critical value for DTPA-extractable copper for barley grown on organic soil. Response to additions of copper would be expected when soil copper levels, as assessed by DTPA-extraction, are below 5.2 $\mu g\ g^{-1}$. This is in accord with the yield data in table 11 and the DTPA-extractable copper concentration values given in table 9. Fertilization with copper, which increased extractable copper in excess of this level, generally increased yields but the responses were small. The critical level of 5.2 μg Cu g^{-1} soil obtained in this study was much below that obtained from the field studies (16 $\mu g\ g^{-1}$). Although the reasons for the variation are not well understood, it is likely that differences in soil temperature between the field and growth chamber studies are largely responsible for the differences between critical levels obtained from the field and growth chamber studies.

It is likely that the higher soil temperature in the growth chamber studies increased plant available soil copper and the plants ability to extract copper. Diffusion of copper to plant roots, root growth and plant copper uptake would be favored by increases in soil temperature (Loneragan 1975).

It appears therefore that attempts to assess plant and soil critical copper levels for organic soils in growth chamber experiments may underestimate the copper requirements of barley grown under field conditions.

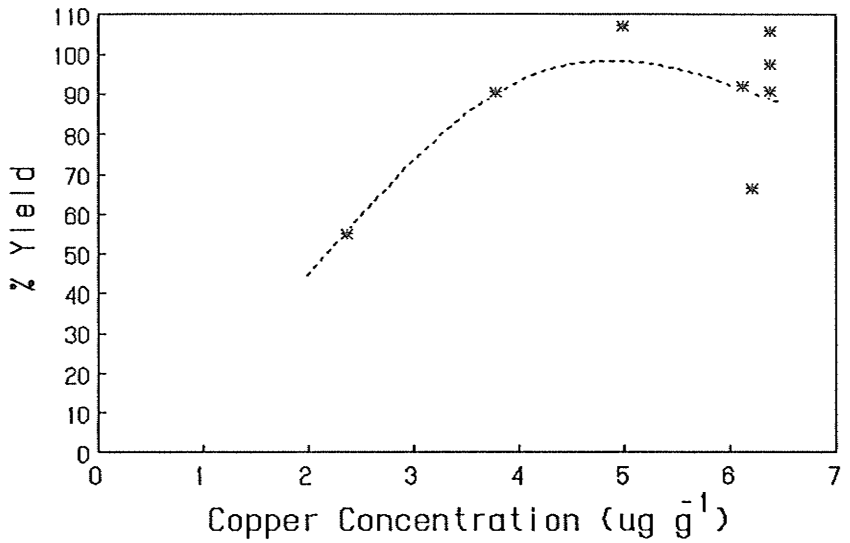


Figure 5. Relationship Between % Grain Yield and Concentration of Copper in Barley at the Heading Stage

$y = x/(7.11 - 1.92x + 0.302x^2)$

$r = 0.75$

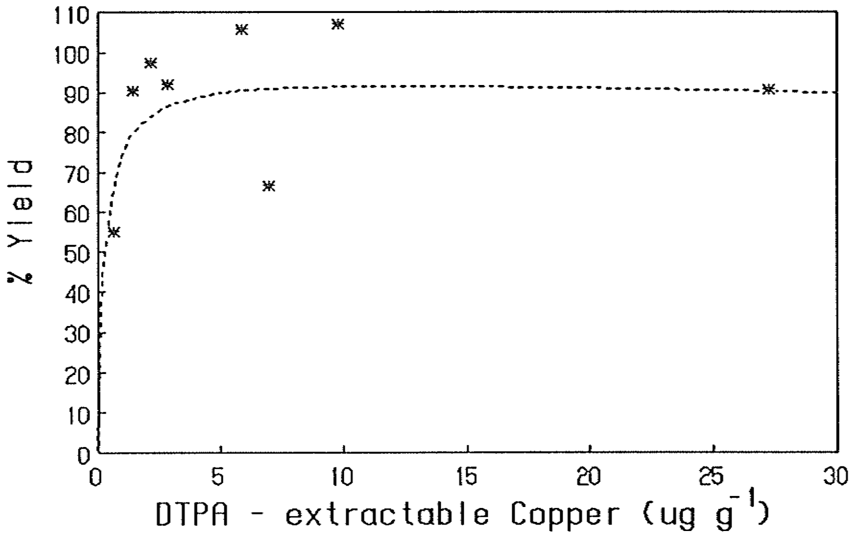


Figure 6. Relationship Between % Grain Yield and DTPA-extractable Copper

$y = x/(0.301 + 1.04x + 0.002x^2)$

$r = 0.64$

Critical soil and plant copper levels ascertained through growth chamber studies tended to be low. Critical levels obtained in the growth chamber were about 1.5 and 3 times lower for plant and soil parameters, respectively, than those obtained from field studies.

4.5 Effect of Copper Source and Rate on Growth and Copper Content of Barley - Growth Chamber Study

Previous research in Manitoba indicated good yield responses to both $CuSO_4$ and Cu-EDTA on organic soils when broadcast and incorporated prior to planting. Barnes and Cox (1973) found no difference in crop response for broadcast and incorporated application of equal rates of $CuSO_4$ and chelated copper. Younts (1964) also indicated that both $CuSO_4$ and Cu-EDTA were equally effective in increasing yield of wheat. In contrast to the findings noted above, chelated forms of copper, due to their greater solubility and mobility, appear to offer increased copper efficiency (Gartrell 1981). Varvel (1983), for example, showed copper chelates to be superior to $CuSO_4$ for almost all methods of application. The cost of chelated copper however, can be as much as three times that of the sulphate form per unit of copper for the year of application. Also, as noted by Karamanos (1986), chelated forms have very little or no residual effects due to the small amounts of copper usually applied in the chelated form.

The intent of the study discussed herein was to compare chelated copper and $CuSO_4 \cdot 5H_2O$ at equal rates of applied copper, as well as to obtain information on the effectiveness of very low rates of copper applied in the chelated form on organic soils.

4.5.1 Materials and Methods

The soil used was from the surface 15 cm of a Terric Mesisol having a history of no prior copper application (table 12). The soil was well mixed and then sealed in polyethylene bags and frozen until use. Prior to potting, the soil was uniformly mixed.

Table 12. Some Characteristics of Soil Used

Organic matter (%)	64
Bulk density (g cm^{-3})	0.12
pH	7.45
Conductivity (ds m^{-1})	0.48
NO_3-N ($\mu g\ g^{-1}$)	111
$NaHCO_3$-extr. P ($\mu g\ g^{-1}$)	10
CH_3COONH_4-extr. K ($\mu g\ g^{-1}$)	478
SO_4-S ($\mu g\ g^{-1}$)	2352
CH_3COONH_4-extr. Ca (mg g^{-1})	14
CH_3COONH_4-extr. Mg (mg g^{-1})	3
DTPA-extr. Cu ($\mu g\ g^{-1}$)	1
DTPA-extr. Fe ($\mu g\ g^{-1}$)	417
DTPA-extr. Mn ($\mu g\ g^{-1}$)	43
DTPA-extr. Zn ($\mu g\ g^{-1}$)	11
Field capacity (% H_2O)	422
Volumetric water content at field capacity (ml cm^{-3})	0.42
Pore space at field capacity ($cm^3\ g^{-1}$)	0.05

A 2000 g sample of moist organic soil at 221% moisture was placed into 5.1 L plastic pots. Basal treatments of nitrogen, phosphorus and potassium were applied as described in section 4.3.1. Copper as $CuSO_4 \cdot 5H_2O$ and Cu-EDTA (7.5% copper, Hampshire Hampene) were dissolved in deionized distilled water, sprayed evenly on the soil which was then thoroughly mixed. Treatments applied consisted of a control (no copper added), Cu-EDTA at rates of 0.25, 0.50 and 5 Kg Cu ha^{-1}, and $CuSO_4 \cdot 5H_2O$ at 5 Kg Cu ha^{-1}. Each treatment was replicated three times and arranged in a completely randomized design in the growth chamber. Fifteen barley seeds (*Hordeum vulgare* var Argyle) were placed in each pot at a soil depth of 1.5 cm and soils watered to field capacity moisture content. Plants were thinned to eight seedlings pot^{-1} shortly after emergence. Soil water content was maintained between 70 and 100% of field capacity during the growth period.

An additional 100 Kg N ha^{-1} as NH_4NO_3 was added 42 d after seeding to each pot in response to an observed nitrogen deficiency.

Temperature, light intensity and other growth conditions in the growth chamber were as previously outlined [section 4.3.1].

The above-ground portion of four plants from each pot was harvested at time of heading, which was 50 d after seeding for all treatments except the control treatment, which was harvested 55 d after seeding. Plants were removed near the soil surface, adhering soil particles were removed and plants were placed in paper bags and air-dried. Plant samples were weighed, finely ground and analyzed. A second harvest of the remaining four plants was conducted 82 d after seeding. Grain and straw yields were obtained and samples of grain and straw were analyzed.

4.6 Results and Discussion

4.6.1 Yield and Copper Content of Plants at Heading

Yield of dry matter at the heading stage varied with treatment (table 13). A significant decrease in yield was noted when 0.25 Kg Cu ha^{-1} as Cu-EDTA and 5 Kg Cu ha^{-1} as $CuSO_4 \cdot 5H_2O$ were added. The lower yields for these treatments appeared to be related to reduced tillering of the plants. The plants grown without added copper tillered profusely whereas plants with added copper did not, and this likely accounts for the high dry matter yield on soil without added copper.

Application of copper significantly increased the concentration of copper in plant tissue harvested at the heading stage when 5 Kg Cu ha^{-1} was added. Rates of 0.25 and 0.50 (chelated form) did not significantly increase plant copper content at the heading stage. The plant copper content, when 5 Kg Cu ha^{-1} was added, was similar regardless of form of copper added.

Plant copper uptake was significantly increased only when 5 Kg Cu ha^{-1} was added as the chelate form. Uptake of copper increased with increasing copper added. The low copper uptake when 5 Kg Cu ha^{-1} as $CuSO_4$ was added is a function of the low dry matter yield of this treatment.

Dry matter yield and plant tissue copper content at the heading stage did not differ significantly between the two forms of copper when equal rates of copper were applied.

Table 13. Effect of Copper Source and Rate on Yield and Copper Content of Barley at the Heading Stage

Treatment (Kg Cu ha^{-1})	Yield (g pot^{-1})	Copper Concentration ($\mu g\ g^{-1}$)	Copper Uptake ($\mu g\ pot^{-1}$)
0	19.4 a‡	4.3 b	83 bc
0.25 as Cu-EDTA	13.8 b	4.4 b	61 c
0.50 as Cu-EDTA	17.1 ab	5.0 b	86 bc
5.0 as Cu-EDTA	18.2 ab	9.5 a	173 a
5.0 as $CuSO_4 \cdot 5H_2O$	13.9 b	8.3 a	115 b

‡ Means followed by the same letter do not differ significantly (P = 0.05).

4.6.2 Yield and Copper Content of Plants at Maturity

Yield of barley grain without added copper was low, being 3.6 g pot^{-1} (table 14). Grain yield was significantly increased by copper application but there were no significant differences among fertilized treatments. Both forms of copper were equally effective in increasing barley grain yield when applied at equal rates.

Table 14. Effect of Copper Source and Rate on Yield and Copper Content of Barley at Maturity

Treatment (Kg Cu ha^{-1})	Grain Yield (g pot^{-1})	Copper Concentration of Grain ($\mu g\ g^{-1}$)	Straw Yield (g pot^{-1})
0	3.6 b‡	1.3 b	19.6 a
0.25 as Cu-EDTA	11.4 a	0.8 c	12.6 b
0.50 as Cu-EDTA	14.2 a	1.1 bc	12.5 b
5.0 as Cu-EDTA	15.9 a	1.4 b	12.1 b
5.0 as $CuSO_4 \cdot 5H_2O$	14.7 a	3.4 a	13.8 b

‡ Means followed by the same letter do not differ significantly ($P = 0.05$).

Straw yield without added copper was 19.6 g pot^{-1} and was significantly greater than straw yield with added copper. Yields of straw declined when copper was added but there was no significant difference among treatments with copper application. The high straw yield of the treatment without added copper was likely a result of excessive tillering. Proliferation of tillering is a common symptom of copper deficiency in barley (Snowball and Robson 1984), and the development of a large number of tillers may compete with developing grain for retranslocated copper (Hill et al. 1978).

The response of grain copper content to copper application was variable. The greatest grain copper content occurred with

5 Kg Cu ha^{-1} as $CuSO_4$. Application of the chelate form resulted in either no significant change or a significant depression of grain copper concentration compared to that of grain grown without added copper. It should be noted that the copper concentration of the grain was very low even when 5 Kg Cu ha^{-1} in the chelated form was added. There appears to be no clear explanation for this occurrence since copper concentrations in plant tissues at the heading stage were very high for this treatment.

Visual inspection of the grain revealed that the plumpness of the kernels varied with treatment. Grain produced with the copper chelate had a shrivelled and shrunken appearance whereas the grain produced with $CuSO_4$ was reasonably plump. Thus, although the chelated copper provided yields similar to that of $CuSO_4$, it appeared that copper from the chelated form was insufficient for production of plump kernels. Even though kernel plumpness was not quantitatively assessed, kernel quality appeared to be strongly related to the copper content of the grain. Only the grain with a relatively high concentration of copper (from the 5 Kg Cu ha^{-1} $CuSO_4$ treatment) was reasonably plump.

In summary, yield for the two copper forms were not significantly different. However, the quality of grain (kernel plumpness) produced with $CuSO_4$ was superior to that produced with Cu-EDTA. Since the chelated form of copper was found to be less effective in producing quality grain than $CuSO_4$, and the literature is contradictory with respect to the relative effectiveness of chelated and inorganic forms of copper, more studies are required to assess the relative effectiveness of these forms of copper.

4.7 Effect of Copper Source and Placement on Growth and Copper Content of Barley - Field Study

The growth chamber studies did not provide definite results on the comparative effectiveness of copper chelates and $CuSO_4$. Also, results obtained in growth chamber studies are not directly applicable to field conditions as was shown in the results obtained for the growth chamber and field studies reported in sections 4.3 and 4.4. Thus field studies were conducted to assess the effectiveness of copper source and placement method on the yield and plant copper content of barley.

Work by Gartrell and Brennan (1979) indicated that some granular copper fertilizer applications may fail in the year of application due to poor plant root-copper contact. Gartrell (1980) suggested that the low plant available copper from banded $CuSO_4$ was a result of limited root-fertilizer copper contact. Root-copper contact is considered to be very important for copper utilization by plants due to the immobility of copper fertilizer after application (Gilkes et al. 1975). Varvel (1983) showed copper chelate to be superior to $CuSO_4$ for almost all methods of application, differences however, were greatest when each source was banded. Varvel (1983) further showed that broadcast $CuSO_4$ was effective in increasing yield of wheat, whereas banded $CuSO_4$ was ineffective. In a later study, Varvel (1984), demonstrated that banded copper chelate was a more effective method of copper placement in terms of increasing wheat yield, than if the chelate form was broadcast.

The field studies previously discussed (sections 4.1 and 4.2) indicated a need for annual applications of copper for maximum yield despite the good residual effects of previously added $CuSO_4 \cdot 5H_2O$. In the field study maximum yields were attained only when 5 Kg Cu ha^{-1} was applied at time of planting to plots previously treated with $CuSO_4$. Yield increase in response to newly added $CuSO_4$ was not always large and in some cases the value of the grain obtained was less than the cost of fertilization. Thus there was a need to investigate a means of increasing crop yield at lower costs of applied copper. Sources and methods of application were considered.

Chelates have been shown to be more effective per unit of copper than $CuSO_4 \cdot 5H_2O$ in increasing yield. Chelates however, usually have a higher cost per unit of copper applied, and at rates normally applied offer no residual benefit. Uptake of copper from fertilizer bands appears to be related to copper source applied. Banded methods of application may be expected to increase plant available copper as a result of reducing soil-copper contact. Limited soil-copper contact would reduce soil organic matter-copper complexation reactions, as opposed to broadcast applications of copper. The decrease in copper-plant root contact, however, would limit plant copper uptake from fertilizer bands. Increasing copper-root area contact is often regarded as the more important factor (Barber 1980; Gartrell 1980; Graham and Nambiar 1981). Therefore, increasing root interception, while limiting soil-copper contact, would likely result in the most plant available copper and the greatest plant uptake of copper.

A field study was conducted to evaluate the effects of band size, placement method, and copper source on the growth of barley. A rate of 2 Kg Cu ha^{-1} was employed to study the relationship of these variables on yield, copper content of plants and uptake of copper.

4.7.1 Materials and Methods

A field site located near Hadashville on the SW ¼ of 33-9-12E on a Terric Mesisol was selected (Table 15). The entire plot was situated within a field sown to barley which acted as a buffer zone. Each treatment plot was 0.3 m by 0.3 m.

Table 15. Some Physical and Chemical Characteristics of Soil Used

Organic matter (%)	72
pH	6.9
NO_3-N* (Kg ha^{-1})	46
$NaHCO_3$-extr. P (Kg ha^{-1})	6
CH_3COONH_4-extr. K (Kg ha^{-1})	29
SO_4-S* ($\mu g\ g^{-1}$)	50
Conductivity (ds m^{-1})	1.3
CH_3COONH_4-extr. Ca (mg g^{-1})	46
CH_3COONH_4-extr. Mg (mg g^{-1})	3
DTPA-extr. Cu ($\mu g\ g^{-1}$)	1.7
DTPA-extr. Fe ($\mu g\ g^{-1}$)	257
DTPA-extr. Mn ($\mu g\ g^{-1}$)	54
DTPA-extr. Zn ($\mu g\ g^{-1}$)	20

* Amount in the 0 to 60 cm depth of soil. Other physical and chemical characteristics are for the 0 to 15 cm depth.

All plots were treated with 75 Kg N ha^{-1} as NH_4NO_3, 20 Kg P ha^{-1} as $NH_4H_2PO_4$ and 40 Kg K ha^{-1} as K_2SO_4 (area basis). Copper was applied as $CuSO_4 \cdot 5H_2O$ (technical grade) or in chelate form (Cu-EDTA, 7.5% copper, Hampshire Hampene) at a rate of 2 Kg Cu ha^{-1}. Nitrogen fertilizer was broadcast at time of seeding, the phosphorus and potassium fertilizer was banded 2.5 cm below and beside the seed row. The various copper fertilizer placements were as follows: (1) Copper fertilizer thoroughly mixed with the surface 7 to 7.5 cm of soil; (2) Copper fertilizer placed 2 to 2.5 cm directly below the seed in a narrow band. The copper was applied dropwise in a single line below the seed; (3) Copper fertilizer sprayed in a 2.5 cm wide band below the seed. This treatment was included to increase root fertilizer contact; (4) Copper fertilizer sprayed in a wide band below the seed. The copper fertilizer in the band was then mixed into the soil to a depth of 2 cm. This resulted in a fertilizer band of 2.5 cm width by 2 cm depth below the seed. This treatment was used to further increase root contact with the applied fertilizer; (5) A treatment consisting of 1 Kg Cu as $CuSO_4$ applied in a narrow band as in (2) above, plus a foliar spray of 1 Kg Cu ha^{-1} as $CuSO_4$ at the 3-4 leaf stage of growth.

Barley (*Hordeum vulgare* var Argyle) was seeded at a rate of forty-five barley seeds per row at a depth of 1.8 to 2 cm. Plants were thinned to twenty-four plants per row after emergence. Seeding and harvest dates are shown in table 16.

Table 16. Seeding and Harvest Dates - Copper Source and Placement Study

	Harvest Dates		
Seeding Date	3 - 4 Leaf	Heading	Maturity
May 18	June 8	July 8	August 12

The field plot was arranged in a randomized complete block design with ten treatments replicated four times. Weed control was accomplished by hand weeding throughout the growing season.

4.7.2 Harvest of Plants

a) <u>3 to 4 Leaf Stage</u>

Entire plants were cut 1.0 cm above the soil surface from each of the two rows for each treatment. The number of plants removed per treatment was varied in order to leave the same number of plants per treatment for harvest at later stages of plant growth. Plant samples were placed in paper bags, air-dried and then finely ground. The chemical composition of the plants was determined as previously described.

b) Heading Stage

Twelve plants were cut from each of two rows of each treatment. Harvest procedures were as outlined above. Yield of dry matter was obtained and plant samples analyzed for nutrient composition.

c) Final Harvest

The remaining twenty-four plants were harvested at maturity. Samples were air-dried and threshed by hand. Grain and straw yields were obtained and samples finely ground. Grain and straw samples were analyzed for nutrient composition.

4.8 Results and Discussion

a) 3 to 4 Leaf Stage

The copper content of plants harvested at the 3 to 4 leaf stage was not significantly affected by treatment (table 17). Copper levels ranged from 3.4 to 6.8 $\mu g\ g^{-1}$. It is interesting to note that for seven of the ten treatments in total, plant copper concentration was below 5 $\mu g\ g^{-1}$, the level of sufficiency most often reported in the literature for barley plants at this stage of growth (Melsted et al. 1969; Akinyede 1977). Only the plants without added copper (control) and plants treated with Cu-EDTA

Table 17. Effect of Copper Source and Method of Placement on Copper Content of Barley - 3 to 4 Leaf Stage

Placement	Copper Concentration ($\mu g\ g^{-1}$)
Control	5.1 ab‡
Narrow band + foliar application	4.6 b
Mixed $CuSO_4$	4.1 b
Mixed chelate	3.9 b
Narrow band $CuSO_4$	3.4 b
Narrow band chelate	4.8 b
Wide band $CuSO_4$	4.6 b
Wide band chelate	5.1 ab
Wide band $CuSO_4$ (mixed)	4.3 b
Wide band chelate (mixed)	6.8 a

‡ Means followed by same letter do not differ significantly ($P = 0.05$)

applied in a wide band and in a 2.5 x 2 cm band (wide band - mixed) contained greater than 5 $\mu g\ g^{-1}$ copper. Although statistically not significant, it should be noted that in all cases copper content of plants grown on plots with a wide band treatment had higher copper contents than plants grown on plots with a narrow band. Also, tissue of plants treated with chelated copper contained more copper than plants treated with $CuSO_4$, except when the fertilizer was thoroughly mixed with soil.

b) Heading Stage

Dry matter yields were significantly increased only by the wide band treatments (table 18). Yield increases with the chelate and sulphate forms were similar. This suggests that for equivalent rates of copper applied, both sources of copper were equally effective in terms of increasing barley yield at the heading stage. As well, no significant difference in yield was apparent between the narrow band treatments for the sulphate and

Table 18. Effect of Copper Source and Method of Placement on Grain and Copper Content of Barley - Heading Stage

Placement	Yield (g treatment^{-1})	Copper Concentration (μg g^{-1})	Copper Uptake (μg treatment^{-1})
Control	49.0 bc‡	3.8 c	184 c
Narrow band + foliar application	51.1 bc	5.1 ab	258 ab
Mixed $CuSO_4$	48.5 c	4.3 bc	209 bc
Mixed chelate	52.2 b	5.6 a	290 a
Narrow band $CuSO_4$	51.6 bc	5.1 ab	261 ab
Narrow band chelate	51.2 bc	5.5 a	285 a
Wide band $CuSO_4$	55.9 a	4.7 abc	262 ab
Wide band chelate	55.6 a	5.7 a	317 a
Wide band $CuSO_4$ (mixed)	56.3 a	4.9 abc	277 ab
Wide band chelate (mixed)	55.7 a	5.4 ab	300 a

‡ Means followed by same letter do not differ significantly (P = 0.05)

chelated form. This would further suggest that both sources of copper were equally effective in enhancing yield at this stage of growth. There was, however, a significantly higher yield with the chelated than with the sulfate form when the fertilizers were thoroughly mixed with the soil.

Copper concentration of barley plants were significantly increased by six of the treatments. $CuSO_4$ did not significantly increase copper content above that of the control except when placed in a narrow band. In contrast, the chelated form of copper significantly increased copper content of barley plants above that of the control in all instances. There was, however, no significant difference in copper content of plants with placement of the chelate. Both the chelate and sulphate copper source, when applied in a narrow band, significantly increased plant copper content over that of the control.

All treatments significantly increased plant copper uptake with the exception of $CuSO_4$ thoroughly mixed with the soil. There were no significant differences in copper uptake among methods of application or form of copper applied.

c) Maturity

Grain yield of barley was significantly increased above that of the control by all treatments except when $CuSO_4$ was added in a narrow band and in a 2.5 x 2 cm band (wide band - mixed) (table 19). The low yield with the $CuSO_4$ applied in a narrow band is likely due to poor root-copper fertilizer contact. Due to the

Table 19. Effect of Copper Source and Method of Placement on Yield and Copper Content of Barley - Maturity

Placement	Grain Yield (g treatment^{-1})	Copper Concentration of Grain (μg g^{-1})	Straw Yield (μg treatment^{-1})	Copper Concentration of Straw (μg g^{-1})	Total Copper Uptake (μg treatment^{-1})
Control	11.8 e[‡]	3.1 ab	17.4 b	2.1 ab	70.1 c
Narrow band + foliar application	22.2 bcd	2.2 b	24.9 ab	2.5 a	112 bc
Mixed $CuSO_4$	18.0 bcd	3.3 ab	19.8 b	2.5 a	108 bc
Mixed chelate	20.1 bcd	2.4 b	23.3 ab	2.3 ab	102 bc
Narrow band $CuSO_4$	16.6 cde	2.8 ab	20.8 b	2.3 ab	92.6 bc
Narrow band chelate	23.2 bc	3.4 ab	23.0 ab	2.5 a	136 b
Wide band $CuSO_4$	26.2 a	4.1 a	31.4 a	2.5 a	184 a
Wide band chelate	24.7 b	3.0 ab	25.9 ab	2.1 ab	129 b
Wide band $CuSO_4$ (mixed)	16.3 de	2.8 ab	19.5 b	2.1 ab	86.4 bc
Wide band chelate (mixed)	24.7 b	2.5 b	29.8 a	2.0 b	120 b

[‡] Means followed by same letter do not differ significantly ($P = 0.05$)

lack of mobility of copper applied as $CuSO_4$ in soil, the method of placement strongly influences plant uptake of copper (Gilkes and Sadleir 1979; Barber 1980). Copper must be placed in an active rooting zone for plant uptake for copper sources which are relatively non-mobile. The low yield with $CuSO_4$ placed in a 2.5 x 2 cm band may have been a result of the applied copper reacting with soil constituents rendering it less plant available (Davies et al. 1969; Ardakani and Stevenson 1972; Gilkes 1981). Although the $CuSO_4$ placed in a 2.5 x 2 cm band provided greater root-soil copper contact, the increase in root interception and copper uptake may have been diminished as a result of the greater soil-copper contact.

Yield among the Cu-EDTA treatments did not differ significantly but were significantly higher than that for the control treatment (table 19). Yield with $CuSO_4$ thoroughly mixed with the soil was similar to that obtained with Cu-EDTA. $CuSO_4$ applied in a 2.5 cm wide band below the seed was the most effective of any treatment in increasing barley grain yield. Yield with $CuSO_4$ applied in a narrow band followed by a foliar application of $CuSO_4$ was similar to yields obtained with Cu-EDTA and $CuSO_4$ thoroughly mixed with the soil.

Copper content of the grain was similar for all treatments. However, copper content of the grain grown on plots treated with $CuSO_4$ applied in a 2.5 cm wide band was significantly greater than the copper content of grain from plots treated with $CuSO_4$ applied in a narrow band plus foliar application, Cu-EDTA thoroughly mixed with the soil, and Cu-EDTA placed in a 2.5 x 2 cm band (wide band - mixed).

Yield of straw was similar for all treatments with yield generally being higher with the copper fertilizers applied in a wide band than with copper fertilizers applied in a narrow band. Application of copper as chelate or $CuSO_4$ provided reasonably similar straw yield. The straw yield with $CuSO_4$ placed in a 2.5 x 2 cm band was significantly less than with Cu-EDTA placed in the same manner.

Application of copper did not significantly increase straw copper content above that of the control. The copper content of the straw appears to be low. However, these values are similar to those obtained in the 1987 field study.

Total copper uptake by barley with $CuSO_4$ applied in a wide band was significantly greater than with any other treatment. Copper uptake with Cu-EDTA in a narrow band, Cu-EDTA in a wide band and Cu-EDTA in a 2.5 x 2 cm band (wide band - mixed) was greater than copper uptake without applied copper. On average, copper placed in wide bands was the most effective treatments in increasing plant copper uptake. This indicates that root-fertilizer interception was increased by placing the fertilizer in a wide band. Increasing the fertilizer band width likely resulted in increased positional availability of applied copper (Barber 1980; Gartrell 1980; Varvel 1983). The poor total uptake of copper when the $CuSO_4$ was placed in a 2.5 x 2 cm band was likely a result of applied copper-soil organic matter complexation reactions. Thus, increasing the band size increased plant available copper, however, exceeding a "critical" band size facilitated copper adsorption to soil, and resulted in reduced plant available copper.

Plant uptake of copper from both copper sources was similar. Placement of $CuSO_4$ in wide bands increased the positional availability of copper from $CuSO_4$ facilitating plant uptake. Placement of $CuSO_4$ in narrow bands resulted in poor plant root-copper contact and limited plant copper uptake. In contrast, due to the relatively greater mobility and solubility of Cu-EDTA than for $CuSO_4$ in soil, placement did not have as large an effect on plant availability as did placement method for $CuSO_4$.

The relative efficiency and effectiveness of $CuSO_4 \cdot 5H_2O$ and Cu-EDTA depended upon the method of placement and stage of plant growth at which an assessment was made. At equivalent rates of application, the Cu-EDTA appeared to provide greater plant available copper early in the growing season. At the later stages of plant growth both $CuSO_4$ and Cu-EDTA appeared to be equally effective in increasing plant copper content and yield. However, placement method which affected the positional availability of copper and copper-soil complexation reactions, strongly affected this relationship.

4.9 Effect of Plant Growth on DTPA-extractable Copper Concentration in Soils - Growth Chamber Study

Soil samples were taken from the field sites during the growing season in 1987 and analyzed for DTPA-extractable copper. The results of the analyses indicated that DTPA-extractable copper concentration in soils increased after planting, approached a maximum at time of heading and then gradually declined. DTPA-extractable copper concentrations at time of crop maturity were somewhat higher than those levels found prior to seeding. The above variation in DTPA-extractable copper during the growing season occurred for both copper fertilized and non-fertilized soils. Determination of soil pH at these times revealed a negative relationship between DTPA-extractable copper and soil pH level. The variations in soil pH and DTPA-extractable copper appeared to be related to the production of crop biomass. Variations in soil pH and DTPA-extractable copper concentration with plant growth noted in the field studies have also been noted by other researchers (Harry and Graham 1981; Jarvis 1981; Linehan et al. 1985).

Thus a growth chamber experiment was conducted to confirm the observations noted from the field studies.

4.9.1 Materials and Methods

The experiments were conducted concurrently with the growth chamber studies reported in sections 4.3 and 4.5. Some of the chemical and physical properties of the soils selected are shown in tables 8 and 12. Methods of potting, treatment application and seeding and harvest procedures were as previously outlined in sections 4.3.1 and 4.5.1.

Soil cores (1.2 cm dia) to a depth of approximately 13 cm were obtained from each pot immediately after each harvest of plant material and at 2 wk intervals for a period of 8 wk following final harvest of grain. The soil was obtained from the rooting zone of the harvested plants, air-dried and well mixed. Soil pH and DTPA-extractable copper concentration were determined as previously outlined.

Statistical analyses of the data obtained in the growth chamber studies was not conducted. Analyses of variance for the results obtained was not possible due to the lack of proper control treatments. A control for each treatment, soil without plants, would have been required to conduct the proper analyses.

4.9.2 Results and Discussion

DTPA-extractable copper concentration increased from time of planting to heading and then decreased with time for soil 7 (figure 7, table 20). The maximum DTPA-extractable copper concentration corresponded to the heading stage of plant growth (47 d from seeding). The increase in DTPA-extractable copper concentration was most likely due to plant root development. Linehan et al. (1985) reported a three-fold increase in soil solution copper concentration during the early stages of

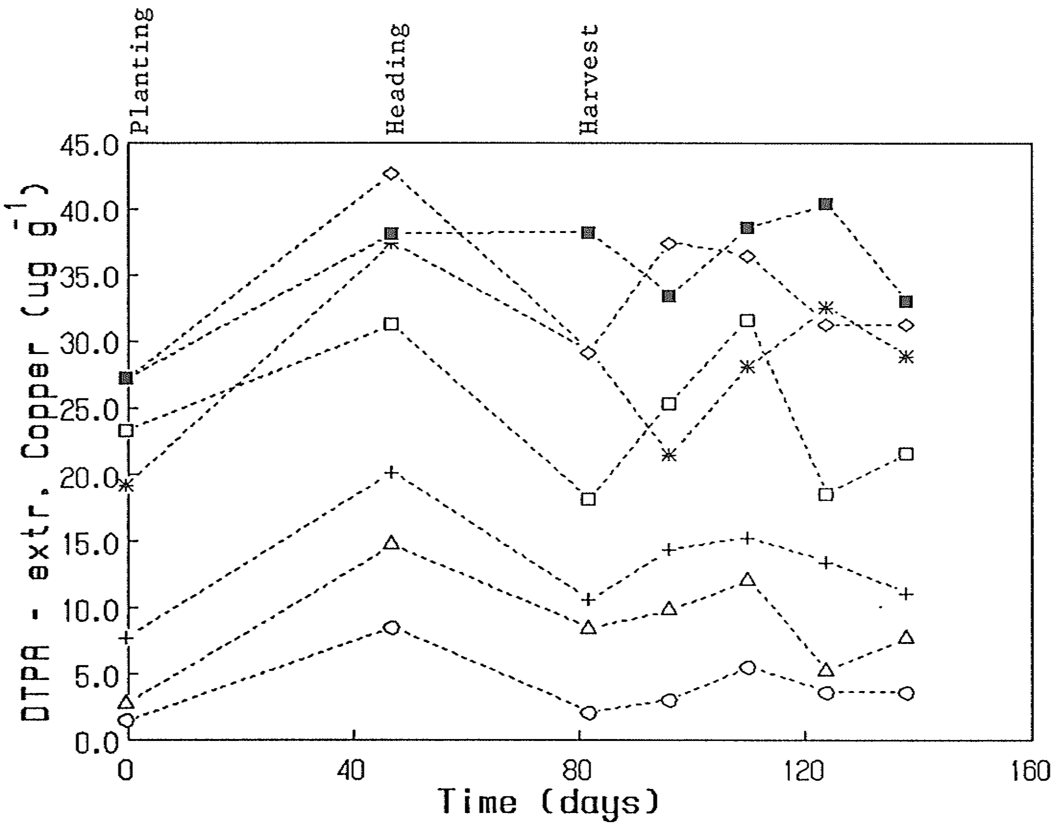


Figure 7. Effect of Time and Plant Growth on DTPA-extractable Copper - Soil 7

○---○ control, △---△ 5 (1986)[1],
□---□ 5 (1987), ✱---✱ 5 (1986) - 5 (1987),
+---+ 10 (1986), ◇---◇ 10 (1986) - 5 (1987),
■---■ 10 (1986) - 5 (1987) - 5 (1987)

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[1] Kg Cu ha^{-1} and year of application

Table 20. Changes in DTPA-extractable Copper and Soil pH as Affected by Plant Growth and Time (Soil 7)

	Copper Treatment and Year of Application (Kg Cu ha^{-1})													
	Control		5 (1986)		5 (1987)		5 (1986) - 5 (1987)		10 (1986)		10 (1986) - 5 (1987)		10 (1986) - 5 (1987) - 5 (1987)	
Time (days)	Soil pH	DTPA-extr. Copper (μg g^{-1})	Soil pH	DTPA-extr. Copper (μg g^{-1})	Soil pH	DTPA-extr. Copper (μg g^{-1})	Soil pH	DTPA-extr. Copper (μg g^{-1})	Soil pH	DTPA-extr. Copper (μg g^{-1})	Soil pH	DTPA-extr. Copper (μg g^{-1})	Soil pH	DTPA-extr. Copper (μg g^{-1})
0	6.85	1.5	7.02	2.9	6.94	23	7.18	19	7.0	7.0	7.02	27	7.02	27
47	5.75	8.0	6.11	15	6.22	31	6.19	38	6.29	20	6.29	43	6.29	38
82	6.47	2.2	6.78	8.5	6.52	18	6.37	29	6.63	11	6.78	29	6.31	38
96	6.16	3.2	6.81	9.9	6.88	26	6.36	22	6.71	15	6.81	38	6.78	34
110	6.67	5.7	7.01	12	7.28	32	7.12	28	6.83	15	7.20	37	7.34	39
124	6.70	3.7	6.81	5.4	7.16	19	7.29	33	7.12	14	7.24	31	7.12	40
138	6.71	3.7	6.81	7.9	7.11	22	7.12	29	7.15	11	7.06	31	7.16	33

rhizosphere development in barley. Their work further indicated that maximum copper concentrations were attained prior to crop heading, followed by a decrease at final grain harvest to concentrations approaching those found prior to seeding. The data for soil 7 parallel their observations.

Rovira and Davey (1974) suggested that mobilization of copper due to crop growth may be a result of the exudation of low molecular weight organic acids by plant roots. Stevenson (1982) indicated that organic acids, which are capable of complexing copper, can occur in concentrations of up to 10^{-5} M in soil solution. Nielson (1976b) showed copper concentration in soil solution to increase due to an increase in organic acid complexing agent content. In an identical study conducted without plant cover, it was found that copper levels remained almost constant. Chaberek and Martell (1959) also suggested that root exudates may directly regulate the uptake of metals from soil.

The data obtained for soil 2 did not show the same variation in copper concentration with time and crop growth stage (figure 8, table 21) as compared to soil 7. For soil 2 the most highly fertilized treatment [10 (1986) - 5 (1987) - (1987)] did, however, show an increase in DTPA-extractable copper concentration at the heading stage of crop growth (52 d). This is most likely due to the newly added copper (5 Kg Cu ha^{-1}) and not wholly due to increases in extractable copper in response to crop rhizosphere development as suggested by Linehan et al. (1985).

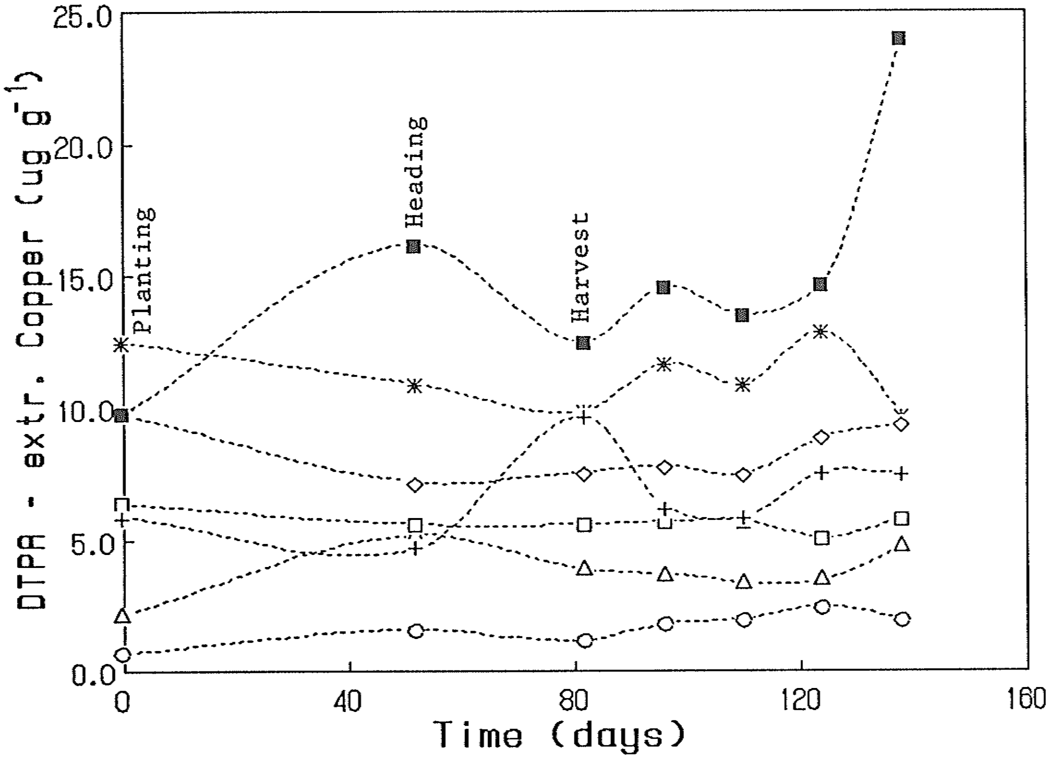

Figure 8. Effect of Time and Plant Growth on DTPA-extractable Copper - Soil 2

○---○ control, △---△ 5 (1986)[1],
□---□ 5 (1987), ✱---✱ 5 (1986) - 5 (1987),
+---+ 10 (1986), ◇---◇ 10 (1986) - 5 (1987),
■---■ 10 (1986) - 5 (1987) - 5 (1987)

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[1] Kg Cu ha^{-1} and year of application

Table 21. Changes in DTPA-extractable Copper and Soil pH as Affected by Plant Growth and Time (Soil 2)

	Copper Treatment and Year of Application (Kg Cu ha^{-1})													
	Control		5 (1986)		5 (1987)		5 (1986) - 5 (1987)		10 (1986)		10 (1986) - 5 (1987)		10 (1986) - 5 (1987) - 5 (1987)	
Time (days)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)
0	7.74	0.7	7.75	2.2	7.95	6.4	7.76	13	7.93	5.9	7.95	9.8	7.95	9.8
52	7.06	1.6	7.26	5.2	7.25	5.6	7.27	11	7.31	4.7	7.26	7.2	7.25	16
82	6.94	1.2	7.41	3.9	7.29	5.6	7.45	10	7.53	9.7	7.28	7.6	7.21	13
96	7.54	1.8	7.53	3.7	7.47	5.7	7.55	12	7.61	6.2	7.67	7.8	7.24	15
110	7.96	2.0	7.84	3.4	7.98	5.7	7.93	11	7.93	5.9	8.01	7.5	8.03	14
124	7.81	2.5	7.96	3.5	8.12	5.1	8.02	13	8.19	7.6	8.23	8.9	8.09	15
138	7.99	2.0	7.97	4.8	8.08	5.8	8.13	9.7	8.11	7.5	8.03	9.4	8.07	24

In both soil 7 and soil 2, a decline in soil pH occurred with plant growth and rhizosphere development (figures 9 and 10, respectively). Soil pH levels declined from time of seeding to the heading stage of crop development and steadily increased as the crop approached maturity. Changes in soil pH were relatively greater for soil 7 than for soil 2. The average initial soil pH for soil 7 was 7.01 and decreased to an average of 6.16 at heading, whereas for soil 2, soil pH values, on average, were 7.86 and 7.24, at time of planting and heading, respectively. For both soil 7 and soil 2 pH levels increased following heading and continued to increase for an 8 wk period following final grain harvest.

Linehan et al. (1985) found that a substantial decline in pH (approx. 1 pH unit) occurred in the early stages of rhizosphere development. The decline in pH however, did not continue into the stage of maximum copper mobilization. Hedley et al. (1982) suggested that pH may be depressed due to H^+ excretion by plant roots. Sanders (1982; 1983) suggested that the pH of the soil solution may be of considerable importance in terms of influencing the solubility and plant availability of copper. Smiley (1974) suggested that root exudates may be responsible for a pH drop of the rhizosphere soil of 1-2 units. The mobilization of copper, however, would appear to not only be influenced by soil pH, but also to be a result of chelation of copper by plant root exudates. Stevenson and Ardakani (1972) stated that the effect of root exudates on increasing copper availability may be attributed to the lowering of pH, and to the production of metal chelates.

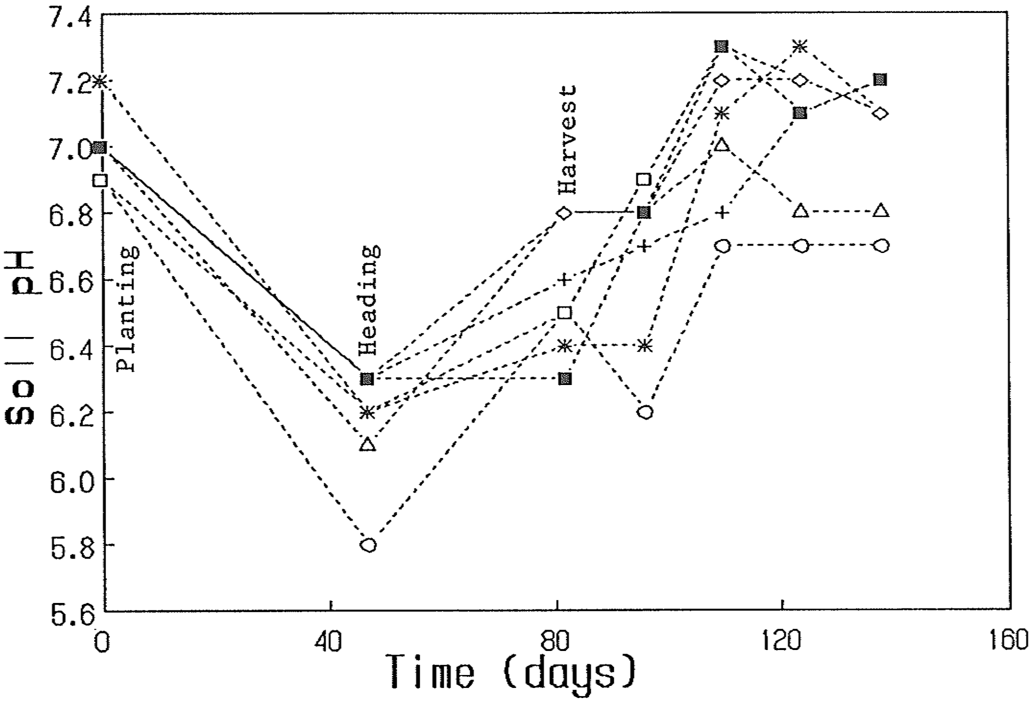

Figure 9. Effect of Time and Plant Growth on Soil pH - Soil 7

○---○ control, △---△ 5 (1986)[1],
□---□ 5 (1987), ✱---✱ 5 (1986) - 5 (1987),
+---+ 10 (1986), ◇---◇ 10 (1986) - 5 (1987),
■---■ 10 (1986) - 5 (1987) - 5 (1987)

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[1] Kg Cu ha^{-1} and year of application

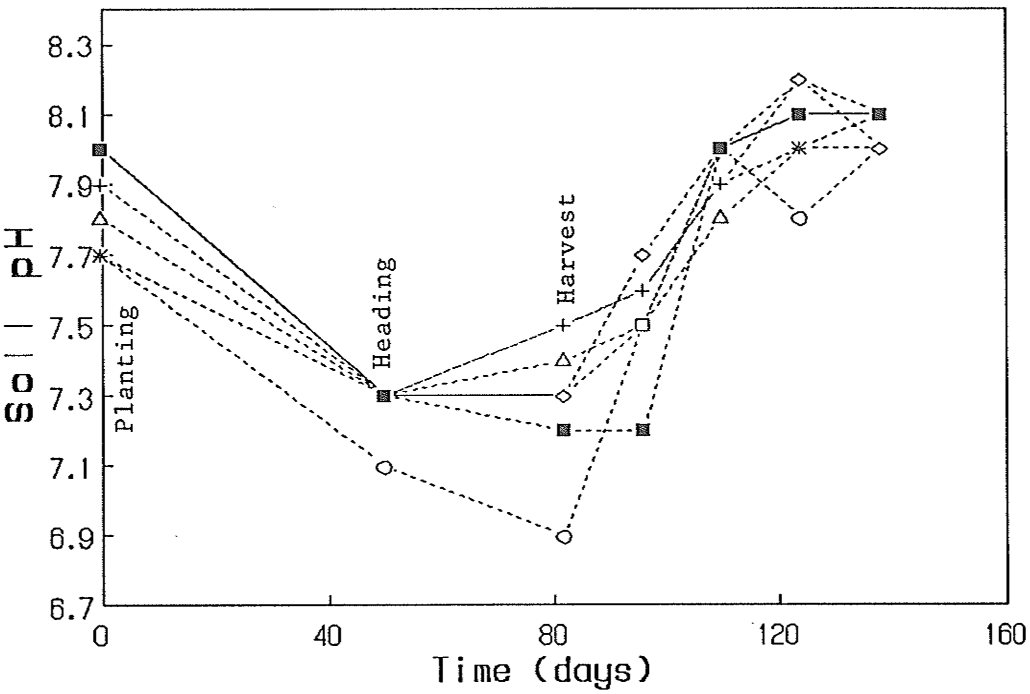

Figure 10. Effect of Time and Plant Growth on Soil pH - Soil 2

○---○ control, △---△ 5 (1986)[1],
□---□ 5 (1987), ✱---✱ 5 (1986) - 5 (1987),
+---+ 10 (1986), ◇---◇ 10 (1986) - 5 (1987),
■---■ 10 (1986) - 5 (1987) - 5 (1987)

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[1] Kg Cu ha^{-1} and year of application

There was a reasonably strong negative relationship between soil pH and DTPA-extractable copper concentration for soil 7. The relationship between soil pH and DTPA-extractable copper was relatively poor for soil 2. Average DTPA-extractable copper levels were 15.5 and 27.5 $\mu g\ g^{-1}$ at time of planting and heading respectively for soil 7, and 6.8 and 7.3 μg Cu g^{-1} soil at time of planting and heading, respectively, for soil 2.

In this study increases and decreases in DTPA-extractable copper concentration were seemingly related to changes in soil pH levels as well as to changes in plant biomass production.

The results obtained in this experiment indicated an increase in DTPA-extractable copper concentration from time of seeding to the heading stage of crop development. The increase in DTPA-extractable copper concentration coincided with plant rhizosphere development and declining soil pH levels. Extractable copper concentration generally declined during crop maturation from the heading stage to final harvest (82 d), and after an 8 wk period of incubation approached levels found prior to seeding.

It is noteworthy that the increase in DTPA-extractable copper concentration with plant growth and time was of a greater magnitude for soil 7 than for soil 2. The copper concentration of the respective soils, as well as the range over which the soil pH change occurred are possible reasons for this occurrence. Soil solution copper exists mainly in a complexed form. Fulvic and humic acids, and a variety of functional groups are largely responsible for copper complexation in organic soils. Soil pH exerts a dominant effect on the ionization of functional groups in soil, and hence copper adsorption-desorption reactions occurring in soils. As previously discussed in the literature review, McLaren and Crawford (1973b) showed copper adsorption to be strongly influenced by soil pH for soils containing high amounts of copper in soil solution (10 $\mu g\ ml^{-1}$). McLaren et al. (1981) found that when soil solution copper was low (0.05 - 0.25 $\mu g\ ml^{-1}$), declining soil pH had little effect on copper adsorption-desorption reactions. It was concluded that adsorption-desorption sites are highly selective and bind copper strongly at low soil solution copper concentrations.

In view of the work of McLaren and Crawford (1973b) and McLaren et al. (1981), desorption of copper from soil organic matter-copper complexes, in response to decreases in soil pH, likely occurred to a relatively greater extent in soil 7, than for soil 2. This, in part, explains the greater increase in DTPA-extractable copper with decrease in soil pH for soil 7 than for soil 2. Decreases in soil pH would cause copper desorption from copper-soil organic matter complexes through the mechanism proposed by Gamble et al. (1970), Schnitzer and Hansen (1970) and Boyd et al. (1981).

The magnitude of pH change as well as the range of pH in which a pH change occurs is also important in adsorption-desorption processes of copper with organic soil constituents. Stevenson (1982) suggested that titration curves for the humic and fulvic acid component of organic soils could be divided into three regions; region 1 (pH 5 - 7) being where COOH and some associated phenolic groups dissociate, region 2 an area for ionization of weak and very weak acid groups (pH 7 - 8), and the third region representing dissociation of phenolic and other weak acid groups (pH 8 - 10). Changes in soil pH for soil 7 were 0.85 pH units (average for all treatments) and likely resulted in greater decomplexation of copper than for soil 2 where the pH change was 0.62 pH units. The greater decomplexation of copper on soil 7 than on soil 2 was reflected in increases in DTPA- extractable copper concentration. However, as noted above, the range over which the change in soil pH occurs is also important in adsorption-desorption processes. For soil 7 the change in soil pH occurred in a range in which a greater number of functional groups capable of complexing copper may exchange Cu^{2+} for H^+. A similar decomplexation reaction likely occurred in soil 2. However, the functional groups capable of exchanging Cu^{2+} for H^+ in this pH range complex copper more strongly (Goodman and Cheshire 1973, 1976).

The analyses of DTPA-extractable copper concentration and soil pH during crop growth, as well as for an 8 wk period following final harvest indicated that DTPA-extractable copper concentration increased over a 47-52 d period following seeding. The increase in DTPA-extractable copper concentration is likely related to crop rhizosphere development and the concurrent decline in soil pH level. DTPA-extractable copper concentrations prior to seeding and at the end of the 8 wk period were similar, the increase in DTPA-extractable copper with plant growth may be a possible explanation for the non-responsiveness of some soils to copper application even though a soil test may indicate a soil copper deficiency. Crop root development and its influence on soil properties such as pH may at times increase plant available copper in soils to sufficient levels.

The information obtained also has implications regarding the time of obtaining soil samples for analysis. For example, if a determination of the correlation between response to copper fertilizer by crops, and DTPA-extractable copper were conducted on samples taken prior to seeding, all samples taken for the assessment of copper fertilizer requirements on other soils should also be taken at this time. Clearly, time of sampling for analysis has to be stipulated.

4.10 DTPA-extractability of Copper Applied as $CuSO_4$ and Cu-EDTA - Growth Chamber Study

4.10.1 Materials and Methods

DTPA-extractable copper was determined for soils treated with Cu-EDTA and $CuSO_4$ and cropped to barley. This experiment was conducted concurrently with the plant growth studies reported in section 4.5. Treatments included a control, 0.25, 0.50 and 5 Kg Cu ha^{-1} added as Cu-EDTA, and 5 Kg Cu ha^{-1} added as $CuSO_4 \cdot 5H_2O$. Soil core samples were obtained following harvest of plant material and at 2 wk intervals as previously described in section 4.9.1.

4.10.2 Results and Discussion

The DTPA-extractable copper concentration of soils treated with 0.25 and 0.50 Kg Cu ha^{-1} as Cu-EDTA were similar to that without added copper at all times of sampling (figure 11, table 22). The DTPA-extractable copper concentration obtained when 5 Kg Cu ha^{-1} was added as Cu-EDTA was usually higher than for the control, but much less than when copper as $CuSO_4$ was added. The DTPA-extractable copper concentrations were 10 and 5.3 μg Cu g^{-1} soil for the $CuSO_4$ and Cu-EDTA treatments, respectively, after 138 days. Thus, the most significant feature of the data obtained was that of the large difference in DTPA-extractable copper concentrations between copper applied as $CuSO_4$ and that applied as Cu-EDTA.

Applications of copper as $CuSO_4 \cdot 5H_2O$ may react rapidly with soil and in essence become a slow release form of copper. Chelated forms (Cu-EDTA), due to their chemical and physical nature, at least initially, are more mobile and less susceptible to copper-soil organic matter binding reactions. Thus, the results obtained were unexpected. Undoubtedly some factor was limiting the extractability of copper from the soil treated with Cu-EDTA. Lindsay and Norvell (1969a, b) suggested that the copper in copper chelate can be displaced by Fe^{+3} and Ca^{+2} in the soil. The extractable iron and calcium levels in the soil were high

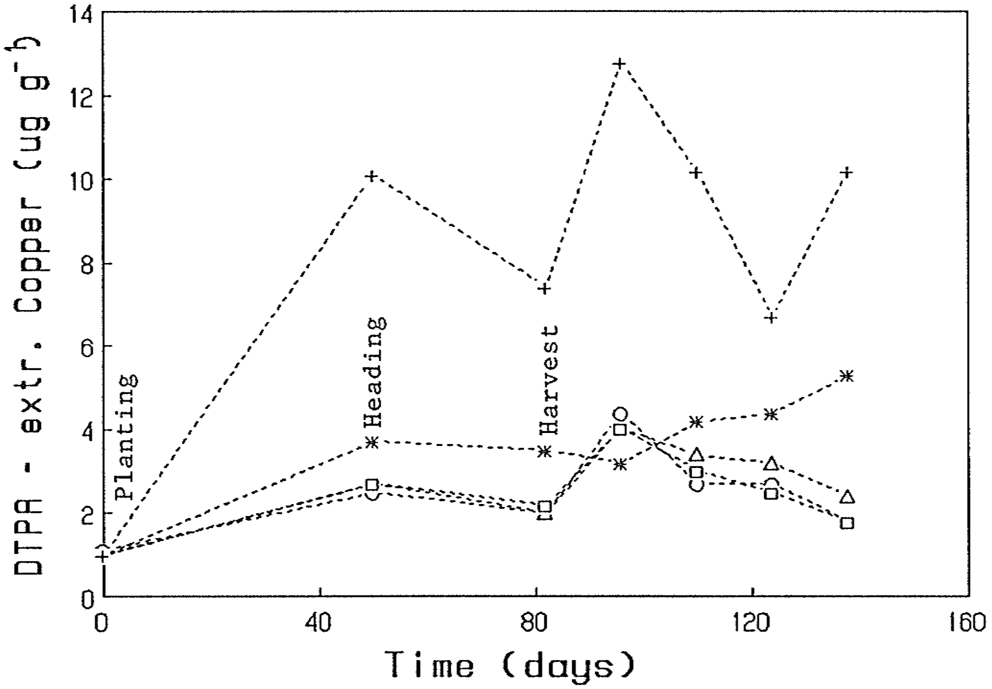

Figure 11. Effect of Time and Copper Source on DTPA-extractable Copper

○---○ control, △---△ 0.25 (Cu-EDTA),
□---□ 0.50 (Cu-EDTA), ✱---✱ 5.0 (Cu-EDTA),
+---+ 5.0 ($CuSO_4 \cdot 5H_2O$)

Table 22. DTPA-extractable Copper and Soil pH as Affected by Plant Growth, Time and Copper Source

Copper Treatment and Copper Source (Kg Cu ha^{-1})

	Control		0.25 (Cu-EDTA)		0.5 (Cu-EDTA)		5.0 (Cu-EDTA)		5.0 ($CuSO_4 \cdot 5H_2O$)	
Time (days)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)	Soil pH	DTPA-extr. Copper ($\mu g\ g^{-1}$)
0	7.45	1.1	7.44	1.0	7.43	1.0	7.46	1.0	7.46	1.0
50*	6.37	2.5	6.64	2.7	6.83	2.7	6.73	3.7	6.76	10
82	6.93	2.0	7.28	2.0	7.13	2.2	6.94	3.5	7.18	7.4
96	6.97	4.4	7.59	4.0	7.89	4.0	7.40	3.2	7.37	13
110	7.36	2.7	7.60	3.4	7.41	3.0	7.68	4.2	7.70	10
124	7.45	2.7	7.43	3.2	7.34	2.5	7.66	4.2	7.68	6.7
138	7.36	1.8	7.21	2.4	7.58	1.8	7.20	5.3	7.63	10

* Control only was harvested at 55 days

(417 and 14,000 $\mu g\ g^{-1}$, respectively, table 12), and this displacement of copper and its subsequent availability for binding reactions with soil organic matter would reduce its extractability. However, Lindsay and Norvell (1978) indicated that the DTPA concentration selected (5×10^{-3} M) for DTPA extraction of copper, was sufficient to remove all extractable copper, iron, magnesium and zinc present, thereby preventing competitive interactions.

The reasons for the low recovery of copper applied as Cu-EDTA via DTPA-extraction, versus that recovered from copper applied as $CuSO_4$ remain unclear. It is possible that the Cu-EDTA, initially being very mobile, moved throughout the soil solution. The copper from the Cu-EDTA may have then been displaced from the Cu-EDTA chelate by competitive ions such as calcium and iron, or released as a result of degradation of the EDTA molecule. If this occurred, the contact between soil and copper fertilizer would be very large and fixation of the copper by soil constituents would be favored. In contrast, due to the low mobility of copper from $CuSO_4$ the contact between copper applied as $CuSO_4$ and the soil may have been relatively more restricted than for Cu-EDTA even though both fertilizers were thoroughly mixed with the soil. Intimate and complete mixing of the $CuSO_4$ with the soil may not have been accomplished. If mixing was incomplete for the applied $CuSO_4$, reactions between soil constituents and copper would be reduced. This would be reflected in increased DTPA-extractability for the applied $CuSO_4$ as compared to Cu-EDTA. Clearly the factors influencing DTPA-extractability, and the fate of copper applied as $CuSO_4$ and Cu-EDTA to organic soils require further study.

Further, it is noteworthy that soil pH levels decreased in a manner similar to that found in the previous study (figure 12). The effect of soil pH and plant growth on DTPA-extractable copper concentration could not be clearly assessed in this study as copper was added just prior to seeding, and extractable copper concentrations would be a function of both fixation reactions of the newly applied copper as well as plant growth effects. Understandably, both soil pH and plant growth effects would be influencing factors in further studies of DTPA-extractability of copper applied as $CuSO_4$ and Cu-EDTA.

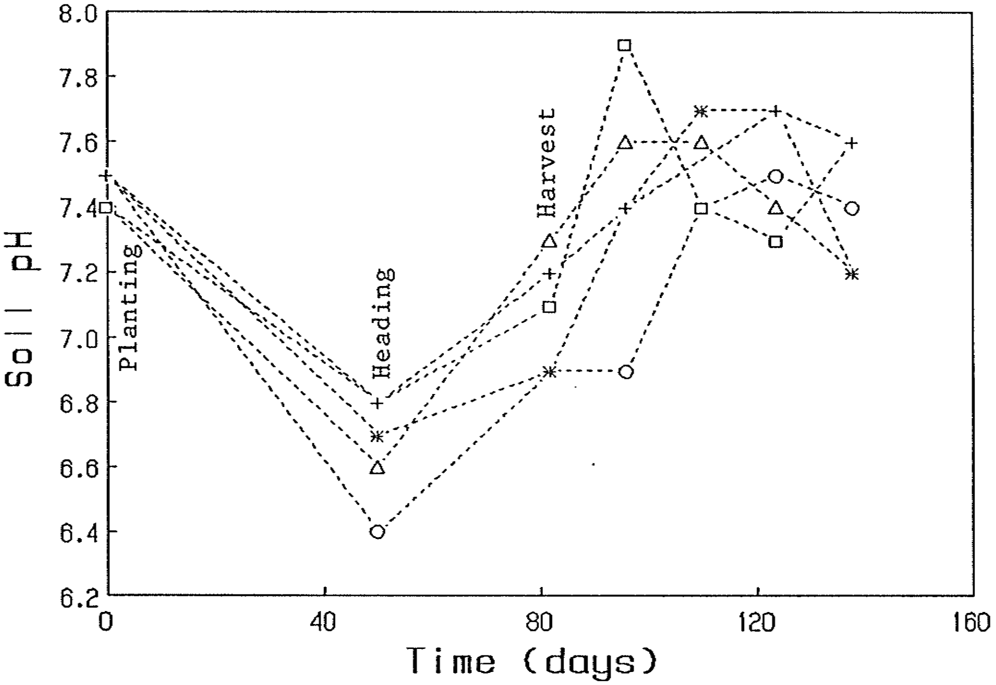

Figure 12. Effect of Time and Copper Source on Soil pH

○---○ control, △---△ 0.25 (Cu-EDTA),
□---□ 0.50 (Cu-EDTA), ✱---✱ 5.0 (Cu-EDTA),
+---+ 5.0 ($CuSO_4 \cdot 5H_2O$)

V. SUMMARY AND CONCLUSIONS

Organic soils in Manitoba generally do not supply adequate plant available copper for normal growth and grain yield of many crops. Cereal crops are often subject to severe copper deficiency when grown on organic soils without the addition of copper fertilizer. Usually copper as $CuSO_4 \cdot 5H_2O$ is applied to alleviate this deficiency. Copper sulphate applied at 5 to 10 Kg Cu ha^{-1} has been shown elsewhere to have good residual effects. Information on the residual effects of $CuSO_4$ applied to organic soils in Manitoba was not available. Also, a means of assessing the copper fertilizer requirements on previously fertilized and non-fertilized soils was not available.

Field studies were conducted in 1987 and 1988 to study the residual effectiveness of various rates of $CuSO_4$ application on organic soils, and to assess the ability of both plant and soil analysis as a means of predicting the need for copper application on these soils. Copper applied as $CuSO_4 \cdot 5H_2O$ at rates of 5 and 10 Kg Cu ha^{-1} increased yields of barley for 3 to 4 yr after initial application, which was the duration of the study. Application of $CuSO_4$ at 5 Kg Cu ha^{-1} to previously fertilized (5 or 10 Kg Cu ha^{-1}) soils resulted in increased grain yields. Yields with 5 Kg Cu ha^{-1} added to soils previously fertilized with copper, were greatly increased in some instances. In other instances yield increases were small and did not justify the cost of copper application.

The relationships between the DTPA-extractable copper concentration of soils and yield, and % yield, were reasonably close

($r = 0.64$, $P = .05$ and $r = 0.58$, $P \leq .01$, respectively). The relationship between concentration of copper in plant tissue at the heading stage and yield, and % yield, were 0.46 ($P = .05$) and 0.60 ($P \leq .01$), respectively. The relationships were sufficiently close that critical levels for DTPA-extractable copper concentration in soils and copper concentration in plant tissue could be established. Critical levels were found to be 5.6 and 16 $\mu g\ g^{-1}$ for plant tissue copper concentration and DTPA-extractable copper concentration in soil, respectively. Both plant tissue analysis and DTPA-extractable copper concentration were judged to be very useful in assessing copper fertilizer requirements. The DTPA-extractable copper soil test would be preferred to plant tissue analysis at the heading stage as a means of predicting the requirement for copper fertilizer applications on organic soils. An assessment of the Cu status of the soil could be made prior to planting.

A growth chamber study using two soils was conducted to augment results obtained from the 1987 and 1988 field studies. Yields of barley grain without added copper were low. A significant increase in grain yield was obtained on both soils when sufficient copper fertilizer was added. The results also showed that soils previously relatively well fertilized with copper would, in some instances, respond to further copper additions. The relationship between % yield and plant tissue copper concentration at the heading stage was somewhat better than the relationship between % yield and DTPA-extractable copper concentration in the soil ($r = 0.75$, $P = .05$ and $r = 0.64$, $P =.05$, respectively). Critical copper levels obtained were 3.8 and 5.2 $\mu g\ g^{-1}$ for plant tissue copper concentration and DTPA-extractable copper concentration, respectively. Critical copper levels obtained in the growth chamber studies for plant tissue copper concentration and DTPA-extractable copper concentration were about 1.5 and 3 times lower than the critical levels obtained from the field studies. This indicated that critical levels obtained in growth chamber studies may not be directly applicable to field conditions.

Although $CuSO_4$ is usually applied to correct copper deficiencies on organic soils, chelated forms of copper are also used. Information on the relative effectiveness of these sources obtained from the literature was contradictory and further studies needed to be conducted to augment information found in the literature. A growth chamber study was conducted to compare the effectiveness of Cu-EDTA and $CuSO_4 \cdot 5H_2O$ in increasing barley grain yield at equal rates of application, as well as at low rates of copper applied in the chelated form. Plant copper content at the heading stage was significantly increased only when 5 Kg Cu ha^{-1} was added either as $CuSO_4$ or as Cu-EDTA. Plant copper uptake was significantly increased only when 5 Kg Cu ha^{-1} was added as the chelate form. Barley grain yield did not differ significantly among the rates or forms of copper added, however quality of grain produced with $CuSO_4$ appeared to be superior to that produced with the chelated form of copper.

A field study was conducted in 1988 to evaluate the effects of copper source, band size and placement method on the growth of barley. Grain yield of barley was significantly increased above that of the control by all treatments except when $CuSO_4$ was added in a narrow band or in a 2.5 cm x 2.0 cm band (wide band - mixed). Copper sulphate applied in a narrow band likely resulted in poor root-copper fertilizer contact, whereas soil-copper complexation reactions likely occurred when $CuSO_4$ was applied in a wide band and mixed with the soil. Yield with Cu-EDTA did not differ significantly with placement method and yields were significantly higher than that of the control treatment. Plant uptake of copper from both copper sources was similar. Placement of $CuSO_4$ in wide bands increased the positional availability of copper from $CuSO_4$, facilitating plant copper uptake. Narrow band placement of $CuSO_4$ resulted in poor plant root-copper contact and limited plant copper uptake. Due to the relatively greater mobility and solubility of Cu-EDTA as compared to $CuSO_4$ in soil, placement method did not greatly affect plant copper availability from this source. On average, wide band treatments were better able to increase barley grain yield and were the most effective treatments in increasing plant copper uptake. At equivalent rates of application, Cu-EDTA appeared to provide greater plant available copper early in the growing season. At the later stages of plant growth both $CuSO_4$ and Cu-EDTA appeared to be equally effective in increasing plant copper content and yield.

Variations in soil pH and DTPA-extractable copper concentration with plant growth were noted in the 1987 field studies. A growth chamber study was conducted using two organic soils to examine the relationships between plant growth, soil pH and DTPA-extractable soil copper. In both soils, a decline in soil pH occurred with plant growth and rhizosphere development. An increase in DTPA-extractable soil copper concentration occurred concomitant with plant rhizosphere development and declining soil pH levels. DTPA-extractable copper concentration increased from time of plant emergence to heading, declined during crop maturation from the heading stage to final harvest, and at time of harvest approached levels found prior to seeding.

The increase in DTPA-extractable copper concentration was likely related to crop rhizosphere development and the concurrent decline in soil pH level. The implications of the results obtained were that the time of sampling of soils needed to be considered in assessing plant available soil copper using the DTPA-extractable copper method. Early spring or late fall were judged to be suitable periods of time for assessing plant available soil copper, and the need for copper application on organic soils.

A growth chamber environment was used to study the DTPA-extractability of copper applied as $CuSO_4$ and Cu-EDTA. DTPA-extractable copper concentrations for soils treated with 0.25 and 0.50 Kg Cu ha^{-1} as Cu-EDTA were similar to that without added copper. The DTPA-extractable copper concentration obtained when 5 Kg Cu ha^{-1} was added as Cu-EDTA was much less than when 5 Kg Cu ha^{-1} as $CuSO_4$ was added. DTPA-extractable copper concentrations were 10 and 5.3 μg Cu g^{-1} soil for the $CuSO_4$ and Cu-EDTA treatments, respectively, after 138 d. It was postulated that copper from Cu-EDTA may have been more subject to soil-copper complexation reactions than copper from $CuSO_4$ when competitive ions displaced copper from the EDTA molecule or when the EDTA molecule was degraded. Due to the greater mobility of copper in soil as Cu-EDTA than that of copper from $CuSO_4$, copper from Cu-EDTA would contact a relatively greater amount of soil than copper from $CuSO_4$

shortly after fertilizer application. Thus, once the copper in the Cu-EDTA molecule was no longer complexed, it may have been more subject to soil-copper complexation reactions than copper from $CuSO_4$.

The studies reported in this manuscript showed that annual applications of copper fertilizer were required to maximize yield despite the very good residual effects of previously added $CuSO_4$. Since annual applications are likely required, and copper fertilizers are costly, it is essential that further research be conducted on increasing the efficiency of copper fertilizers. Additionally, further research on the fate and bioavailability of copper from $CuSO_4$ and chelated copper with time is required to clarify some of the observations noted for these copper fertilizer sources in this manuscript.

APPENDIX

Table 1a. Seeding and Harvest Dates (1987)

Site No.	Seeding Date	Heading Harvest	Final Harvest
1	May 18	July 8[+]	August 18
2	May 15	July 8	August 8
3	May 21	July 15	August 18
4	May 13	July 2	August 7
5	May 27	July 15	August 18
6	May 27	July 15/July 24[6]	August 18
7	May 21	July 16	August 25

[+] control (unfertilized) treatment did not survive to heading stage

[6] control treatment sampling date.

Table 1b. Seeding and Harvest Dates (1988)

Site No.	Seeding Date	Heading Harvest	Final Harvest
1	May 17	July 16	August 17
2	June 2	July 19	August 19
3	June 2	July 19	August 19
4	May 26	July 11	August 12
5	May 18	July 14	August 18
6	May 18	July 12	August 18
7	May 23	July 15	August 16

Table 1c. Effect of Copper Fertilization on the Iron, Manganese and Zinc Concentration of Barley at the Heading Stage (1987) ($\mu g\ g^{-1}$)

	Initial Application Rate* (Kg Cu ha^{-1})																	
	0						5						10					
	New Application Rate (Kg Cu ha^{-1})																	
	0			5			0			5			0			5		
Site No	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn
1	——	——	——	74.6	30.7	27.6	--	--‡	--	--	--	--	115.9	16.6	21.4	165.3	17.3	25.6
2	169.6	24.5	12.0	134.1	11.7	8.2	196.3	23.0	12.1	152.7	17.3	13.4	144.4	21.2	12.0	147.4	12.9	9.2
3	73.8	84.4	24.9	47.7	55.4	20.4	--	--	--	--	--	--	47.3	54.5	17.0	78.3	63.1	23.1
4	77.8	22.0	20.2	61.3	22.3	19.6§	64.4	24.1	27.6	59.9	22.5	23.1	60.1	22.4	27.9	34.8	24.6	30.4
5	100.9	14.8	19.1	77.4	9.6	18.9	--	--	--	--	--	--	44.6	12.0	16.2	45.6	9.2	19.6
6	87.4	8.4	19.7	--	--	--	--	--	--	--	--	--	64.3	16.6	27.6	--	--	--
7	88.1	33.6	26.3	81.5	23.6	27.5	74.8	33.6	27.5	77.0	31.8	27.3	65.6	33.4	27.4	81.0	28.3	31.2

* Date of Initial Application - Sites 1, 3 and 5: Spring 1985
Sites 2, 4 and 7: Spring 1986
Site 6: Spring 1984.

-- Treatment not included in experiment.

§ Application rate in 1987 20 Kg Cu ha^{-1}.

Table 1d. Effect of Copper Fertilization on the Iron, Manganese and Zinc Concentration of Barley at the Heading Stage (1988) ($\mu g\ g^{-1}$)

	Initial Application Rate* (Kg Cu ha^{-1})																	
	0						5						10					
	New Application Rate (Kg Cu ha^{-1})																	
	0			5			0			5			0			5		
Site No	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn
1	96.2	36.5	23.3	90.3	26.1	31.1	--	--	--	--	--	--	152.7	20.7	25.9	111.0	15.5	26.9
2	107.3	36.3	13.3	103.8	20.4	11.3	102.3	42.8	18.8	98.8	22.5	12.8	74.1	27.8	12.4	101.0	21.9	13.3
3	99.9	56.3	28.5	61.6	44.1	28.8	--	--	--	--	--	--	55.5	30.9	22.3	63.0	26.3	28.0
4	89.7	28.4	27.1	66.5	25.5	31.3§	78.9	31.6	30.3	71.3	41.0	31.0	71.7	30.4	28.6	70.0	49.1	30.7
5	123.4	16.1	16.1	99.0	12.3	25.0	--	--	--	--	--	--	90.0	11.9	24.8	80.2	10.2	24.9
6	92.8	25.3	31.3	--	--	--	--	--	--	--	--	--	74.4	17.8	29.3	--	--	--
7	115.9	20.0	22.6	76.8	18.1	24.1	71.6	24.4	23.6	76.4	21.3	22.5	81.5	25.7	21.5	55.9	24.6	22.2

* Date of Initial Application - Sites 1, 3 and 5: Spring 1985
Sites 2, 4 and 7: Spring 1986
Site 6: Spring 1984.

-- Treatment not included in experiment.

§ Application rate in 1987 20 Kg Cu ha^{-1}.

LITERATURE CITED

Akinyede, F. A. 1977. Effect of rate and method of placement of $CuSO_4$ on dry matter yield and nutrient uptake of barley (*Hordeum vulgare L.* var. Conquest). M.Sc. thesis, Dept. Soil Sci., Univ. of Manitoba, Winnipeg, Manitoba.

Allison, L. E. 1965. Organic carbon. In: *Methods of Soil Analysis: Chemical and Microbiological Properties*. C. A. Evans, J. C. White, L. E. Enominger and F. E. Clark (Eds.), pp. 1367-1378. American Society of Agronomy, Spec. Pub. No. 9. Madison, Wisconsin.

Ardakani, M. S. and F. J. Stevenson. 1972. A modified ion-exchange technique for the determination of stability constants of metal organic matter complexes. Soil Sci. Amer. Proc. 36: 884-890.

Barber, S. A. 1980. Soil-plant interactions in the phosphorous nutrition of plants. In: *The Role of Phosphorus in Agriculture*. E. C. Sample and E. J. Kamprath (Eds.), pp. 591-615. American Society of Agronomy, Madison, Wisconsin.

Barnes, J. S. and F. R. Cox. 1973. Effects of copper sources on wheat and soybeans grown on organic soils. Agron. J. 65: 705-708.

Barrow, N. J. and N. A. Campbell. 1972. Methods of measuring residual value of fertilizers. Aust. J. Exp. Agric. Anim. Husb. 12: 502-510.

Bates, T. E. 1971. Factors affecting critical nutrient concentrations in plants and their evaluation: a review. Soil Sci. 112: 116-130.

Blevins, R. L. and H. F. Massey. 1958. Evaluation of two methods of measuring available soil copper and the effects of soil pH and extractable aluminum on copper uptake by plants. Soil Sci. Soc. Amer. Proc. 23: 296-298.

Bloom, P. R. and M. B. McBride. 1979. Metal ion binding and exchange with hydrogen ions in acid washed peat. Soil Sci. Soc. Am. J. 43: 687-692.

Boischot, P. and P. Quillion. 1952. Fixation of copper in calcareous soils. Ann. Agron. 3: 350-363.

Boyd, S. A., L. E. Sommers and D. W. Nelson. 1981. The mechanism of copper (II) binding by humic acid: An electron spin resonance study of a copper (II) - humic acid complex and some adducts with nitrogen donors. Soil Sci. Soc. Amer. J. 45: 745-749.

Bray, R. H. 1948. Requirements of successful soil testing. Soil Sci. 66: 83-89.

Brennan, R. F., J. W. Gartrell, and A. D. Robson. 1980. Reactions of copper with soil affecting its availability to plants. I. Effect of soil type with time. Aust. J. Soil Res. 18: 447-459.

Brennan, R. F., J. W. Gartrell, and A. D. Robson. 1983. Reactions of copper with soil affecting its availability to plants. II. Effects of soil pH, soil sterilization and organic matter on the availability of applied copper. Aust. J. Soil Res. 21: 155-163.

Brennan, R. F., J. W. Gartrell and A. D. Robson. 1986a. The decline in availability to plants of applied copper fertilizer. Aust. J. Agric. Res. 37: 107-113.

Brennan, R. F., J. W. Gartrell and A. D. Robson. 1986b. The effect of successive crops of wheat on the availability of copper fertilizers to plants. Aust. J. Agric. Res. 37: 115-124.

Caldwell, T. H. 1971. Copper deficiency in crops. I. Review of past work. In: *Trace Elements in Soils and Crops*. pp. 62-72. Tech. Bull. 21, Min. of Agric., Fisheries and Food, U.K.

Caldwell, T. H. 1976. In: *Trace Elements in Soils and Crops*. p. 73. Tech. Bull. 21, Min. of Agric., Fisheries and Food, U.K.

Chaberek, S. and A. E. Martell. 1959. Organic sequestering agents. p. 616. John Wiley & Sons, Inc., New York.

Chaudry, F. M. and J. F. Loneragan. 1970. Effect of nitrogen, copper and zinc fertilizers on the copper and zinc nutrition of wheat plants. Aust. J. Agric. Res. 21: 865-879.

Cox, F. R. and E. J. Kamprath. 1972. Micronutrient soil tests. In: *Micronutrients in Agriculture*. J. J. Mortvedt, P. M. Giordano and W. L. Lindsay (Eds.), pp. 289-317. Soil Sci. Soc. Amer., Madison, Wisconsin.

Davies, R. I., M. V. Cheshire and J. Graham-Bryce. 1969. Retention of low levels of copper by humic acid. J. Soil Sci. 20: 65-71.

DeMumbrum, L. E. and M. L. Jackson. 1965. Copper and zinc exchange from dilute neutral solutions by soil colloidal electrolytes. Soil Sci. 81: 353-357.

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

Elgawhary, S. M., W. L. Lindsay and W. D. Kemper. 1970. Effect of complexing agents and acids on the diffusion of zinc to a simulated root. Soil Sci. Soc. Amer. Proc. 34: 211-214.

Ennis, M. T. and J. C. Brogan. 1966. The availability of Cu from copper-humic acid complexes. Irish J. Agric. Res. 1: 35-42.

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

Gamble, D. S. 1970. Titration curves of fulvic acid: the analytical chemistry of a weak acid polyelectrolyte. Can. J. Chem. 48: 2662-2669.

Gamble, D. S., M. Schnitzer and I. Hoffman. 1970. Cu^{2+} - fulvic acid chelation equilibrium in 0.1 M KCl at 25°C. Can. J. Chem. 48: 3197-3204.

Gartrell, J. W. 1980. Residual effectiveness of copper fertilizer for wheat in Western Australia. Aust. J. Exp. Anim. Husb. 20: 370-376.

Gartrell, J. W. 1981. Distribution and correction of copper deficiency in crops and pastures. In: *Copper in Soils and Plants*. J. F. Loneragan, A. D. Robson and R. D. Graham (Eds.), pp. 313-349. Academic Press: Sydney.

Gartrell, J. W. and R. F. Brennan. 1979. Copper deficiency in wheat. West. Aust. Dep. Agric. Bull. Agdex 112/632, South Perth, West. Aust.

Geering, H. R. and J. F. Hodgson. 1969. Micronutrient cation complexes in soil solution: III. Characterization of soil solution ligands and their complexes with Zn^{2+} and Cu^{2+}. Soil Sci. Soc. Amer. Proc. 33: 54-59.

Gilkes, R. J. 1981. Behaviour of Cu additives-fertilizer. In: *Copper in Soils and Plants*. J. F. Loneragan, A. D. Robson and R. D. Graham (Eds.), pp. 97-117. Academic Press: Sydney, Australia.

Gilkes, R. J. and S. B. Sadleir. 1978. The availability to wheat of copper from various sources including copper super phosphate. Aust. J. Soil Res. 16: 113-120.

Gilkes, R. J. and S. B. Sadleir. 1979. The influence of placement geometry on the effectiveness of copper superphosphate. Aust. J. Soil Res. 17: 1-8.

Gilkes, R. J., R. C. Young and J. P. Quirk. 1975. Leaching of copper and zinc from trace element superphosphate. Aust. J. Soil Res. 13: 33-41.

Gladstones, J. S., J. F. Loneragan and W. J. Simmons. 1975. Mineral elements in temperature crop and pasture plants. III. Copper. Aust. J. Agric. Res. 26: 113-126.

Goodman, B. A. and M. V. Cheshire. 1973. Electron paramagnetic resonance evidence that copper is complexed in humic acid by the nitrogen of porphyrin groups. Nature, New Biol. 244: 158-159.

Goodman, B. A. and M. V. Cheshire. 1976. The occurrence of copper-porphyrin complexes in soil humic acids. J. Soil Sci. 27: 337-347.

Gosh, K. and M. Schnitzer. 1981. Fluorescence excitation spectra and viscosity behavior of a fulvic acid and its copper and iron complexes. Soil Sci. Amer. J. 45: 25-29.

Graham, R. D. and Nambiar, E. K. S. 1981. Advances in research on copper deficiency in cereals. Aust. J. Agric. Res. 32: 1009-1037.

Gupta, V. C. 1979. I. Ecological cycling. In: *Copper in the Environment*. J. O. Nriagu (Ed.), p. 255. John Wiley and Sons, Inc., New York.

Gupta, V. C. and L. B. McLeod. 1970. Response to copper and optimum levels in wheat, barley and oats under greenhouse and field conditions. Can. J. Soil Sci. 50: 373-378.

Guy, R. O., C. L. Chakrabarti and L. L. Schramm. 1975. The application of a simple chemical model of natural waters to metal fixation in particulate matter. Can. J. Chem. 53: 661-669.

Haq, A. U. and M. H. Miller. 1972. Predictions of available soil Zn, Cu and Mn using chemical extractants. Agron. J. 64: 779-782.

Harper, H. J. 1924. The accurate determination of nitrate in soils. Ind. Eng. Chem. 16: 180-183.

Harry, S. P. and R. D. Graham. 1981. Tolerance of triticale, wheat and rye to copper deficiency and to low and high pH. J. Plant Nutr. 3: 721-730.

Hedley, M. J., P. H. Nye and R. E. White. 1982. Plant induced changes of rape (*Brassica napus* var. Emerald) seedlings. II. Origin of the pH change. New Phytol. 91: 31-44.

Hill, J., A. D. Robson and J. F. Loneragan. 1978. The effect of copper and nitrogen supply on the retranslocation of copper in four cultivars of wheat. Aust. J. Agric. Res. 29: 925-939.

Hodgson, J. F., H. R. Geering and W. A. Norvell. 1966. Micronutrient cation complexes in soil solution: partition between complexed and uncomplexed forms by solvent extraction. Soil Sci. Soc. Amer. Proc. 29: 665-669.

Isaac, R. A. and J. D. Kerber. 1971. Atomic absorption and flame photometry. Techniques and uses in soil, plant and water analysis. In: *Instrumental Methods for Analysis of Soils and Plant Tissues*. L. M. Walsh (Ed.), pp. 17-37. Soil Sci. Soc. Amer., Madison, Wisconsin.

James, R. O. and N. J. Barrow. 1981. Copper reactions with inorganic components of soils including uptake by oxide and silicate minerals. In: *Copper in Soils and Plants*. J. F. Loneragan, A. D. Robson and R. D. Graham (Eds.), pp. 47-68. Academic Press: Sydney.

Jarvis, S. C. 1981. Copper sorption by soils at low concentrations and relation to uptake by plants. J. Soil Sci. 32: 257-270.

Jarvis, S. C. and D. C. Whitehead. 1981. The influence of some soil and plant factors on the concentration of copper in perennial rye grass. Plant and Soil 23: 275-286.

Jeffery, J. J. and N. C. Uren. 1983. Copper and zinc species in the soil solution and the effects of soil pH. Aust. J. Soil Res. 21: 479-488.

Jones, J. B. Jr. 1972. Plant tissue analysis for micronutrients. In: *Micronutrients in Agriculture*. J. J. Mortvedt, P. M. Giordano and W. L. Lindsay (Eds.), pp. 319-346. Soil Sci. Soc. Amer., Madison, Wisconsin.

Karamanos, R. E. 1986. Copper - role, deficiency and practical solutions. Proc. Fertilizer Forum, Mornville, Alberta. Nov. 21, 1986. Agricultural Sciences and Technology, Agricultural Chemical Technology Division. Esso Chemical Canada, Lethbridge, Alberta, pp. 1-33.

Karamanos, R. E., G. A. Kruger and J. W. B. Stewart. 1986. Copper deficiency in cereal and oilseed crops in Northern Canadian Prairie soils. Agron. J. 78: 317-323.

Kerven, G. L., D. G. Edwards and C. J. Asher. 1982. The determination of active ionic copper concentrations and copper complexation in peat soil extracts. Soil Sci. 137(2): 91-98.

Kiekens, L. and A. Cottenie. 1981. Behavior of copper in soils: adsorption and complexation reactions. In: *Copper in Animal Wastes and Sewage Sludge*. Proc. EEC Workshop. P. L'Hermite and J. Dehandtschulter (Eds.), pp. 85-101. Bordeaux, France, D. Reidel Publ. Co. Dordrecht, Holland.

King, P. M. and A. M. Alston. 1975. Diagnosis of trace element deficiencies in wheat on Eyre Peninsula, South Australia. In: *Trace Elements in Soil-Plant-Animal Systems*. D. J. D. Nicholas and A. R. Egan (Eds.), pp. 339-352. Academic Press: New York.

Kline, J. R. and R. H. Rust. 1966. Fractionation of copper in neutron activated soils. Soil Sci. Soc. Amer. Proc. 30: 188-192.

Kruger, G. A., R. E. Karamanos and J. P. Singh. 1985. The copper fertility of Saskatchewan soils. Can. J. Soil Sci. 65: 89-99.

Lazrus, A. J., K. C. Hill and J. P. Lodge. 1965. A new colorimetric microdetermination of sulfate ion. Automation in analytical chemistry. Technicon Symposium, Mediad. pp. 291-293.

Lindsay, W. L. 1979. Chemical equilibria in soils. John Wiley and Sons, Inc., New York.

Lindsay, W. L. and W. A. Norvell. 1967. A new soil test for the simultaneous determination of available zinc and iron. Meeting of the Western Soil Sci. Soc., Los Angeles, California, June 1967. Cited In: Lindsay, W. L. and W. A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sci. Soc. Amer. J. 42: 421-428.

Lindsay, W. L. and W. A. Norvell. 1969a. Equilibrium relationships of Zn^{2+}, Fe^{+3}, Ca^{+2} and H^{+} with EDTA and DTPA in soils. Soil Sci. Soc. Amer. Proc. 33: 62-68.

Lindsay, W. L. and W. A. Norvell. 1969b. Reactions of EDTA Complexes of Fe, Zn, Mn and Cu with soils. Soil Sci. Soc. Amer. Proc. 33: 86-91.

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

Linehan, D. J., A. H. Sinclair and M. C. Mitchell. 1985. Mobilisation of Cu, Mn and Zn in soil solutions of barley rhizospheres. Plant and Soil 86: 147-149.

Loneragan, J. F. 1975. The availability and absorption of trace elements in soil-plant systems and their relation to movement and concentrations of trace elements in plants. In: *Trace Elements in Soil-Plant-Animal Systems*. D. J. D. Nicholas and A. R. Egan (Eds.), pp. 109-134. Academic Press, New York.

Loneragan, J. F., A. D. Robson and R. D. Graham. (Eds.) 1981. Copper in Soils and Plants. Academic Press: Sydney, Australia.

Loneragan, J. F., K. Snowball and A. D. Robson. 1976. Remobilization of nutrients and its significance in plant nutrition. In: *Transport and Transfer Processes in Plants*. J. F. Wardlaw and J. B. Passioura (Eds.), pp. 463-469. Academic Press: New York.

Loneragan, J. F., K. Snowball and A. D. Robson. 1980. Copper supply in relation to content and redistribution of copper among organs of the wheat plant. Ann. Bot. 45: 621-632.

Louw, H. A. and D. M. Webley. 1959. The bacteriology of the root region of the oat plant grown under controlled pot culture conditions. J. Appl. Bacteriol. 22: 216-226.

Lucas, R. E. and J. F. Davis. 1961. Relationship between pH values of organic soils and the availability of 12 plant nutrients. Soil Sci. 92: 177-182.

Lucas, R. E. and B. D. Knezek. 1972. Climatic and soil conditions promoting micronutrient deficiencies in plants. In: *Micronutrients in Agriculture*. J. J. Mortvedt, P. M. Giordano and W. L. Lindsay (Eds.), pp. 265-288. Soil Sci. Soc. Amer., Madison, Wisconsin

McAndrew, D. W. 1979. Copper and zinc nutrition of cereal and oilseed crops in Manitoba. M.Sc. thesis, Dept. Soil Sci., Univ. of Manitoba, Winnipeg, Manitoba.

McBride, M. B. 1978. Transition metal bonding in humic acid. An ESR Study. Soil Sci. 126: 200-209.

McBride, M. B. 1981. Forms and distribution of copper in solid and solution phases of soil. <u>In</u>: *Copper in Soils and Plants.* J. F. Loneragan, A. D. Robson and R. D. Graham (Eds.)., pp. 25-45. Academic Press: New York.

McBride, M. B. and J. J. Blasiak. 1979. Zinc and copper solubility as a function of pH in an acid soil. Soil Sci. Soc. Amer. J. 43: 866-870.

McGregor, W. R. 1972. A study of copper and zinc status of some Manitoba soils. M.Sc. thesis, Dept. Soil Sci., University of Manitoba, Winnipeg, Manitoba.

McLaren, R. G. and D. V. Crawford. 1973a. Studies on soil copper. I. The fractionation of copper in soils. J. Soil Sci. 24: 172-181.

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

McLaren, R. G. and D. V. Crawford. 1974. Studies on soil copper. III. Isotopically exchangeable copper in soils. J. Soil Sci. 25: 111-119.

McLaren, R. G., Swift, R. S. and J. G. Williams. 1981. The adsorption of copper by soil materials at low equilibrium solution concentrations. J. Soil Sci. 32: 247-256.

McLaren, R. B., J. G. Williams and R. S. Swift. 1983. Some observations on the desorption and distribution behavior of copper with soil components. J. Soil Sci. 34: 325-331.

Melstead, S. W., Motto, H. L. and T. R. Peck. 1969. Critical plant nutrient composition values useful in interpreting plant analysis data. Agron. J. 61: 17-20.

Mengel, K. and E. A. Kirkby. 1979. Principles of Plant Nutrition. International Potash Inst., Switzerland.

Mortvedt, J. J. 1977. Micronutrient soil test correlations and interpretations. <u>In</u>: *Soil Testing: Correlating and Interpreting the Analytical Results.* T. R. Peck (Ed.), pp. 99-117. American Society of Agronomy Spec. Pub. No. 29. Madison, Wisconsin.

Murphy, J. and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analy. Chem. Acta. 27: 31-36.

Murphy, L. S. and L. M. Walsh. 1972. In: *Micronutrients in Agriculture*. J. J. Mortvedt, P. M. Giordano and W. L. Lindsay (Eds.), pp. 347-387. Soil Sci. Soc. Amer., Madison, Wisconsin.

Nambiar, E. K. S. 1976a. Genetic differences in the copper nutrition of cereals. I. Differential responses of genotypes to copper. Aust. J. Agric. Res. 27: 453-463.

Nambiar, E. K. S. 1976b. Genetic differences in the copper nutrition of cereals. II. Genotypic differences in response to copper in relation to copper, nitrogen and other mineral contents of plants. Aust. J. Agric. Res. 27: 465-477.

Nambiar, E. K. S. 1977. The effects of drying of the topsoil and of micronutrients in the subsoil on micronutrient uptake by an intermittently defoliated rye grass. Plant and Soil 46: 185-143.

Nelson, L. G., K. C. Berger and H. J. Andries. 1956. Copper requirements and deficiency symptoms of a number of field and vegetable crops. Soil Sci. Soc. Amer. Proc. 20: 69-72.

Nielsen, N. E. 1976a. A transport kinetic concept of ion uptake from soil by plants. III. Test of the concept by results from water culture and pot experiment. Plant and Soil 45: 659-677.

Nielsen, N. E. 1976b. The effect of plants on the copper concentration in the soil solution. Plant and Soil 45: 679-687.

Norvell, W. A. and W. L. Lindsay. 1972. Reactions of DTPA chelates of iron, zinc, copper and manganese with soils. Soil Sci. Soc. Amer. Proc. 36: 778-783.

Nriagu, J. O. 1979. In: *Copper in the Environment. I.* Ecological Cycling. J. O. Nriagu (Ed.). John Wiley & Sons, Inc., New York.

Oliver, S. and S. A. Barber. 1966. Mechanisms for the movement of Mn, Fe, B, Cu, Zn, Al and Sr from one soil to the surface of soybean roots (*Glycine max.*). Soil Sci. Soc. Amer. Proc. 30: 468-470.

Olsen, S. R., C. W. Cole, F. S. Watanabe and L. A. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U. S. Dept. Agric. Arc. 939.

Osiname, O. H., E. E. Schulte and R. B. Corey. 1973. Soil tests for available copper and zinc in soils of western Nigeria. J. Sci. Food Agric. 24: 1341-1349.

Petruzzelli, G. and G. Guidi. 1976. Influence of soil organic matter on copper availability to plants. Z. Pflanzenernaehr. Bodenkd. Heft. 6: 679-684.

Piper, C. S. 1942. Soil and plant analysis. Hassel Press, Adelaide, Australia. p. 219.

Pizer, N. H., T. H. Caldwell, G. R. Burgess and J. L. O. Jones. 1966. Investigations into copper deficiency in crops in East Anglia. J. Agric. Sci. 66: 304-314.

Posner, A. M. 1964. Titration curves of humic acid. Int. Congr. Soil Sci., Lyons. 8th II. 17: 161-174.

Preston, C. M., S. P. Mathur and B. S. Rauthan. 1980. The distribution of copper, amino compounds and humus fractions in organic soils of different copper content. Soil Sci. 131: 344-352.

Reid, J. M. 1982. Availability of manganese and effects of soil temperature on availability of manganese to plants grown on organic soils. M.Sc. thesis, Dept. Soil Sci., University of Manitoba, Winnipeg, Manitoba.

Reith, J. W. S. 1968. Copper deficiency in crops in northeast Scotland. J. Agric. Res. 70: 39-45.

Reuter, D. J. 1975. The recognition and correction of trace element deficiencies. In: *Trace Elements in Soil-Plant-Animal Systems*. D. J. D. Nicholas and A. R. Egan (Eds.), pp. 291-324. Academic Press: New York.

Reuther, W. and C. K. Labanauskas. 1966. Copper in diagnostic criteria for plants and soils. H. D. Chapman (Ed.), pp. 157-179. Univ. of California, Division of Agricultural Sciences.

Robson, A. D. and D. J. Reuter. 1981. Diagnosis of copper deficiency and toxicity. In: *Copper in Soils and Plants*. J. F. Loneragan, A. D. Robson and R. D. Graham (Eds.), pp. 287-312. Academic Press: Sydney.

Rovatt, J. W. and H. Katznelson. 1961. A study of the bacteria on the root surface and in the rhizosphere soil of cropped plants. J. Appl. Bacteriol. 24: 164-171.

Rovira, A. D. and C. B. Davey. 1974. Biology of the rhizosphere. In: *The Plant Root and Its Environment*. E. W. Carson (Ed.), pp. 153-191. University Press of Virginia, Charlottsville.

Sanders, J. R. 1982. The effect of pH upon the copper and cupric ion concentrations in soil solutions. J. Soil Sci. 33: 679-689.

Sanders, J. R. 1983. The effect of pH on the total and free ionic concentrations of manganese, zinc and cobalt in soil solutions. J. Soil Sci. 34: 315-323.

Sanders, J. R. and C. Bloomfield. 1980. The influence of pH, ionic strength and reactant concentrations on copper complexing by humified organic matter. J. Soil Sci. 31: 53-63.

Schnitzer, M. and E. H. Hansen. 1970. Organo-metallic interactions in soils: 8. An evaluation of methods for the determination of stability constants for metal-fulvic acid complexes. Soil Sci. 109: 333-340.

Schnitzer, M. and S. U. Khan. 1972. Humic substances in the environment. Mercel Dekker Inc., New York, New York.

Schuman, G. E., M. A. Stanley and D. Knudson. 1973. Automated total nitrogen analysis of soil and plant samples. Soil Sci. Soc. Amer. Proc. 37: 480-481.

Sequi, P., G. Guidi and O. G. Petruzzelli. 1975. Influence of metals on solubility of soil organic matter. Geoderma 13: 153-161.

Singh, R. R. and B. Prasad. 1986. Selection of suitable extractant for predicting the response of barley (*Hordeum vulgare L.*) to copper application in calcareous soils. Plant and Soil 23: 211-222.

Smiley, R. W. 1974. Rhizosphere pH as influenced by plants, soils and nitrogen fertilizers. Soil Sci. Soc. Amer. Proc. 38: 795-799.

Smith, P. F., G. K. Rasmussen and G. Hrnciar. 1962. Leaching studies with metal sulphates in light sandy citrus soils in Florida. Soil Sci. 94: 235-238.

Snowball, K. and A. D. Robson. 1984. Comparison of the internal and external requirements of wheat, oats and barley for copper. Aust. J. Agric. Res. 35: 359-365.

Soltanpour, P. N., A. Khan and W. L. Lindsay. 1976. Factors affecting DTPA extractable Zn, Fe, Mn and Cu from soils. Comm. in Soil Sci. and Plant An. 7: 791-821.

Soltanpour, P. N., A. Khan and A. S. Shawb. 1979. Effect of grinding variables on the NH_4HCO_3-DTPA soil test for Fe, Zn, Mn, Cu, P and K. Comm. in Soil Sci. and Plant An. 10: 903-909.

Soon, Y. K. and M. H. Miller. 1977. Changes in rhizosphere due to NH_4^+ and NO_3^- fertilization and phosphorus uptake by corn seedlings (*Zea mays L.*) Soil Sci. Soc. Amer. J. 41: 77-80.

Steenbjerg, F. 1951. Yield curves and chemical plant analysis. Plant Soil 3: 97-109.

Stevenson, F. J. 1982. Humus Chemistry, Genesis Composition, Reactions. John Wiley, New York.

Stevenson, F. J. and M. S. Ardakani. 1972. Organic matter reactions involving micronutrients in soils. In: *Micronutrients in Agriculture*. J. J. Mortvedt, P. M. Giordano and W. L. Lindsay (Eds.), pp. 79-114. Soil Sci. Soc. Amer., Madison, Wisconsin.

Stevenson, F. J. and A. Fitch. 1981. Reactions with soil organic matter. In: *Copper in Soils and Plants*. J. F. Loneragan, A. D. Robson and R. D. Graham (Eds.), pp. 69-95. Academic Press: Sydney.

Tokarchuk, J. M. 1982. Availability of copper to plants and methods of evaluating plant available copper in organic soils. M.Sc. thesis, Dept. Soil Sci., Univ. of Manitoba, Winnipeg, Manitoba.

Varvel, G. E. 1983. Effect of banded and broadcast placement of Cu fertilizers on correction of Cu deficiency. Agron. J. 75: 99-101.

Varvel, G. E. 1984. A copper-phosphorus interaction in wheat. J. Plant Nutr. 7(11): 1623-1634.

Vedy, J. C. and S. Bruckert. 1979. Soil solution: Composition and pedogenic significance. In: *Constituents and Properties of Soils*. M. Bonneau and B. Souchier (Eds.), pp. 184-213. Academic Press, London.

Viets, F. J. Jr., and W. L. Lindsay. 1973. Testing soils for zinc, copper, manganese and iron. In: *Soil Testing and Plant Analysis*. L. M. Walsh and J. D. Beaton (Eds.), pp. 153-172. Soil Sci. Soc. Amer., Madison, Wisconsin.

Wallace, A. and H. Heimadin. 1962. Use of EDDHA as a means of testing soils for available iron. In: *A Decade of Synthetic Chelating Agents in Inorganic Plant Nutrition*. A. Wallace (Ed.), pp. 131-134. Edward Brothers Inc., Ann Arbor, Michigan.

Ward, R. C., D. A. Whitney and D. G. Westfall. 1973. Plant analysis as an aid to fertilizing small grains. In: *Soil Testing and Plant Analysis*. L. M. Walsh and J. D. Beaton (Eds.), pp. 329-348. Soil Sci. Soc. Amer., Madison, Wisconsin.

Whitney, D. A. 1975. Micronutrient tests. North Dakota Agricultural Experimental Station. Bull. 499: 22-23.

Younts, S. E. 1964. Response of wheat to rates, dates of application, and sources of copper and to other micronutrients. Agron. J. 56: 266-269.