COPPER FERTILIZATION OF WHEAT AND METHODS EVALUATING AVAILABILITY OF COPPER TO WHEAT GROWING ON MINERAL SOILS

by

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ABSTRACT

Two growth chamber and two field experiments were conducted to study the effect of copper fertilization on yield and chemical composition of wheat grown on various mineral soils from Manitoba. The studies included an evaluation of five chemical extractants for soil copper as means of assessing plant available soil copper.

In one field study conducted at two sites, application of 15 kg Cu/ha significantly increased the copper concentration of wheat harvested at the boot stage but did not influence dry matter yields. Copper concentration of wheat harvested at the boot stage of wheat from Haywood was lower than some reported critical levels for Cu in wheat. Grain yield was not influenced by application of 15 kg Cu/ha. Copper concentration of grain was above the critical levels reported in literature and soil copper at these two sites was thought to be adequate.

The effect of copper fertilization on yield and chemical composition of wheat growing on ten mineral soils under controlled environmental conditions was studied. Grain yield was increased significantly on six out of ten soils by application of 38 mg Cu/pot containing 5 kg of soil. Copper concentrations in wheat harvested at the boot stage were increased to adequate levels on all soils except the Treherne soil. One molar HCl extractable copper was found to be best related to percent grain yield of wheat when soils were sampled before seeding. For soil samples taken at crop maturity, 1.0 M HCl and DTPA extractable

copper were found to be almost equally well related to percent grain yield of wheat. A critical level of 2.0 ppm was delineated for the 1.0 M HCl extractable soil copper.

The second field experiment was set up to investigate the effect of copper and phosphorus fertilization on yield and chemical composition of wheat at Haywood. Dry matter yield of wheat harvested at the boot stage was not influenced by application of 15 kg Cu/ha. Dry matter yield was increased by P fertilization when no copper was applied. Copper fertilization did not influence grain yield at this site. Although high rates of phosphate fertilizer did not influence grain yield, a combination of copper and P fertilization increased grain yield significantly. The Cu concentration of grain was increased significantly by application of fertilizer copper. An additional 0.5 kg Cu/ha foliar effectively increased grain copper concentration above comparable treatments. Foliar application of fertilizer copper was therefore found to be the most efficient method for supplying Cu to wheat.

A rate experiment was conducted in the growth chamber with five levels of copper in order to study the response curve more closely and to evaluate five chemical extractants for soil copper as means of assessing availability of soil copper to plants. Soil from Haywood site was used. The Na_2DP (ethylene diamine di (O-hydroxyphenyl acetic acid) disoldium salt) extraction procedure reflected the amounts of applied copper best while 0.1 M HCl was the poorest extractant.

One molar HCl extractable Cu determined from soil samples taken at seeding was correlated best to grain yield of wheat. Extractable Cu determined by 0.01 M Na_2EDTA (ethylene diamine tetracetic acid disodium salt) from soil samples taken at maturity was best correlated to grain yield. This indicated that the choice of an extraction procedure depends on the sampling time.

Both grain copper concentration and total Cu uptake at maturity were better correlated to extractable soil copper than grain yield. Generally, it was found that sampling at final harvest gave better relationships than sampling at seeding time.

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I INTRODUCTION

The copper fertility status of many Manitoba mineral soils has not been extensively researched because available copper levels have generally been thought adequate for most crops. Studies conducted in growth chambers however, have indicated that some mineral soils may be marginal or even deficient in available soil copper for growth of plants (McGregor, 1972). Those that may be deficient include well drained sandy soils. Experiments were thus initiated to study the effect of copper fertilization on yield and chemical composition of wheat growing on mineral soils and to evaluate methods for assessing availability of soil copper to wheat.

Various chemical extractants have been used to estimate copper availability to plants. Calibration of these soil tests for plant available soil copper have been limited and soil copper critical levels that are used as guides to the copper status of mineral soils in Manitoba have not been well calibrated. Robson et al (1980) have indicated the need for local verification of soil tests for available soil copper. Experiments reported in this manuscript were therefore intended to evaluate the suitability of 0.1 M HCl, 1.0 M HCl, 0.01 M Na_2DP, 0.01 M Na_2EDTA, and 0.005 M DTPA as means of assessing availability of soil copper to wheat. The 1.0 M HCl, 0.1 M HCl, 0.01 M Na_2DP, 0.01 M Na_2EDTA and 0.005 M DTPA extraction procedures were also evaluated on the basis of their ability to reflect amounts of applied copper. The effect of soil carbonate content, organic matter, and pH on the ability of each extractant to assess available copper was also investigated.

Numerous workers have shown that high rates of fertilizer P, applied on soils with marginal levels of soil copper may induce copper deficiency (Olsen 1972; Touchton et al 1980; Modestus 1984). An experiment to study the effect of copper and P fertilization on yield and chemical composition of wheat is also reported in this manuscript.

II LITERATURE REVIEW

1. THE ESSENTIAL NATURE OF COPPER FOR GROWTH OF HIGHER PLANTS

The essentiality of copper for the proper growth of higher plants was first demonstrated by Lipman et al (1931). In their experiment, flax plants growing in a solution culture containing 0.125 ppm copper applied as $CUSO_4$ were found to blossom much better than flax plants growing on copper deficient culture. They also found that none of the copper deprived plants produced any seed capsule or seed. In a subsequent experiment, barley plants were unable to produce seed without the presence of a small amount of copper in the root medium. Since then, many workers have demonstrated the importance of copper in various physiological functions in higher plants. Principal among the major functions is its role in plant reproduction (Brown and Clark 1977).

The criteria put forward by Arnon (1950) for determining essentiality of micronutrients to plants have been shown to apply for copper. Copper has been shown to be necessary for reproduction processes especially in the development of viable pollen and therefore meets the requirement stipulated by Arnon that an essential element is one whose deficiency prevents the plant from completing its vegetative and reproductive stage in its life cycle. (Lipman et al 1931; Graham 1975; Brown and Clark 1977).

2. FUNCTIONS OF COPPER IN PLANTS

The functions of copper in higher plants have been broadly divided into: (1) carbohydrate metabolism; (2) nitrogen metabolism; (3) cell wall metabolism and water relations; and (4) reproductive processes of plants (Graham et al 1981). Copper has been shown to be an important constituent of many plant proteins and enzymes such as plastocyanin, cytochrome oxidase and ascorbate oxidase (Baszynski et al 1978; Walker and Loneragan 1981). Deficiency of copper affects the activity of these copper containing proteins and enzymes and thus influences plant growth and reproduction (Walker et al 1981).

Baszynski et al (1978) studied the effect of copper deficiency on the photochemical reactions of oat and spinach chloroplasts. They found that apart from having fewer chloroplasts, the copper deficient plants had reduced rates of photosynthesis as measured by the amount of O_2 evolved. Brown et al (1958) exposed wheat plants to ^{14}C labelled carbon dioxide and under the same conditions found that copper deficient plants fixed less ^{14}C into sugars than did plants adequately supplied with copper. Subsequently Brown et al (1977) found that copper sufficient wheat plants accumulated more reducing sugars than copper deficient plants. They concluded that this was due to reduced photosynthesis which was also responsible for the poor grain filling that they observed.

Cartwright et al (1970) found that at low copper supply, the copper content of tissues and the activity of copper containing enzymes cytochrome oxidase and ascorbate oxidase were greatly reduced in subterranean clover plants. They found that the low activity of these enzymes reduced nitrogen fixation. Snowball et al (1980) also concluded that copper was required for nitrogen fixation. The need for copper in nitrogen metabolism processes has also been indicated from experiments by Brown et al (1958) and Walker et al (1981).

Graham 1976(a) showed that copper deficiency led to decreased lignification of xylem vessels, epidermis, collenchyma and phloem cells. He also showed that this was due to low levels of phenoloxidase enzymes which were involved with lignification. He therefore concluded that wilting commonly found on copper deficient plants is due to structural weakness of plant tissue. Dekock et al (1971b), also found that the characteristically white tipped leaves of copper deficient oat plants had ruptured chloroplasts.

By far, the most important function of copper has been its influence on the reproductive processes of plants. Lipman et al (1931) observed that copper deficient barley was unable to develop any seed. Later, work by Graham (1975) showed that non-viability of pollen in copper deficient plants was the primary source of failure to set grain. In that experiment, copper deficient plants developed small anthers and their pollen did not stain with iodine indicating non-viability. Hill et al (1979a) also indicated that the necessity of copper in reproduction is through its effect on pollen formation. Reuter et al (1981) showed that reduction in yield of copper deficient clover plants was due to poor flower set, and also due to decreased pollen fertility. Other workers have demonstrated the importance of copper in the formation of viable pollen (Brown et al 1977; Dell 1981). It is apparent from the preceding discussion that copper influences plant growth through many plant processes. It therefore affects the general condition of the plant, and its deficiency results in poor growth and yield of plants.

3. DIAGNOSIS OF COPPER STATUS OF SOILS

Two approaches have been used in assessing the plant availability of soil copper, and varying degrees of success achieved with both (Robson et al 1981). Firstly, various methods of determining soil copper have been used to estimate the amount of plant available copper in soil. Secondly, plant tissue has been analysed to determine the total copper concentrations. With both approaches, results of analysis are calibrated against plant factors such as yield. The underlying assumption of either approach is that a positive relationship exists between soil and plant copper concentration and plant performance (Munsion et al 1973). Basically, the aim is to determine critical levels for both soil copper and plant copper concentration.

3.1. SOIL TESTING

Cox et al (1972) stated that the major objective of a micronutrient soil test is to separate a deficient from a non-deficient soil. Robson et al (1981) also stated that the objective of any diagnostic test for soil copper is to separate nutritional adequacy from both deficiency and toxicity. The choice of a good soil test will therefore involve selecting the best extracting method and calibrating it against plant response such as yield, nutrient uptake or concentration. Viets and Lindsay (1973) discussed the attributes of a good soil test. They indicated that it should extract nutrients from the same labile pool that plants do and that it should be cheap and reproducible in different laboratories.

The total content of a micronutrient in the soil has been conveniently placed into five pools that may or may not be in equilibrium with one another (Viets 1962). He suggested placing the various forms of micronutrient into the following pools: (1) water soluble; (2) exchangeable; (3) adsorbed chelated and complexed; (4) micronutrient in secondary minerals; and (5) micronutrient in primary minerals. McLaren et al (1973) also distinguished five pools of soil copper on the basis of extractability with different reagents. These authors suggested that the first three pools were in equilibrium with one another and constitute the available pools of micronutrient cations. Pools (4) and (5) were deemed to be unavailable to plants and are therefore of little value for soil testing purposes. Bray (1948) proposed that a good soil test should extract all or a proportionate part of the available forms of a micronutrient from soils with variable properties. In this respect therefore, a good soil test for copper should extract pools (1), (2) and (3) or their proportionate amounts to give a measure of availability of soil copper to plants. These three pools would represent the intensity and capacity factors of the soils ability to supply plants with copper.

3.1.1. WATER AND SALT EXTRACTANTS

Hot water extraction has been used to measure availability of soil copper to higher plants. Kruglova (1962) compared boiling water, acid solutions and neutral salts as extractants for soil copper and found that the hot water method was the best as a measure of available copper for cotton. Fiskell et al (1967) found that the water extractable copper reflected amounts of added copper. However he preferred the use of NH_4OAc extractable copper as a measure of available copper for citrus roots. Nishita and Haug (1974) found that extractability of soil copper was greatest when the soil was heated to 200 C but they did not relate the extractable copper to plant response. Water has not been widely used probably because it extracts only small amounts of the available copper, and these are difficult to detect.

NH_4OAc has been used widely as an extractant for available soil copper. Fiskell (1965) compared I.O N NH_4OAc at pH 4.8 with NH_4NO_3 and found that it extracted more copper than NH_4NO_3. He suggested that 5 ppm copper in soil as determined by this method may indicate toxicity while 0.2 ppm or lower may indicate deficiency. Grewal et al (1969) found that responses of maize and wheat grown in pot experiments to copper was better estimated by NH_4OAc than by a chelating agent. In an experiment with jowar plants, Neelkantan et al (1961) found a high correlation between the amount taken up by the jowar plants and copper extracted by neutral I.O N NH_4OAc. Fiskell et al (1967) also found a good correlation coefficient (0.807) between 1.0 N NH_4OAc extractable copper and citrus root copper concentration. McKenzie (1966) found contrasting results when using I.O N NH_4OAc. He found a poor correlation between extractable soil copper and copper deficiency in Australian soils.

Dolar et al (1971) found I.O N $Mg(NO_3)_2$ extractable copper not to be as well correlated with plant uptake of copper as was 0.1 M HCl or 0.001 M EDTA. The correlation coefficient between soil copper determined by I.0 N $MgNO_3$ and the copper uptake by oats was significant at 1 percent but was much lower than the values for the other extractants. McGregor (1972) evaluated 1.0 N NH_4NO_3 on predominantly sandy soils and found that it extracted very small quantities of copper which could not be measured accurately. Tills et al (1983) found 0.2 M ammonium oxalate to be a poor method for estimating availability of soil copper to wheat.

Generally, water and salt extractants would be expected to remove solution and exchangeable copper in the soil as postulated by McLaren et al (1973). They suggested that because of the small amount of exchangeable copper, it is unlikely that it can maintain adequate plant growth under intensive systems of cropping. This would suggest that despite some success, water and salt extractants are not suitable for estimating plant available copper.

3.1.2. ACID EXTRACTANTS

Dilute acids have been used to measure availability of copper to plants with limited success. As Robson et al (1981) point out, these extractants may underestimate the available copper status of soils. Dilute HCl and HNO_3 are the two commonest acids that have been used for assessing copper availability to plants. Nelson et al (1956) used 0.1 M HCl as an extractant for soil copper and found little correlation between extractable copper and the response of oats to copper. Cheng et al (1953) also found a poor correlation between 0.1 M HCl extractable copper and crop response. Martens (1968) however found a good relationship between 1.0 M HCl extractable copper and the copper uptake of corn, but only when other soil variables were included in the relationship. McGregor (1972) found no significant relationship between copper uptake by flax and copper extracted by 0.1 M HCl.

Oien et al (1967) found a good relationship between plant copper concentration and 0.43 N HNO_3 extractable copper. Henkens (1961) compared chemical methods of extraction with a bioassay method (*Aspergillus niger* method) and found I.0 N HNO_3 extractable copper to be effective as a measure of availability of copper to oats. Recently, Tills et al (1983) evaluated dilute HNO_3 as a measure of plant available copper in soils. They found a highly significant relationship between dilute HNO_3 extractable copper and copper uptake by wheat. They also found a significant relationship between 0.1 M HCl extractable copper and copper uptake by wheat. These authors found that inclusion of other soil variables in the relationships between soil copper and plant copper uptake greatly improved the relationship.

Martens (1968) suggested that dilute acids extract the organically bound copper in soils. This was confirmed by the fact that there was a significant positive correlation coefficient between I.O N HCl extractable copper and soil organic matter. On the basis of the importance of organically held copper these dilute acids would be expected to better estimate available copper than the salt extractants (Robson et al 1981). Acids have also been shown to be inappropriate for estimating plant available copper in calcareous soils. Tills et al (1983) found a negative correlation between I.O M HCl extractable copper and calcium carbonate equivalence in soil. This was accounted for by the fact that a lot of acid was neutralized by the carbonates. They thus recommended that acid extractants are not suitable for calcareous soils.

3.1.3. CHELATE EXTRACTANTS

Many workers have recognized the importance to plants of organically bound copper and have attributed the success of chelate extractants to their ability to extract the organically bound copper (Robson et al 1981; Graham et al 1981). McLaren et al (1973) found that the bulk of reserves of soil copper which may be made available to plants through equilibrium exchange was organically bound.

EDTA is one of the most widely used extractants for assessing plant available soil copper. It has been used in different concentrations and with various other chemicals with varying degrees of success. Mitchell et al (1957) compared total copper, acetic acid soluble copper and EDTA extractable copper, and found EDTA extractable copper a satisfactory diagnostic technique for identification of copper deficient mineral soils. In Scotland, Reith (1968) used 0.05 M EDTA as an extractant for soil copper and found a significant correlation between extractable copper and yield response of spring sown oats and barley. He suggested a critical level of 0.75 ppm for soil copper determined by this method.

Many more workers have had a measure of success with 0.02 M EDTA as an extractant for soil copper (Henriksen 1956; McKenzie 1966; Oien and Semb 1967; Grewal et al 1969). McGregor (1972) also evaluated 0.02M Na_2EDTA and NH_4OAc at pH7 and found it to be a good estimator of available soil copper for both flax and wheat. In the same experiment, 0.01 M Na_2EDTA extractable copper could only correlate with copper content of flax. Pizer (1966) evaluated the ammonium salt of EDTA at pH4 and found it a good extractant. He suggested a critical level of 1.3 ppm for this method. Henriksen (1957) used this method and concluded that there is unlikely to be a crop response if soil extractable copper is above 3 ppm and 4 ppm for oats and wheat, respectively. Tills (1983) evaluated several methods as means of assessing copper availability to plants. The disodium EDTA method proved a much better method for estimating available copper than the DTPA method and also had better correlations with plant response than the DTPA method. Edlin et al (1983) also showed that EDTA was better able to predict Cu uptake by flax than was DTPA.

Another major chelating agent widely used to assess plant availability of soil copper is DTPA (diethylene triamine pentaacetic acid). The extractant as used by Lindsay and Norvell

(1978) consists of 0.005 M DTPA mixed with 0.1 M triethanolamine and 0.01 M $CaCl_2$ and is used at a pH of 7.3. In their study, Lindsay and Norvell suggested a critical level of 0.2 ppm for soil copper. McGregor (1972) evaluated the method on mineral soils and found it an adequate extractant for assessing availability of soil copper to flax. In the same experiment however, DTPA could not estimate the availability of soil copper to wheat. Haynes (1983) evaluated both the DTPA and the EDTA methods of estimating available soil copper. He concluded that 0.005 M DTPA is not a suitable method for use over a wide range of soil pH values, mainly because pH determines amounts of extractable copper substantially. Follet and Lindsay (1971) suggested that DTPA was useful in monitoring the availability of both native and fertilizer copper. Dolar et al (1971) also found DTPA extractable copper to be highly correlated to copper uptake by oats (r^2=0.77) grown for 23 days in 36 soils. Makarim et al (1982) used the DTPA method of Soltanpour and Schwab (1977) and found significant relationships between extractable copper and the percent yield of wheat. They suggested a critical value of 0.53mg Cu L^{-1}. The DTPA method for assessing plant available soil copper has not been successful with some workers (Haq and Miller 1972; Haynes 1983). In their experiment, Haq et al (1972) found very poor correlations between extractable copper by EDTA and DTPA and concentration of copper in 16-day-old corn.

McGregor (1972) evaluated Na_2DP [ethylene diamine - di (- O - hydroxylphenol acetic acid) disodium salt]. Among 14 extractants that he tested as extractants for soil copper, Na_2DP best reflected the plant uptake by flax. He suggested a critical value of 1.3 ppm for this method used as 0.01 M Na_2DP and I.0 M NH_4 OAc at pH7.

As has been indicated by preceding discussions, chelating agents have had greatest success for evaluating soil copper availability to plants. DTPA has actually been adopted in many States of the U.S.A. as the best extractant for soil copper. Viets (1973) indicated that chelated metal that accumulates in solution during extraction with a chelating agent is a function of both the initial activity of the metal ions in solution (intensity factor) and the ability of the soil to replenish those ions (capacity factor). In this way, chelating agents tend to simulate the removal of nutrients by roots and their replenishment from surrounding soil. What is required now is to calibrate these chelating extractants for a wide range of soils and crops.

3.1.4. FACTORS AFFECTING AVAILABILITY OF SOIL COPPER TO PLANTS

Cox and Kamprath (1972) observed that there has been relatively little research on field calibration and interpretation of micronutrient soil tests. They indicated that many micronutrient soil test methods have proved successful only in localized areas which may necessitate inclusion of other soil properties in order to improve the relationships. As Dolar and Keeney (1971) note, the practice of including soil properties in an equation relating plant response and extractable soil nutrients improves the predictability of plant uptake of these nutrients. In their experiment involving several extractants, they found that copper uptake by oats was best predicted by inclusion of soil pH together with the EDTA extractable copper in the regression equations. DTPA was also shown to extract more copper at low pH. They therefore concluded that if availability of copper is to be predicted by chelating agents, soil pH would exert a significant negative relationship.

The pH of the soil determines both the solubility of the micronutrient and the stability of the metal - chelate complexes (Haynes 1983). Hence changes in pH affect the extractability of soil copper by affecting these two factors. Joshi et al (1983) found that extractability of copper by DTPA was significantly negatively correlated with soil pH (r = -.545). Tills et al (1983) evaluated eight soil tests for plant available copper and found a negative relationship between extractable soil copper and soil pH. They however found a positive relationship between copper concentration in wheat and the soil pH. This had earlier been reported by McGregor (1972). He found that wheat growing on calcareous soils had a higher copper content than that growing on non-calcareous soils.

The effect of soil pH on availability of copper has not been conclusive. However, more workers have found a negative relationship between soil pH and plant copper concentration or uptake indicating that heavy liming may induce copper deficiency.

Martens (1968) found a poor correlation between I.O N HCl extractable copper and copper uptake by corn, and yet found a significant relationship when he included soil pH, organic matter and clay content. However he found that organic matter and extractable copper were the main factors determining availability of soil copper to corn. The amount of copper extracted increased with the organic matter content of the soil suggesting that organic matter was the main source of copper extracted by I.O N HCl. The overall effect was that the amount of copper extracted from the soils by 0.1 N HCl increased with an increase in soil pH and organic matter and with a decrease in the soil clay content. Joshi et al (1983) studied the effect of organic matter and DTPA extractable copper and found a positive relationship between them. Dragun et al (1982) found no relationship between DTPA extractable copper and the organic matter content of the soil, while Tills et al (1983) found that DTPA extractable copper decreased as the organic matter content of the soil increased. Hodgson et al (1966) found that over 98 percent of copper in displaced solutions from 20 calcareous soils was complexed with organic matter. This bound copper would have to be dissociated from the organic ligand before it can be taken up by plants. Shuman et al (1979) also found that clay and organic matter held most of the copper in the soil with the organic matter having the more dominant role in coarse textured soils. These observations account for the importance of organic matter in determining availability of soil copper especially in coarse textured soils. However, as McGregor (1972) points out, the effect of organic matter may not be conclusive especially in low organic matter soils.

Tills et al (1983) evaluated the effect of clay on the copper concentration of wheat and found a positive relationship with all the extractants considered. Dragun *et al* (1982) found a contrasting result in that there was a negative relationship between extractable copper content and percent clay and silt. Osiname *et al* (1973a) also found no appreciable benefit by including the clay content of the soil together with extractable copper. The effect of clay content on the availability of copper would be through its effect on the CEC (cation exchange capacity) of the soil.

The carbonate content of mineral soils has also been used together with extractable soil copper to predict plant response to soil copper. McGregor (1972) found a significant relationship between the copper content of flax and the carbonate content of the soil. The carbonate content of the soil did not affect the copper concentration in wheat. Joshi *et al* (1983) also studied the effect of carbonate content on the extractability of copper by DTPA and found no significant relationship between extractable copper and the soil carbonate content. The relationship between carbonate content and plant availability of copper is not therefore clearly defined.

3.2. PLANT ANALYSIS

Plant analysis to diagnose copper deficiency depends on the relationship between copper concentration in the plant and plant performance. Jones (1972) points out that the nutrient element content of the plant is a reflection of the soils available nutrient status. The major objective of developing a relationship between plant performance and plant nutrient concentration is to delineate a critical level for the nutrient and plant part sampled. Munsion and Nelson (1973) define the critical level as that nutrient concentration below which growth, yield, and quality declines significantly. Ulrich and Hills (1967) suggested the critical level to be the nutrient concentration at which yield reduction is 10 percent of the potential yield. Under this definition, the concentration of the nutrient at maximum yield is defined as the optimum concentration.

In establishing the critical levels for a particular crop, the same plant part at the same level of maturity must be used for all subsequent comparisons because nutrient concentrations vary depending on the plant part sampled and the stage of growth (Reuter et al 1981b; Robson et al 1981). For example, to enable comparison, the whole aerial portion of small grains is harvested at the boot stage and analysed for the nutrient required.

3.2.1. CRITICAL LEVELS OF COPPER IN PLANTS

In a glasshouse experiment Gupta et al (1970) found the critical concentration of copper in wheat to be 3.3 ppm for the above ground portion harvested at the boot stage. Melsted et al (1969) evaluated thousands of plants analysis data and came up with critical levels for various plants and plant parts. They suggested a critical level of 5 ppm for the whole shoot of wheat harvested at the boot stage. McAndrew (1980) suggested a critical level of 4.9 ppm for wheat shoots at the boot stage. A range of 3.0 to 3.8 ppm copper concentration in the aerial portion of wheat was found sufficient for the nutritional needs of wheat grown in soils of North Carolina and harvested prior to heading (Younts 1964).

Grain copper concentration has also been used to diagnose copper deficiency. King et al (1975a) found that grain containing less than 2.5 ppm copper may indicate deficiency. Davies et al (1971), and Caldwell (1971) considered grain copper concentration of less than 2 ppm to indicate deficiency while concentrations above 3 ppm to indicate adequacy. However, Nambiar (1976) has shown that some wheat genotypes may have less than 2 ppm copper in the grain and yet not respond to copper application.

The critical values indicated above show that there is no standard curve relating copper concentration in the plant and plant performance. The critical level determined depends on numerous factors, many of which are discussed in an extensive review by Bates (1972). Brown et al (1955) reported genotypic differences in copper nutrition among cereals. Nambiar (1976) found significant differences in the critical levels of copper between some Australian wheat varieties sampled at mid-tillering. He also reported variation among six varieties in time of appearance of foliar copper deficiency symptoms.

Another major limitation of using the critical concentration in the plant to diagnose soil nutrient availability is that the concentration of copper in the plant increases as the degree of deficiency increases from marginal to severe (Andrew and Thorne 1962; Steenbjerg et al 1963). It is therefore important to know what part of the response curve one is dealing with to ensure correct interpretation.

The age of tissues sampled has also been shown to be critical (Bates 1972; Loneragan et al 1980; Reuter et al 1981b). Reuter et al (1981b) showed that the concentration of copper in young leaf blades of subterranean clover remained relatively constant regardless of age of the plant. They therefore suggested the use of young leaves to diagnose copper status of the plant.

Nutrient supply has also been found to determine the critical level of copper in whole shoots (Thiel and Finck 1973). Bates (1973) indicated that other factors such as nutrient interactions and environmental factors such as moisture supply may appreciably affect the nutrient concentration in plants and so may affect the interpretation of the plant analysis. This indicates that critical concentration data should be considered very carefully before any interpretations are done.

III GENERAL MATERIALS AND METHODS

Several studies are reported in this thesis, and different experimental procedures were used for the different studies. It was therefore found necessary to include the experimental procedures for each individual study, with the results obtained. However, the general analytical procedures common to all the studies are described in this section.

1. SOIL ANALYSIS

Soil collected for copper analysis from all the experiments was thinly spread on plastic material and air-dried for 7 days. Plant debris were removed and the soils were put into paper containers. A subsample from these soils was then hand ground with a porcelain pestle and mortar and passed through a 2 mm sieve. This was stored to await copper analysis.

Soil samples for general characterization of soil were also spread out on plastic material and air-dried for 7 days. Debris were removed and the soil passed through an electric grinder and ground to 2 mm size. This was placed into paper containers to await laboratory analysis. Soil texture was determined from an unground portion of the soil.

(a) Soil pH

Soil pH was measured electrometrically using a glass-colomel electrode pH meter. The pH for general characterization of the soil was measured in water while the pH values to be used in multiple regression relationships were obtained by equilibrating 25 g of soil in 50 ml of 0.01 M $CaCl_2$ solution for 30 minutes and then measuring with the colomel electrode pH meter. pH in water was measured after equilibrating 25 g of soil with 25 ml of distilled water for 30 minutes.

(b) Electrical Conductivity

Conductivity was measured on the same paste as pH in water, using a model CDM 2e conductivity meter.

(c) Organic Matter

Organic matter was determined by oxidation with excess chromic acid in the presence of Conc H_2SO_4 as described by Walkley and Black (1934). Unreacted chromic acid was determined by backtitrating with 0.5 M $FeSO_4 \cdot 7H_2O$ using barium diphenyl-sulphonate as an indicator.

(d) Inorganic Carbon (Carbonates)

A one g sample of soil was treated with 0.1 M HCl in a digestion flask for 10 minutes and the evolved CO_2 absorbed by ascarite. The change in weight due to absorbed CO_2 was determined, from which the inorganic carbonate content was calculted.

(e) Nitrate-Nitrogen

The colorimetric determination of NO_3-N involved treating 10 g of soil in 50 ml of extracting solution consisting of a mixture of 0.02 M $CuSO_4$ and 0.06% $AgSO_4$. The soil-solution mixture was shaken for 15 minutes then 0.16 g $Ca(OH)_2$ (heated in a muffle furnace at 750 degrees C for 2 hours) was added. The solution was shaken for 5 minutes and 0.5 g $MgCO_3$ was added. The mixture was then shaken again for 15 minutes and filtered through

#1 filter paper. A 25 ml sample was taken from the extract and evaporated to dryness at 50 degrees C. The dried material was dissolved in 2 ml phenoldisulphonic acid and diluted with water. NH_4OH was then added to develop color and the solution made up to 100 ml. The NO_3-N was determined colorimetrically using a Bauch-lomb UV-VIS spectrophotometer at 415 nm.

(f) Extractable Phosphorus

A modification of the Olsen et al (1965) method was used to determine extractable phosphorus. Five g of soil was extracted with 100 ml of 0.5 M $NaHCO_3$ buffered at pH 8.5 for 30 minutes. The suspension was filtered through #42 filter paper and a 10 ml aliquot drawn. A drop of 2.4 dinitrophenol was added and then, concentrated H_2SO_4 was added dropwise until the solution became clear. Color was developed by adding a mixed reagent consisting of ammonium paramolybdate - antimony solution and ascorbic acid. The P concentration in the solution was then determined on a Bauch-lomb UV-VIS spectrophotometer at 885 nm.

(g) Sulfate-Sulfur

Sulfate sulfur was determined by shaking 25 g of soil in 50 ml of 0.01 M $CaCl_2$ solution for 30 minutes. An aliquot of the filtrate was passed through Auto Analyzer II. In the Auto Analyzer II the extract was passed through a cation exchange resin and reacted with a solution containing 1.526 g/l $BaCl_2$ and 0.236 g/l methylthymol blue at a pH of 2.5-3.0. Excess $BaCl_2$ reacted with methylthymol blue to form a blue colored chelate at a pH of 12.5 to 13.0. The amount of uncomplexed methylthymol blue (gray color) measured at 460 nm on Auto Analyzer II reflected the amount of sulfur in the sample.

(h) Extractable K

Extractable K was determined by extracting 5 g of air-dry soil with 100 ml of a solution containing I.0 N NH_4OAc + 250 ppm lithium on an auto stirrer for one hour. The solution was then filtered through Whatman #42 paper. The K concentration in the filtrate was then determined using a Perkin-Elmer 560 Atomic Absorption Spectrophotometer.

(i) Mechanical Analysis

The pipette method of particle size analysis was used. A 10 g sample of air-dry soil was weighed into a flask. Organic matter was destroyed using 30% H_2O_2 and boiled until all is destroyed. Calgon solution was added and the suspension stirred in a mechanical stirrer for 15 minutes. The separation of the sand fraction was done by placing a 300 mesh sieve into a funnel and passing the suspension through the sieve. The silt and clay content of the remaining suspension was determined by drawing aliquots at a depth of 10 cm immediately after shaking and 380 minutes after shaking for silt and clay determination, respectively.

(j) Extractable Soil Copper

Five chemical extractants were evaluated as methods for assessing availability of soil copper to wheat. These five extractants were:

(i) DTPA

This extractant consisted of 0.005 M DTPA (diethylenetriamine pentaacetic acid), 0.01 M $CaCl_2$ and 0.1 M TEA

(Triethanolamine) at pH 7. The extractant was prepared by first placing 3.930 g DTPA in about 50 ml of deionized water. A 29.8 g sample of TEA (Triethanolamine) was then added and the mixture stirred to dissolve the DTPA. The solution was then added to another solution containing 2.22 g of $CaCl_2$ and more deionized water was added to make a total solution of approximately 2 litres. pH was then adjusted to 7 by using dilute HCl and volume made up to 2 litres.

(ii) Na_2DP

The extractant consisted of 0.01 M Na_2DP [Ethylenediamine di(O-hydroxyphenyl acetic acid) di-sodium salt] and 1 M NH_4OAc at pH 7. This extractant was prepared from ethylene-diamine di (O-hydroxyphenol acetic acid) disodium salt (EDDHA) which was 90% pure. One part of impure EDDHA was shaken with 5 parts methanol (reagent grade) for 30 minutes. After shaking, the suspension was centrifuged at 500 rpm for 30 minutes. The supernatant was decanted and the remaining compound dried and ground. To prepare one litre of extracting solution, 0.8 g of NaOH was dissolved in 650 ml of distilled water. A 3.6 g sample of purified EDDHA was then added and stirred vigorously with a magnetic stirrer for 30 minutes. Seventy-seven g of NH_4OAc was then added and the mixture stirred until all dissolved. The undissolved material was filtered out using Whatman #42 filter paper. The solution was then made up to 950 ml and pH adjusted to 7.0 using dilute HCl. The solution was then made up to 1 litre. A new solution was made up each day.

(iii) Na_2EDTA

The extractant consisted of 0.01 M Na_2EDTA (ethylenediamine tetraacetic acid di-sodium salt) and 0.67 M $(NH_4)_2CO_3$ at pH 8.65. This extractant was prepared by dissolving 3.74 g of Na_2EDTA in approximately 50 ml of deionized water. A 64.38 g sample of $(NH_4)_2CO_3$ were then added to the solution and more deionized water added. After all material was dissolved, the solution was made up to approximately 975 ml then pH was adjusted to 8.65 using dilute HCl. The solution was then made up to 1 litre.

(iv) 1.0 M HCl

The 1.0 M HCl was prepared from 12.1 N HCl using deionized water.

(v) 0.1 M HCl

The 0.1 M HCl was prepared from 12.1 N HCl using deionized water.

With the DTPA method, 25 g of air-dried soil was shaken for 2 hours with 50 ml of extracting solution. In all the other methods, 5 g of air-dried soil was shaken for 1 hour with a mechanical stirrer. The suspensions were then filtered through Whatman #42 filter paper. Copper concentration was determined using a Perkin-Elmer 560 Absorption spectrophotometer.

2. PLANT TISSUE ANALYSIS

Harvesting was done at the boot stage and at maturity in all growth chamber and field experiments. Harvesting procedures specific to each experiment are described under each experiment. Plant material harvested at the boot stage from both the growth chamber and field experiments was rinsed twice with deionized water, air-dried for 4 days and then oven-dried at 70 degrees C for 2 days. The material was then finely ground and stored in polythene bags. For the two growth chamber experiments, all the plant material was harvested at maturity. The material was oven-dried at 70 degrees C for 2 days after which grain was separated from straw. A sample of each was ground and stored in polythene bags.

At maturity, a sample of plant material was harvested from each field plot and air-dried for 7 days. Grain was then separated from straw. A sample of grain and one of straw were taken and ground, then stored in polythene bags.

Two g of finely ground plant material was placed in a micro-Kjedahl flask and an acid mixture consisting of 5 ml concentrated HNO_3 and 2.5 ml of 70% $HClO_3$ was added. The mixture was digested by heating on a micro-Kjeldahl unit until clear. The digested material was filtered through #42 filter paper into 25 ml volumetric flasks and diluted to volume using deionized water. Concentrations of Cu, Zn, Mn, and Fe were determined by aspirating a portion of solution into a Perkin-Elmer 560 Atomic Absorption spectrophotometer. Concentrations of Ca, Mg, and K were determined by taking a 1.0 ml aliquot, adding 2.5 ml of 2500 ppm $LiNO_3$ solution and diluting to 25 ml using deionized water. A portion of this solution was then aspirated into a Perkin-Elmer 560 Atomic Absorption spectrophotometer.

Total phosphorus concentration was determined from the undiluted extract. A 0.5 ml aliquot was diluted to 10.5 ml using deionized water. A 0.5 ml aliquot of the dilute extract was diluted again to 10.5 ml and 2.0 ml of a solution containing 250 g/l L-ascorbic acid and 7.5 g/l ammonium molybdate was added. Phosphorus concentration was measured using a Bauch-lomb UV-VIS spectrophotometer set at 885 nm.

Concentration of sulfur in the plant digest was determined as described by Lazrus et al (1966). A 0.2 ml aliquot of the original digest was diluted to 30 ml and sulfur was determined using Auto Analyzer II at 460 nm as described previously.

Plant N was determined using the modified Kjedahl-Gunning method described by Jackson (1958). A 0.5 g of ground material was weighed and a catalyst consisting of 5 g K_2SO_4 and 5 mg selenium was added. Six ml of Conc H_2SO_4 and boiling chips were added. The mixture was heated and digested for one hour. After digestion, the flasks were cooled and 25 ml of distilled water was added. Distillation and titration was then done on a Kjeltech Auto-Kjedahl machine.

IV RESULTS AND DISCUSSION

A. FIELD EXPERIMENT I - The effect of copper fertilization on yield and chemical composition of wheat grown on four field soils and methods of evaluating availability of soil copper to wheat

1. INTRODUCTION

Many of the experiments conducted to test the relationships between extractable soil copper and plant performance have been done under controlled environmental conditions. Further, many of these experiments have been done using several soils with similar properties. It has therefore been found that soil tests that have been developed using greenhouse data may not apply under field conditions, where environmental conditions and soil properties may vary appreciably. This experiment was intended to study relationships between extractable soil copper and wheat performance on different soil types. Specifically, this experiment was designed to:

(i) determine the effect of copper fertilization on yield, copper uptake and copper concentration of wheat harvested both at the boot stage and at maturity.

(ii) evaluate the suitability of five chemical extractants for assessing plant available copper in mineral soils. The five extractants used were 0.005 M DTPA, 0.01 M Na_2DP, 0.01 M Na_2EDTA, 1 M HCl and 0.1 M HCl. Detailed procedures for each extractant are described in the General Materials and Methods.

(iii) study the influence of organic matter, carbonate content and pH on the ability of each extractant to predict plant performance by using these variables and extractable soil copper as independent variables.

2. EXPERIMENTAL METHODS

The experiment was set up as a completely randomized block design with two treatments and twelve replicates. Treatment (1) was a control with no copper applied while in treatment (2), 15 kg Cu/ha was applied as $CuCl_2.2H_2O$. The $CuCl_2.2H_2O$ was dissolved in water, sprayed on the surface and rototilled to a depth of 10 cm. Each plot was 6.1 M x 2.1 M.

Four sites were selected for this field experiment. Three were selected on the basis of the low soil copper content determined from preliminary soil samples while the fourth soil was selected for having a high soil copper content and thus providing a low to high range in extractable soil copper. The experiment was however discontinued on two sites, one due to non-uniform germination of wheat, and the other due to flooding. Hence, only characteristics for soils located at Sifton and Haywood are provided (Table 1). The soil characteristics were determined from samples taken adjacent to each block of 4 plots at each of the two sites to a depth of 120 cm. The soil at Sifton is a Gilbert sandy loam developed on coarse textured lacustrine deposits as described by Ehrlich et al (1959). The soil at Haywood is an Almasippi sand developed on coarse textured lacustrine material as described by Ehrlich et al (1953).

For each soil, all plots received uniform treatment in terms of N, P, K and S. At Haywood, 100 kg N/ha as ammonium nitrate, ammonium phosphate and ammonium sulfate was applied. 79 kg P/ha as ammonium phosphate, 83 kg K/ha as potassium chloride and 15 kg S/ha as ammonium sulfate were also broadcast and roto-tilled to 10 cm. At Sifton, 150 kg N/ha as ammonium nitrate and ammonium phosphate, 44 kg P/ha as ammonium phosphate and 83 kg K/ha as potassium chloride were broadcast on all plots and roto-tilled to 10 cm. After rototilling, soil samples were taken from five points within each plot to a depth of 15 cm. These soil samples were handled as described in the General Materials and Methods.

Wheat (<u>Triticum aestivum</u> Var Columbus) was seeded at 100 kg/ha at both sites. Seeding at Haywood was done on 21st May 1982 while seeding at Sifton was done on June 4, 1982. Wheat samples were taken at the boot stage and at maturity and at each time soil samples were taken. The plant and soil samples were handled as described in the General Materials and Methods section.

3. RESULTS AND DISCUSSION

(a) Soil Characteristics

The soil at Haywood had a zinc content of 0.94 ppm which according to the literature would be considered low (Lindsay and Norvell, 1978). (Table 1). It also had lower levels of manganese and iron than the DTPA extractable critical levels of 1.0 and 4.5 ppm respectively, determined by Lindsay and Norvell (1978). Extractable P of this soil was also low.

Table 1: Physical and chemical characteristics of the soils used in field experiment I

Soil Characteristic	Soil Location: Sifton	Soil Location: Haywood
Texture	loamy fine sand	loamy fine sand
Soil pH	8.0	8.1
Carbonate Content (%)	7.3	6.1
Organic Matter Content (%)	1.3	5.1
Salinity dSm^{-1}	0.3	0.5
*1 NO_3-N kg/ha	13	127
Extractable P kg/ha	16	13
Extractable K kg/ha	68	104
SO_4-S kg/ha	60	111+
*2 Zn (ppm)	0.59	0.94
Cu (ppm)	0.34	0.42
Mn (ppm)	1.78	0.87
Fe (ppm)	3.55	3.91

Note: *1 Values for NO_3-N, and SO_4-S were determined to a depth of 60 cm. All other values are for the 0-15 cm only.

*2 DTPA extraction for Zn, Cu, Mn and Fe.

The DTPA extractable Zn and Fe for the Sifton soil were lower than the critical levels for these two micronutrients determined by the same authors. Both the NO_3-N and extractable P were low.

(b) Yield and Chemical Composition of Wheat at the Boot Stage

(i) Sifton

Dry matter yields of wheat at the boot stage of growth ranged from 1926 kg/ha for the control to 1983 kg/ha for the copper treated plots at Sifton (Table 2). This increase in dry matter yield was not significant. The lack of response to applied copper was probably due to a sufficient supply of soil copper noting that, the concentration of copper in plant tissue from the control plots was higher than the critical level of 3.3 ppm suggested by Gupta et al (1970). However, the copper concentration of tissue from untreated plots was below the critical level of 4.9 ppm determined by McAndrew (1980) and wheat would therefore have been expected to respond to applied copper. The fact that copper fertilization increased copper concentration in the tissue significantly without increasing dry matter yield significantly indicates that copper was not limiting yield and the critical level of copper at this stage was probably lower than that of 4.9 ppm determined by McAndrew (1980).

Copper fertilization increased copper uptake at Sifton from 8.7 g/ha for the control plots to 12.1 g/ha for the copper treated plots (Table 2). Due to extreme variability in copper uptake of individual plots, this increase in copper uptake between the control and treated plots was not significant.

Copper fertilization at Sifton had no significant effect on the chemical composition of wheat tissue apart from increasing the copper concentration (Tables 2 and 3). However, there were slight insignificant decreases in other element concentrations in the tissue from copper treated plots compared to the control. The manganese content of both treated and untreated plots was lower than the critical level of 30 ppm determined by Melsted et al (1969) and this could have limited dry matter yields at this site. All other nutrients were in the adequate range judging from critical values determined by Melsted et al (1969).

(ii) Haywood

The dry matter yield of wheat at Haywood ranged from 3768 kg/ha for the control to 4007 kg/ha for the copper treated plots (Table 4). This increase in dry matter was not significant although the copper concentration in the tissues was increased significantly when 15 kg Cu/ha was applied. The copper concentration of tissues from the untreated plots was below the critical level of 3.3 ppm determined by Gupta et al (1970). Application of 15 kg Cu/ha increased the copper concentration above this critical level but was still lower than the critical level of 4.9 ppm suggested by McAndrew (1980). From these observations, it can be concluded that copper was not limiting yield at this site.

Table 2: Dry matter yield, copper concentration and copper uptake of wheat at the boot stage as affected by copper fertilization at Sifton---field experiment 1

Treatment	Dry Matter kg/ha	Copper Concentration ppm	Copper Uptake g/ha
0 kg cu/ha	1926 a[1]	4.5 a	8.7 a
15 kg cu/ha	1983 a	6.1 b	12.1 a

[1]Students paired t test. Values followed by the same letter within columns are not significantly different at P = 0.05.

Table 3: The nutrient composition of wheat shoots harvested at the boot stage as affected by copper fertilization at Sifton---field experiment I

Treatment	Concentration in %						Concentration ppm		
	N	P	K	S	Ca	Mg	Zn	Mn	Fe
0 kg Cu/ha	3.1a[1]	0.30a	2.5a	0.20a	0.53a	0.20a	17.1a	13.9a	62.0a
15 kg Cu/ha	2.9a	0.30a	2.1a	0.19a	0.48a	0.18a	17.5a	12.7a	57.7a

[1]Students paired t test. Values followed by same letter are not significantly different at (P = 0.05).

Copper fertilization significantly increased copper uptake by wheat at Haywood from 10.2 g/ha for the control to 17.2 g/ha for the fertilized plots (Table 4). As there was no significant increase in dry matter yield, this significant increase in copper uptake can be explained by the increase in tissue copper concentration between control and treated plots.

Copper fertilization did not significantly affect the concentration of other elements in the tissues at Haywood (Table 5). The concentration of P for both treatments was lower than the critical level of 0.30% determined by Melsted et al (1969). The zinc concentration in tissues from the copper treated plots was below the critical level of 15 ppm determined by the same authors. Yields at this site were much higher than those at Sifton and although P and Zn concentrations in the tissues were low, they do not appear to influence yields. The high yields at Haywood are probably due to relatively higher fertility level of soil at this site compared to Sifton (Table 1). The NO_3-N, extractable K and SO_4-S were all considerably higher at Haywood than at Sifton and probably contributed to the wide yield difference between the two sites.

(c) Yield and Chemical Composition of Grain at Final Harvest

(i) Sifton

Grain yield at Sifton was not affected by copper fertilization though there was a slight insignificant decrease in grain yield when 15 kg Cu/ha was applied (Table 6). However, the copper concentration in the grain was increased significantly from 3.3 ppm for the control to 5.5 ppm. On application of 15 kg

Table 4: Dry matter copper concentration and copper uptake of wheat at the boot stage as affected by copper fertilization at Haywood---field experiment 1

Treatment	Dry Matter kg/ha	Copper Concentration ppm	Copper Uptake g/ha
0 kg cu/ha	3768 a[1]	2.7 a	10.2 a
15 kg cu/ha	4007 a	4.3 b	17.2 b

[1]Students paired t test. Values followed by the same letter within columns are not significantly different at P = 0.05.

Table 5: The nutrient composition of wheat shoots harvested at the boot stage as affected by copper fertilization at Haywood---field experiment I

Treatment	Concentration in %						Concentration ppm		
	N	P	K	S	Ca	Mg	Zn	Mn	Fe
0 kg Cu/ha	2.2a[1]	0.20a	1.7a	0.20a	0.43a	0.24a	15.9a	19.7a	64.9a
15 kg Cu/ha	2.1a	0.19a	1.8a	0.19a	0.50a	0.23a	14.8a	18.0a	63.3a

[1]Students paired t test. Values followed by same letter are not significantly different at (P = 0.05).

Cu/ha, copper uptake was increased from 11.0 g/ha for the control to 16.6 g/ha for the treated plots. Despite no increase in yield from the application of 15 kg Cu/ha, the crop recovered a significant amount of applied copper manifested in increased copper concentration and uptake.

Apart from increases in copper concentration, the chemical composition of grain was not significantly affected by copper fertilization at Sifton (Tables 6 and 7). There were slight insignificant reductions in the concentrations of Mn and Fe on application of 15 kg Cu/ha. The concentrations of Zn and P increased insignificantly while the concentrations of N, K and S remained constant when 15 kg Cu/ha was applied. The manganese content of grain from this site was very much lower than that for grain from Haywood and may have limited grain yield at this site.

(ii) Haywood

Grain yield at Haywood ranged from 2242 kg/ha for the treated plots to 2382 kg/ha for the control (Table 8). Hence there was a small but insignificant reduction in grain yield when 15 kg Cu/ha was applied. The copper concentration in the grain was not significantly affected by copper fertilization and was higher than the critical level of 2.5 ppm suggested by King (1975). The zinc concentration of wheat tissue harvested from the copper treated plots at the boot stage of wheat was observed to be lower than the critical level of 15 ppm determined by Melsted et al (1969). The concentration of P in the boot stage of wheat tissue was also lower than the critical level of 0.30% determined by the same authors. Marginal concentrations of P and

Table 6: Grain yield, copper concentration and total copper uptake of wheat at maturity as influenced by copper fertilization at Sifton---field experiment I

Treatment	Grain Yield kg/ha	Copper (Grain) Concentration ppm	Total Copper Uptake g/ha
0 kg Cu/ha	1435 a[1]	3.3 a	11.0 a
15 kg Cu/ha	1330 a	5.5 b	16.6 b

[1]Students paired t test. Values followed by same letter are not significantly different at P = 0.05.

Table 7: The chemical composition of grain as affected by copper fertilization at Sifton---field experiment I

Treatment	Concentration (ppm)			Concentration (%)			
	Zn	Mn	Fe	N	P	K	S
0 kg Cu/ha	27.2 a[1]	16.0 a	31.3 a	2.8 a	0.47 a	0.40 a	0.13 a
15 kg Cu/ha	28.0 a	15.1 a	31.1 a	2.8 a	0.48 a	0.40 a	0.13 a

[1]Students paired t test. Values followed by the same letter are not significantly different at (P = 0.05).

Zn together may have limited grain yield but probably only to a small extent because grain yield at this site was high when compared to Sifton.

The total copper uptake at maturity was increased from 17.7 g/ha for the untreated plots to 20.7 g/ha for the copper treated plots (Table 8). This increase in copper uptake was not significant and reflects an increase in the copper concentration of grain.

The chemical composition of grain from Haywood was unaffected by copper fertilization (Table 9). Nonsignificant decreasing trends were noted for most nutrients and for grain yield when 15 kg Cu/ha was applied. In view of this, it is not likely that the decreasing trends in nutrient concentrations could be accounted for by biological dilution.

(d) Regression Relationships

Of the original four sites, the two that were continued were low in soil copper. This, therefore, did not give the wide range in extractable soil copper that would be required in order to establish regression relationships between extractable copper and plant performance. Combining the values from fertilized and unfertilized plots resulted in two distinct groupings of data when plotted and thus this approach was found unsuitable with the set of data that was available. Interpretations about plant performance between these two sets of groupings could not be made and any regression relationships generated from such data would not have been useful.

Both simple, and multiple regression relationships were

Table 8: Grain yield, grain copper concentration and total copper uptake of wheat at maturity as influenced by copper fertilization at Haywood---field experiment I

Treatment	Grain Yield kg/ha	Copper (grain) Concentration ppm	Total Copper Uptake g/ha
0 kg Cu/ha	2382 a[1]	4.6 a	17.7 a
15 kg Cu/ha	2242 a	5.1 a	20.7 a

[1]Students paired t test. Values followed by the same letter are not significantly different at 5% level of probability.

Table 9: The chemical composition of grain as affected by copper fertilization at Haywood---field experiment I

Treatment	Concentration (ppm)			Concentration (%)			
	Zn	Mn	Fe	N	P	K	S
0 kg Cu/ha	28.9 a[1]	26.5 a	31.9 a	2.7 a	0.42 a	0.40 a	0.16 a
15 kg Cu/ha	25.2 a	21.8 a	28.9 a	2.8 a	0.38 a	0.36 a	0.14 a

[1]Students paired t test. Values followed by the same letter are not significantly different at (P = 0.05).

intended to be generated using extractable soil copper and other soil factors such as organic matter, carbonate content and pH as independent variables. Many researchers have found that including soil factors such as organic matter, pH, and carbonate content in regression relationships relating soil copper to plant performance improves the relationships significantly (Martens 1968; McGregor 1972; and Dolar and Keeney 1971b). These factors affect the availability of soil copper to plants, and their influence on the availability of soil copper to wheat under field conditions is of prime importance. Due to the discontinuation of the experiment at two sites, the range of values for soil organic matter, pH and carbonate content was narrow and the data too few to be useful for this type of relationship to be generated. These types of relationships would be useful where soils vary appreciably in organic matter content, pH and carbonate content, etc. Due to extreme variability in environmental conditions in the field, these factors may improve the relationships only slightly. This was however not verified because the data was found unsuitable for this type of regression analysis.

B. GROWTH CHAMBER EXPERIMENT I - The effect of copper fertilization on yield and chemical composition of wheat grown on ten different soils and methods of evaluating availability of soil copper to wheat

1. INTRODUCTION

This experiment was designed to study the effect of copper fertilization on wheat grown on ten mineral soils. In Field Experiment I, the effect of copper fertilization on yield and chemical composition of wheat was studied. The results from the field experiment were restricted by having only two sites both low in extractable soil copper. It was therefore felt that in order to have a better range in extractable soil copper, more soils were required. Ten sites were selected on the basis of low soil copper determined at the Manitoba Provincial Soil Testing Laboratory and soils were taken from these sites in the fall of 1982 (Table 10).

The specific objectives of this experiment were to:

(a) determine the effect of copper fertilization on yield and chemical composition of wheat grown on ten Manitoba soils;

(b) evaluate five extraction methods as means of assessing availability of soil copper to wheat under controlled environmental conditions;

(c) study the effect of texture, organic matter, pH, and carbonate content on the ability of five chemical extractants to assess the plant availability of soil copper;

(d) determine the critical levels of copper in soil for each of the five chemical extractants;

(e) determine the critical level of copper in plant tissue at

Table 10: Location and description of soils used in growth chamber experiment I

Soil Location	Legal Description	Soil Series
Poppleton*1	---	---
Haywood A	16-7-6W	Almasippi Sand
Sandilands	NE27-4-9E	Sandlands Sand
Dauphin	18-24-8W	Edwards Alluvial Clay Loam
Graysville	14-7-6W	Almasippi Loamy Fine Sand
Steinbach	7-6-7E	Pelan (Shallow phase) Fine Sand
Sifton	NE4-28-19W	Gilbert Sandy Loam
Haywood B	25-8-6W	Almasippi Sand
Treherne	29-8-9W	Almasippi Sand
Cowan	SW1-35-21W	Spearhill Substrate Phase

*1Actual legal description not known.

the boot stage and the critical level of copper in grain.

2. EXPERIMENTAL METHODS

Fifty kg of soil was obtained from the Ap horizon of each of 10 Manitoba soils. The 10 soils (Table 11) were selected on the basis of low to high extractable soil copper indicated by data from Manitoba Provincial Soil Testing Laboratory. The experiment consisted of 2 treatments: 1. no copper, 2. Cu at 38 mg Cu/pot. Treatments were replicated 3 times, thus there were a total of 60 pots. The experimental design was a completely randomized design.

The soils were air-dried, and sieved to remove debris. Percent moisture of each was determined by drying a sample at 105 degrees C to constant weight. Air-dried soil equivalent to 5.25 kg of oven dry soil was then weighed into 6 litre plastic pots lined with polythene material.

Copper was applied at the rate of 38 mg Cu/pot as $CuCl_2.2H_2O$ to pots receiving the copper treatment. All the pots received 100 ppm N as ammonium nitrate, and monoammonium phosphate, 100 ppm K as potassium sulfate and 40 ppm P as monoammonium phosphate. All nutrients were supplied in solution form and thoroughly mixed throughout the soil.

When mixing was done, a soil sample equivalent to 250 gm of oven dry soil was drawn from each pot and air dried, later to be analysed for initial soil copper. Extractable Cu from these samples was designated initial extractable soil copper. The pots were then arranged in a completely randomized design in the growth chamber. The soils were then watered to approximately 50%

Table 11: Physical and chemical characteristics of the soils used in the growth chamber experiment I

Soil Location	Textural Class	CONCENTRATION (ppm) pH	NO_3-N	Extractable P	Extractable K	SO_4-S	Cu	Zn	Mn	Fe	Carbonate Content%	Organic Matter %
Poppleton	loamy fine sand	7.3	1.5	4.6	34.4	5.5	0.26	1.22	1.74	2.82	0.3	2.61
Haywood A	fine sand	7.0	1.2	10.2	110.4	0.14	0.22	1.04	1.20	2.54	0.2	1.74
Sandilands	fine sand	6.3	1.1	24.5	54.4	458	0.38	0.56	0.98	4.70	0.2	1.45
Dauphin	silty clay	7.1	4.3	17.6	473.0	6.3	2.32	7.76	4.98	6.86	0.2	6.37
Graysville	loamy fine sand	7.1	3.6	47.7	211.8	4.3	0.38	2.34	2.8	3.52	0.5	3.68
Steinbach	loamy fine sand	7.4	1.5	33.8	46.2	4.2	0.34	1.48	2.14	5.12	0.3	1.56
Sifton	loamy fine sand	7.4	5.1	9.0	64	8.3	0.34	1.12	1.34	2.94	7.3	3.25
Haywood B	fine sandy loam	7.7	5.9	4.1	31.2	5.6	0.40	0.88	0.62	3.70	6.1	4.55
Treherne	fine sand	6.8	3.5	7.4	70.2	2.8	0.32	0.90	1.68	2.06	0.4	2.07
Cowan	fine sand	6.7	0.4	9.9	22.4	1.7	0.16	0.72	2.94	6.68	0	1.76

field capacity. Ten wheat (Triticum aestivum Var. Columbus) seeds were sown at 2 cm deep. After emergence, the plants were thinned to five plants per pot. The plants were subjected to a 16 hr. day and 8 hr. night regime at 21 degrees C and 17 degrees C, respectively. The relative humidity maintained was 70% and 40% during the night and day, respectively. Plants were watered to field capacity every two days.

At the boot stage of wheat, one plant chosen at random was harvested, washed and handled as described in the General Materials and Methods section. The remaining four plants were taken to maturity and harvested. Grain and straw were weighed and handled as described in the General Materials and Methods section.

After harvesting the plants at maturity, the soil from each pot was retained and handled as described in the General Materials and Methods section. These samples were designated as final samples and extractable soil copper determined from them designated as final extractable soil copper.

3. RESULTS AND DISCUSSION

3.1. COPPER CONCENTRATION OF WHEAT HARVESTED AT THE BOOT STAGE

The copper concentration of wheat grown on the untreated soils ranged from 2.5 ppm to 4.2 ppm (Table 12). This is below the critical level of 5 ppm and 4.9 ppm determined by Melsted _et al_ (1969) and McAndrew (1980), respectively. Tissue copper concentrations of whole plants on three soils (Sandilands, Graysville and Sifton) were below the critical level of 3.3 ppm

Table 12: The influence of copper fertilization on tissue copper concentration of wheat harvested at boot stage---growth chamber experiment I

Soil Location	Mean Copper Concentration of Tissue (ppm)[2]	
	No Cu added	38[1] Mg Cu added/pot
Poppleton	4.2	7.8 **
Sandilands	2.7	8.6 **
Haywood A	3.3	8.5 **
Graysville	2.5	7.3 **
Sifton	2.6	7.8 **
Dauphin	3.7	5.2 **
Treherne	3.7	4.7
Haywood B	3.5	7.9 **
Steinbach	4.0	10.0 **
Cowan	3.8	6.5 *

1 38 mg Cu/pot equivalent to 15 kg Cu/ha

2 mean of 3 replicates

**Concentration significantly higher than that of untreated pots at (P = 0.01).

*Concentration significantly higher than that of untreated pots at (P = 0.05).

determined by Gupta et al (1970). The aforementioned soils could therefore be suspected of being low in plant available copper as determined by previous researchers. It is however interesting to note that only the Cowan soil had a copper content lower than the critical level of 0.2 ppm suggested by Lindsay and Norvell (1978) for DTPA extractable soil copper. It is likely, therefore, that the critical level of 0.2 ppm for DTPA extractable copper suggested by Lindsay and Norvell (1978) is low and the critical level of 0.5 ppm determined by Edlin et al (1983) is probably more realistic.

Application of 38 mg Cu/pot increased the copper concentration of wheat tissue on all soils but not significantly on Treherne soil. Tissue concentrations of copper were greater than the critical value of 5.0 ppm established by Melsted et al (1969) except for tissue from the Treherne soil.

3.2. FINAL HARVEST

Grain yields for the non-treated soils ranged from 0 to 19.7 g/pot (Table 13). Two soils (Sandilands and Cowan) had zero grain yield indicating that wheat growing on them was severely deficient in copper.

Grain yield from the copper treated plots ranged from 13.2 g/pot to 22.6 g/pot (Table 13). Application of 38 mg Cu/pot significantly increased yield on six soils including the two soils in which there was no yield on the control. Of those soils on which wheat did not respond significantly to applied copper, grain yield either remained constant or increased slightly.

Copper deficiency has been known to prevent formation of

Table 13: The effect of copper fertilization on grain yield of wheat grown on ten soils---growth chamber experiment I

Soil Location	Mean Grain Yields gm/pot [1]		Percent Yield
	No Cu added	38 mg Cu added	
Poppleton	13.9	17.7	79
Sandilands	0	20.7 *	0
Haywood A	3.8	17.1 *	22
Graysville	3.9	20.4 *	19
Sifton	15.7	19.2 *	82
Dauphin	18.1	22.6	80
Treherne	13.3	17.1 *	78
Haywood B	12.2	13.2	92
Steinbach	19.7	19.7	100
Cowan	0	21.4 *	0

[1] Mean of 3 replicates

*Significant response in grain yield to applied copper at (P = 0.05) viable pollen which normally results in sterile heads. The two soils (Sandilands and Cowan) that had zero grain yield had sterile heads and had earlier exhibited the typical symptoms of copper deficiency in which leaf tips coil and die. These two soils could be suspected to be severely deficient of available soil copper although extractable copper for the Sandilands soil was above the critical level of 0.2 ppm suggested by Lindsay and Norvell (1978) (Table 11).

Percent grain yield was used to determine the responsiveness of the ten soils to applied soil copper. Percent yield was determined as follows :

$$\% \text{ yield} = \frac{\text{grain yield from untreated soil}}{\text{grain yield from Cu treated soil}} \times 100.$$

Two soils (Sandilands and Cowan) were the most responsive with a percent yield of 0 while another two soils (Haywood B and Steinbach) were the least responsive with percent yields of 92 and 100 respectively (Table 13). Apart from the soils that had zero percent yield, the next two most responsive soils (Haywood A and Graysville) had large increases in grain copper concentration when 38 mg Cu/pot was applied (Tables 13 and 14). The Steinbach and Haywood B soils had large increases in grain copper concentration when 38 mg Cu/pot were applied but little grain increases were observed. This suggests that the increase in grain copper concentration was not reflected in percent grain yield to be obtained when 38 mg Cu/pot was applied.

Copper concentration in grain from six soils was significantly increased by application of 38 mg Cu/pot (Table 14). Of the remaining four soils, there was no grain yield from the

Table 14: The influence of copper fertilization on grain copper concentration and uptake---growth chamber experiment I.

Soil Location	Mean Copper Concentration in Grain (ppm)[1]	
	No. Cu added	38 mg Cu added/pot
Poppleton	1.4	5.6 *
Sandilands	-	6.5
Haywood A	1.0	4.7 *
Graysville	0.5	3.8 *
Sifton	1.5	5.5 *
Dauphin	3.1	3.2
Treherne	1.2	2.5
Haywood B	1.4	4.4 *
Steinbach	1.3	5.7 *
Cowan	-	5.1

Soil Location	Copper Uptake ug/pot[1]	
	No Cu added	38 mg Cu added/pot
Poppleton	112	264 **
Sandilands	97	292 **
Haywood A	89	219 **
Graysville	122	212 *
Sifton	88	246 **
Dauphin	113	166 *
Treherne	71	160 **
Haywood B	84	146 **
Steinbach	100	263 **
Cowan	73	262 **

[1]Mean of 3 replicates

*Significantly higher than for the untreated pots at (P = 0.05)

**Significantly higher than for untreated pots at (P = 0.01) control pots of two soils. The copper concentration in grain from the remaining two soils increased slightly but not significantly when 38 mg Cu/pot was applied. In all cases except for the Dauphin soil, the grain copper concentration of untreated soils was below the critical levels of 2.0 and 2.5 ppm determined by Davies (1971) and King (1975) respectively. The fact that wheat did not respond to applied copper even when grain copper concentration increased means that there is no simple relationship between grain yield and grain copper concentration. These findings agree with Nambiar (1976) who showed that wheat may not respond to copper fertilization even when grain copper concentration is below 2.0 ppm.

The total copper uptake of wheat harvested from the untreated soils ranged from 71 ug/pot to 122 ug/pot (Table 14). The range in total copper uptake for the treated soils was between 146 ug/pot and 292 ug/pot. The application of 38 mg Cu/pot increased the copper uptake of wheat significantly on all the soils. The Dauphin soil had the least increase in copper uptake between treated and untreated soil. This is probably due to high levels of plant available soil copper in the untreated soil with the result that application of 38 mg Cu/pot increased dry matter yield and plant (both grain and straw) copper concentration only slightly. The Cowan and Sandilands soils which showed the largest response in grain yield to applied copper also responded most in terms of copper uptake resulting from copper application (Table 14).

The zinc concentration of grain from the untreated soils ranged from 14.8 ppm to 58.4 ppm while the range for grain from the copper treated soils was between 11.9 ppm and 39.3 ppm (Table 15). Zinc concentration in the grain decreased significantly on four soils when 38 mg Cu/pot was applied. Zinc concentration in grain from the other six soils showed decreasing trends when 38 mg Cu/pot were applied although these were not significant. A negative interaction between Cu and zinc has been reported by many workers (McGregor 1972, Akinyende 1978). Grain yield from Steinbach soil was not significantly affected by copper fertilization yet there was a significant decrease in zinc concentration as a result of copper fertilization. This would suggest a negative interaction between copper and zinc concentration in the grain. On some soils in which there was a significant increase in grain yield, the decrease in zinc concentration may partly be due to a dilution effect. It is also interesting to note that the significant decrease in grain zinc concentration when 38 mg Cu/pot was applied occurred on soils with high levels of extractable soil zinc.

Manganese concentration in the grain from untreated soils ranged from 14.5 ppm to 53.0 ppm (Table 16). The range for the treated soils was between 8.6 ppm to 54.2 ppm. The manganese concentration in the grain from five soils decreased significantly when 38 mg Cu/pot was applied. This is consistent with data of Tokarchuk (1982) who found that manganese concentration of the above ground portion of wheat decreased substantially when high levels of copper were applied. He attributed low grain yields of

Table 15: The influence of copper fertilization on zinc concentration in grain---growth chamber experiment I.

Soil Location	Mean Zinc Concentration ppm[1]	
	No. Cu added	38[2] mg Cu added/pot
Poppleton	27.53	20.4 *
Sandilands	-	27.4
Haywood A	48.4	21.4 *
Graysville	58.4	31.6 *
Sifton	19.3	15.3
Dauphin	53.7	39.3
Treherne	21.1	17.5
Haywood B	14.8	12.9
Steinbach	19.3	11.9 *
Cowan	-	21.2

[1] Mean of 3 replicates.
[2] 38 mg Cu/pot equivalent to 15 kg Cu/ha.
* Concentration significantly different from that of untreated pots at ($P = 0.05$).

Table 16: The effect copper fertilization on manganese and iron concentration in grain---growth chamber experiment I

Soil Location	Mean Manganese Concentration in grain (ppm)[1]	
	No. Cu added	38[2] mg Cu added/pot
Poppleton	28.20	18.3 *
Sandilands	-	54.2
Haywood A	53.0	28.9 *
Graysville	53.0	23.4 *
Sifton	14.5	8.6 *
Dauphin	27.5	19.6 *
Treherne	28.9	20.6
Haywood B	28.3	24.5
Steinbach	21.3	16.6
Cowan	-	37.5

Soil Location	Mean Iron Concentration in grain (ppm)[1]	
	No. Cu added	38 mg Cu added/pot
Poppleton	41.0	32.9 *
Sandilands	-	34.5
Haywood A	68.6	39.0 *
Grayville	63.5	37.6 *
Sifton	34.9	10.2 *
Dauphin	42.3	37.2
Treherne	40.4	40.3
Haywood B	40.9	34.8 *
Steinbach	39.5	34.0
Cowan	-	33.9

1 Mean of 3 replicates.
2 38 mg Cu/pot equivalent to 15 kg Cu/ha.
* Concentration significantly different from that of untreated pots at (P = 0.05).

wheat at one site to this negative interaction in which high levels of applied copper accentuated manganese deficiency. Reid (1982) found that copper fertilization decreased the manganese concentrations in barley and attributed the decrease to a dilution effect. For some soils reported here, there were significant decreases in the manganese concentration which were not accompanied by increases in grain yield. This would suggest that the decrease in manganese concentration would not be due to a dilution effect. The grain from the Sifton soil had very low levels of manganese compared to other soils and this may have limited grain yield on this soil.

Iron concentration of grain from untreated soil ranged from 34.9 ppm to 68.6 ppm (Table 16). The range of iron concentration in grain grown on the treated soils was between 10.2 ppm to 40.3 ppm. There was a significant decrease in iron concentration of grain from soils when 38 mg Cu/pot was applied. Akinyende (1978) found the same trend with the Fe concentration of barley tissue when large amounts of copper were applied to the soil. He attributed this decrease to a dilution effect. He also concluded that the low iron uptake that he observed at high levels of applied copper was due to zinc limiting growth. Results reported here show that iron concentration of grain from some soils (Haywood B and Poppleton) decreased significantly yet there was no grain yield increase. This would also suggest that the decrease in iron concentration may not all be attributed to a dilution effect.

3.3. RELATIONSHIPS BETWEEN COPPER CONCENTRATION IN THE SOIL AS MEASURED BY VARIOUS EXTRACTANTS AND PERCENT GRAIN YIELD OF WHEAT

Linear regression relationships were generated relating percent yield and extractable soil copper for each of five extractants. The variables for the Dauphin soil were excluded from the regression analysis and considered as outliers because the extractable soil copper was several fold higher than that for the other soils. It was also felt that the soil copper level was sufficiently high that it would mask any differences in plant Cu content that might have resulted from application of copper.

The relationships between percent yield and initial (samples taken before seeding) extractable soil copper (Table 17) indicate that 1.0 M HCl is the only extractant in which extractable soil copper was significantly related to the percent grain yield of wheat. The derived relationship could account for 76% of variation in percent yield. One-tenth molar HCl was the poorest extractant for soil copper and its extractable soil copper could account for only 5% of the variation in percent yield. This is not surprising because 0.1 M HCl is a weak extractant for soil copper and does not remove consistent amounts of soil copper from the soils. It is possible that inorganic carbonates may neutralize 0.1 M HCl and thus render it ineffective as an extractant for soil copper. One molar HCl on the other hand is a strong extractant and could extract consistent amounts of soil copper. The problem with strong extractants is that they tend to over-estimate available copper status of soils (Robson _et al_, 1981). The soils in this study were mostly sandy with low levels of copper and there was therefore little possibility of over-estimating the available soil copper. The r^2 values for the chelate extractants (0.43 for DTPA and Na_2DP and 0.24 for Na_2EDTA) were much higher than for 0.1 M HCl (r^2 = 0.05) but much lower than that for 1.0 M HCl (r^2 = 0.76).

Relationships between final extractable copper (samples taken after maturity) and percent yield, indicate that DTPA and 1.0 M HCl are almost equally good as extractants for available soil copper with r^2 values of 0.59 and 0.58, respectively (Table 18). There was no significant relationship between percent yield and final extractable soil copper determined by any of the other three extractants (0.1 M HCl, Na_2EDTA, and Na_2DP). It is however to be noted that the r^2 value for DTPA improved between initial and final sampling while for the others, virtually no relationships were found at final sampling.

From the preceding discussion, it was observed that 1.0 M HCl is a better extractant at initial sampling than at final sampling while DTPA is better at final sampling than at initial sampling. Therefore based on the data that was available, the best time of sampling is determined by the extractant used.

Linear regression analysis between yield, copper concentration and uptake as dependent variables and extractable soil copper as an independent variable were attempted. However, the extractable copper data was found to consist of two distinct groups (measurements from treated and untreated pots) separated by a gap in the data. This data was therefore found unsuitable for this type of regression analysis.

Table 17: Relationship between percent yield and initial soil copper as determined by five extractants---growth chamber experiment I

Extractant	Equation	F	r^2
DTPA	Y = 11.6 + 117.7 X	5.4	0.43
0.1 M HCl	Y = 39.3 + 69.3 X	0.4	0.05
1.0 M HCl	Y = -11.7 + 45.4 X	22.5	0.76 **
Na_2DP	Y = -19.0 + 67.7 X	5.21	0.43
Na_2EDTA	Y = -3.2 + 36.5 X	2.3	0.24

Y = Percent Yield
X = Extractable copper in ppm
** = Significant at 1% level of probability

Table 18: Relationship between percent yield and final soil copper as determined by five extractants---growth chamber experiment I

Extractant	Equation	F	r^2
DTPA	Y = -86.0 + 659.1 X	10.1	0.59 *
0.1 M HCl	Y = 57.8 - 58.0 X	0.2	0.02
1.0 M HCl	Y = -23.6 + 57.4 X	9.7	0.58 *
Na_2DP	Y = 44.9 + 12.6 X	0.1	0.01
Na_2EDTA	Y = 43.2 + 9.3 X	0.1	0.01

3.4. CRITICAL LEVELS IN SOIL AND PLANT MATERIAL

The critical level of copper in the soil was determined by the method described by Cate and Nelson (1965). This method is recommended for data generated from soils having different yeild potentials. As this experiment employed ten different soils, this method was the most applicable. The method involves first plotting percent yield against extractable soil copper for each extraction method. Two perpendicular lines--one parallel to the horizontal axis and the other parallel to the vertical axis are drawn so that there is a minimum number of observations in the upper left hand and lower right quadrants. The intersection of the vertical line with the X axis is the critical level. As described above, this method essentially separates those soils which are responsive from those that are sufficient. To ensure that the definition of the critical level as being the extractable soil copper below which yield is reduced by 10% holds, the horizontal line is fixed at 90%. The vertical line was then moved back and forth until there were a minimum number of observations in the lower right and upper left quadrants.

Before the data was subjected to the Cate and Nelson (1965) method, a linear regression analysis was conducted and only those extractants that had significant relationships were used in the Cate and Nelson (1965) method of determining the critical levels. Of the five chemical extractants used, the only significant relationship between initial extractable copper and percent yield was obtained with 1.0 M HCl. A critical level of 2.0 ppm was determined for soil copper extracted with 1.0 M HCl

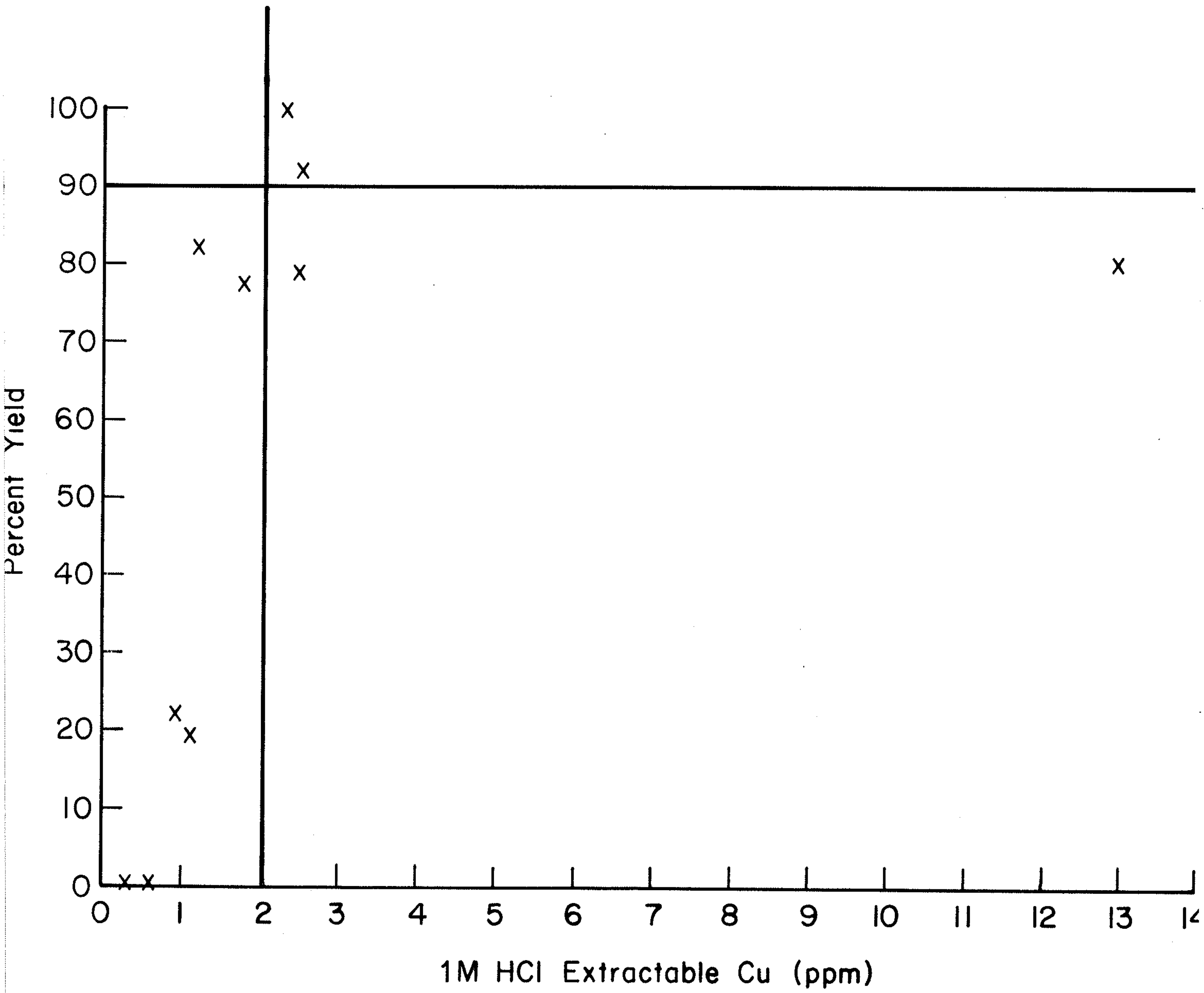
(Figure 1).

The determination of the critical levels for grain copper concentration and for wheat tissue harvested at the boot stage was attempted but there was no significant relationship between either of them and percent grain yield.

In summary, significant grain yield increases were obtained on six out of ten soils when 38 mg Cu/pot was applied. In the other four remaining soils, grain yield remained constant or increased slightly when 38 mg Cu/pot was applied. Copper uptake was however increased on all soils by application of 38 mg Cu/pot. Copper concentration of tissues harvested at the boot stage increased significantly on nine out of the ten soils. The striking feature of these results is that yield responses in the greenhouse were obtained on a soil that did not give yield increases under field conditions (Sifton soil). It was felt that yield responses to copper were obtained in the growth chamber because plants growing on the untreated soil could only exploit a small volume of soil and copper may therefore be limiting yield. This problem would not be present in the field because the plants have a greater volume of soil to exploit. Demands for soil copper may also be greater in the growth chamber where conditions of growth are controlled and more ideal for plant growth than field conditions.

Despite grain yield increases when 38 mg Cu/pot was applied to soils, relationships between extractable soil copper and percent grain yield were generally poor. However, 1.0 M HCl extractable copper from initial samples (samples taken before

Figure 1: The relationship between percent yield and initial soil copper as determined by 1.0 M HCl---growth chamber experiment I



seeding) was significantly related to percent grain yield. Hence the critical level of 2.0 ppm that was determined for 1.0 M HCl extractable copper from initial soil samples seems reasonable.

C. FIELD EXPERIMENT II - The effect of Cu and P fertilization on yield and chemical composition of wheat

1. INTRODUCTION

In Field Experiment I, copper was applied to plots that received high rates of P (79 kg P/ha at Haywood and 44 kg P/ha at Sifton). Researchers have found however, that high P fertilization may affect uptake or utilization of copper by wheat. Touchton _et al_ (1980) found that increasing phosphate fertilizer rates decreased the copper concentration in wheat plants from acceptable to marginal levels. Olsen (1972) attributed this reduction in the concentration of copper in plants to reduced absorption and transport of applied copper. This experiment was conducted to:

(a) study the effect of copper fertilization on dry matter and grain yield of wheat under field conditions;

(b) Evaluate possible interactions between high levels of applied fertilizer P and micronutrient nutrition of wheat.

2. EXPERIMENTAL PROCEDURES

This experiment was conducted on an Almasippi loamy fine sand at Haywood. This soil had been identified as marginal in copper for wheat (Growth Chamber Experiment I). Characteristics of the plot soil are shown in Table 19. The experiment was designed as a completely randomized block with five treatments and six replicates. Plots were 2.3 M x 7.6 M. The five treatments were:

Treatment 1 0 kg Cu/ha + 22 kg P/ha

Treatment 2 15 kg Cu/ha + 22 kg P/ha

Treatment 3 15 kg Cu/ha + 87 kg P/ha

Treatment 4 15 kg Cu/ha + 87 kg P/ha + 0.5 kg Cu/ha foliar

Treatment 5 0 kg Cu/ha + 87 kg P/ha

Fertilizer copper was applied in form of $CuCl_2.2H_2O$ which was dissolved in water, and sprayed on the surface. All plots received 130 kg N/ha as NH_4NO_3 and $NH_4H_2PO_4$, 42 kg K/ha as KCl and 15 kg S/ha as $CaSO_4.2H_2O$. All these fertilizers were broadcast on the surface. Also, all those plots receiving high rates of P (87 kg P/ha) had 65 kg P/ha broadcast. All broadcast fertilizers and the $CuCl_2.2H_2O$ sprayed on the surface were incorporated by rototilling to a depth of 10 cm.

Wheat (Triticum aestivum Var Benito) was seeded at the rate of 100 kg/ha together with 22 kg P/ha as $NH_4H_2PO_4$ on June 1, 1983. At the five leaf stage, 0.5 kg Cu/ha as $CuCl_2.2H_2O$ was sprayed on the plants receiving foliar copper (treatment 4). At the boot stage, tissue samples were taken from each plot for yield determination and for chemical analysis. The samples were prepared as described in the general materials and methods section. Samples were also taken at maturity to determine grain yield, total dry matter yield and the chemical composition of grain. Micronutrient composition of grain was determined on individual samples whereas the concentration of N, P, K, and S was determined on bulked samples from each treatment.

RESULTS AND DISCUSSION

(a) Soil Characteristics

The soil from Haywood on which this experiment was conducted was low in NO_3-N and extractable P (Table 19). The soil was marginal in extractable K and SO_4-S and according to the critical level suggested by Lindsay and Norvell (1978), the soil was marginal in available soil copper. The soil was also low in Zn, having a Zn concentration lower than the critical level of 1.0 ppm suggested by the same authors.

(b) Yield and Chemical Composition of Wheat at the Boot Stage

Dry matter yield at the boot stage of wheat varied between 1410 kg/ha and 1707 kg/ha (Table 20). The highest yield of 1707 kg/ha was obtained from plots receiving high rates of P (87 kg P/ha) and no copper, indicating that these soils responded to applied P and not to copper application. The application of 15 kg Cu/ha together with 0.5 kg Cu/ha as a foliar treatment reduced dry matter yield significantly over the treatment receiving 87 kg P/ha and no copper. The above discussion indicates that there was no response to the application of 15 kg Cu/ha at both levels of applied P. Only the application of 15 kg Cu/ha together with 0.5 kg Cu/ha applied as a foliar treatment significantly reduced dry matter yields relative to the treatment receiving 87 kg P/ha and no copper. Plant available copper was therefore adequate in this soil.

Copper concentration of tissue harvested at the boot stage varied between 2.7 ppm and 23.8 ppm (Table 20). Application of 15 kg Cu/ha did not influence the copper concentration of tissue harvested at the boot stage. However application of 15 kg Cu/ha in addition to 0.5 kg Cu/ha applied as a foliar treatment significantly increased the copper concentration of tissue above

Table 19: Characteristics for soil used in the field experiment II

Soil Characteristics	Value
Texture	loamy fine sand
Soil pH	7.5
Carbonate Content (%)	0.22
Organic Matter Content (%)	1.1
Salinity dSm^{-1}	0.19
NO_3-N kg/ha	11.7
Extractable P kg/ha	16.5
Extractable K kg/ha	214
Sulfate-Sulfur kg/ha	29.1
*1 Zn ppm	0.52
Cu ppm	0.26
Mn ppm	1.27
Fe ppm	2.60

Note: Values for NO_3-N, and SO_4-S were determined to a depth of 60 cm. All other values are for the 0-15 cm depth only.

*1 Zn, Cu, Mn and Fe determined by the DTPA method of Lindsay and Norvell (1978).

Table 20: The influence of copper and P fertilization on yield, nutrient concentration and uptake of wheat harvested at the boot stage---field experiment II

TREATMENT	DRY MATTER YIELD	CONCENTRATION						UPTAKE				
		ppm				PERCENT		g/ha			kg/ha	
	kg/ha	Cu	Zn	Mn	Fe	N	P	Cu	Zn	Mn	N	P
1. 0 kg Cu/ha + 22 kg P/ha	1410c[1]	2.7b	12.2a	29.5cb	86.4a	2.47a	0.17a	3.7b	16.9ab	41.1c	34.5a	2.4a
2. 15 kg Cu/ha + 22 kg P/ha	1475bc	5.0b	12.3a	26.8c	80.4a	2.11b	0.19a	7.4b	17.9a	38.8c	30.7a	2.8a
3. 15 kg Cu/ha + 87 kg P/ha	1592ab	4.7b	9.8b	30.9ab	83.2a	2.15b	0.18a	7.3b	15.3b	48.8b	33.8a	2.9a
4. 15 kg Cu/ha + 87 kg P/ha + 0.5 kg Cu/ha foliar	1493bc	23.8a	10.4b	30.4b	79.1a	2.18b	0.19a	35.0a	15.3b	44.9bc	32.1a	2.8a
5. 0 kg Cu/ha + 87 kg P/ha	1707a	3.4b	10.8b	33.5a	86.9a	2.11b	0.19a	5.7b	18.2a	56.4a	35.5a	3.2a

[1] Duncans multiple range test: values followed by the same letter are not significantly different at (P = 0.05).

all the other treatments.

Copper concentration of tissue from one treatment (treatment 1) was lower than the critical level of 3.3 ppm determined by Gupta et al (1970) while the copper concentration of tissue from two other treatments (treatments 3 and 5) was lower than the critical value of 4.9 ppm determined by McAndrew (1980). Interpretation of critical levels in the literature would indicate that tissue from three out of the five treatments would be classified as deficient. However, because there was no response to applied copper, in this experiment it is most unlikely that Cu could have limited yield.

Total copper uptake of wheat at the boot stage varied from 3.7 g/ha for the plots receiving no Cu and low P (22 kg/ha) to 35 g/ha for plots receiving the foliar copper treatment (Table 20). At both levels of applied P, copper fertilization resulted in increasing trends in copper uptake but these were not significant. Foliar applied copper increased the Cu uptake of wheat significantly above all other treatments. This would suggest that foliar application of copper is a more efficient method of applying copper to wheat than applying it to soil.

The concentration of Zn in the tissues varied from 9.8 ppm to 12.3 ppm (Table 20). There was no significant effect of copper fertilization on the Zn concentration of tissue harvested at the boot stage. However, the high rate of P (87 kg P/ha) significantly decreased the zinc concentration of tissue at the two levels of copper. Uptake data for zinc (Table 20) indicate that there was a significant decrease in zinc uptake when a high rate of P was applied at the higher level of copper. This decrease in the zinc uptake occurred together with a significant decrease in zinc concentration of wheat tissue indicating a negative interaction between zinc and P. Similar negative interactions have been reported by other researchers (Olsen 1972). This interaction did not occur where no copper was applied even though zinc concentration of tissue decreased significantly. In all the treatments, the zinc concentration of tissue was lower than the critical level of 15 ppm determined by Melsted et al (1969). This could have limited dry matter yields at this site.

Manganese concentration in tissue varied from 26.8 ppm to 33.5 ppm (Table 20). Copper fertilization did not influence manganese concentration significantly at any of the two levels of applied P. The high rate of P (87 kg P/ha) increased the Mn concentration of tissue significantly at the two levels of copper. The uptake of Mn was also increased by high P application at both levels of copper (Table 20). These observations could not be explained and no literature was available in which phosphate fertilization increased both the concentration and uptake of Mn in plant tissue.

There were no treatment effects on the concentrations of Fe and P (Table 20). It was surprising that the P concentration of tissue was not increased by high rates of P. It was however observed that high rates of P increased dry matter yield although slightly, and this increase in dry matter could have diluted the P concentration in the plant. This is so because uptake of P was not affected by either copper or P fertilization (Table 20).

The N content of tissue ranged from 2.11% to 2.47% (Table 20). Application of 15 kg Cu/ha at the low rate of P (22 kg P/ha) decreased the N content of tissue significantly. Also, application of high rate of P and/or copper reduced the N concentration of wheat significantly compared to the treatment receiving 22 kg P/ha and no applied copper. The decrease in N concentration due to copper and P fertilization could partially be due to a dilution effect noting that the N uptake (Table 20) was not influenced by the treatments while dry matter yield was increased by P fertilization.

(c) Yield and Chemical Composition of Wheat at Maturity as Influenced by Copper and P Fertilization

Grain yield in field experiment II ranged from 1380 kg/ha to 1563 kg/ha (Table 21). Application of copper at 15 kg Cu/ha did not significantly increase grain yields compared to the no copper treatments in combination with low levels of P (22 kg P/ha) or high rate of P (87 kg P/ha). Application of 87 kg P/ha did not significantly increase yield compared to 22 kg P/ha rate when copper was applied at 0 or 15 kg Cu/ha. Higher yields were obtained from the application of 15 kg Cu/ha and 87 kg P/ha relative to where 22 kg P/ha alone was applied. Foliar application of copper at tillering stage had no significant effect on the grain yield.

The grain yield at this site was low compared to grain yield on a similar soil (field experiment I) in the previous crop year. Marginal levels of Zn, P and K that were observed in the tissue harvested at the boot stage may have reduced grain yield

Table 21: The influence of copper and phosphorus fertilization on grain yield, nutrient concentration and uptake---field experiment II

TREATMENT	GRAIN YIELD	CONCENTRATION						UPTAKE					
		ppm				PERCENT		g/ha				kg/ha	
	kg/ha	Cu	Zn	Mn	Fe	N	Mg	Cu	Zn	Mn	Fe	N	Mg
0 kg Cu/ha + 22 kg P/ha	1380b[1]	3.2c	26.0a	33.2b	40.6b	3.45a	0.15b	4.5b	36.3a	45.6c	56.0b	47.5a	2.02c
15 kg Cu/ha + 22 kg P/ha	1437ab	5.2ab	25.2a	32.7b	41.1b	3.41b	0.15b	7.5a	36.3a	46.7bc	59.4ab	48.8a	2.12bc
15 kg Cu/ha + 87 kg P/ha	1563a	4.6b	20.2c	34.8b	40.1b	3.30c	0.15b	7.3a	31.8b	53.8a	62.6ab	51.5a	2.40a
15 kg Cu/ha + 87 kg P/ha + 0.5 kg Cu/ha foliar	1504ab	5.9a	20.4c	35.3b	39.8b	3.33bc	0.15b	9.2a	31.0b	52.6ab	59.9a	50.0a	2.31ab
0 kg Cu/ha + 87 kg P/ha	1459ab	2.5c	22.6b	38.9a	45.6a	3.44a	0.16a	3.7b	33.1ab	56.4a	66.2ab	50.0a	2.31ab

[1] Duncans multiple range test: values followed by the same letter are not significantly different at (P = 0.05).

at this site in field experiment II. Late planting coupled with a hot dry spell at heading probably combined to limit yield.

The concentration of copper in the grain ranged from 2.5 ppm to 5.9 ppm (Table 21). Application of 15 kg Cu/ha increased the concentration of copper in the grain from 3.2 ppm to 5.2 ppm at the low rate of P (22 kg P/ha). Fifteen kg Cu/ha also increased the copper concentration from 2.5 ppm to 4.6 ppm at the high rate of P (87 kg P/ha). Application of an additional 0.5 kg Cu/ha as a foliar spray together with 15 kg Cu/ha and 87 kg P/ha increased the copper concentration significantly over plots not receiving foliar copper. Application of the high rate of P at the low level of copper (0 kg Cu/ha) did not influence the copper concentration in the grain significantly. Application of high rate of P at the high rate of copper (15 kg Cu/ha) also did not effect the copper concentration of grain significantly. Although this interaction was not demonstrated, high P levels have been shown to decrease Cu concentration in wheat. Touchton et al (1980) found that increasing phosphate fertilizer rates, added to a high phosphate fixing soil decreased copper concentration in wheat from acceptable to marginal levels. Modestus (1984) observed the same trend when large amounts of phosphate fertilizer were applied. Olsen (1972) suggested that the reduced copper concentration when large amounts of P were applied was a result of reduced absorption and transport of applied copper.

The total copper uptake into grain at maturity ranged from 3.7 g/ha and 9.2 g/ha (Table 21). Application of 15 kg Cu/ha increased the copper uptake significantly. Copper uptake into grain was not influenced by high rates of applied P. The most dramatic increase in copper uptake was after application of 15 kg Cu/ha on the soil and 0.5 kg Cu/ha as a foliar application. This would be expected due to the very high concentration of Cu in grain resulting from the foliar application.

The zinc concentration in the grain ranged from 20.2 ppm to 26.0 ppm (Table 21). Zinc concentration in the grain was significantly reduced when 87 kg P/ha was applied compared to the lower rate of 22 kg P/ha at both levels of copper. A further significant reduction in zinc concentration occurred when 15 kg Cu/ha was applied in addition to 87 kg P/ha. This interaction has been reported by other workers and high levels of P supply are known to induce zinc deficiency in areas with marginal levels of available zinc (Olsen 1972). The zinc uptake into grain was either decreased significantly or was not affected by high P fertilization (Table 21). As there was no significant yield response to applied phosphorus, this decrease in Zn concentration cannot be explained by dilution effect. Olsen (1972) reviewed the effects of applied P on Zn concentration and suggested that this interaction could arise due to the effect of applied P on uptake, translocation and utilization of Zn in plants.

Mn concentration of grain ranged from 32.7 ppm to 38.9 ppm (Table 21). In contrast to the effect of P on Zn and Cu concentration, the high rate of P significantly increased the concentration of Mn in the grain where no copper was applied. This was also observed at the boot stage of wheat. The Mn uptake into grain was also significantly increased by high levels of P at each of the two levels of copper (Table 21). Mn^{++} being the most important Mn form in the soil, application of high rates of phosphate would be expected to reduce the Mn concentration in plant tissue just as it does for similar cations such as Cu^{++} and Zn^{++}. No literature was found in which Mn concentration was increased by high rates of P. The Mn concentration of grain was reduced significantly by application of 15 kg Cu/ha at the high rate of P. There was no significant effect of applied copper on the Mn concentration at the low rate of P. The observed reduction in Mn concentration would be expected due to competition of Mn^{2+} and Cu^{2+} during uptake.

The Fe concentration in grain ranged from 39.8 ppm to 45.6 ppm (Table 21). Application of the high rate of P (87 kg P/ha) with no copper increased the Fe concentration in the grain significantly from 40.6 ppm to 45.6 ppm. The Fe uptake of grain was not affected by high rate of P at any of the two levels of copper. This interaction could not therefore be due to dilution. Fe is mainly taken up as Fe^{++} and due to its similarity with Zn^{++} would have been expected to decrease in concentration at high levels of P just like Zn. The reduction in Fe concentration in plants due to copper fertilization has been observed (Lingle et al 1963), although this was not observed in this experiment.

The Mg concentration in grain was significantly increased from 0.15% to 0.16% by high rate of P where no copper was applied (Table 21). At either of the two levels of P, copper fertilization at 15 kg Cu/ha did not affect the concentration of Mg. The Mg uptake into grain was significantly increased by high phosphate application at the two levels of copper. This observation in which P fertilization increases the concentration of Mg in grain has not been reported in the literature.

The N concentration in grain was between 3.3% to 3.45% (Table 21). Copper fertilization at the higher level of P (87 kg P/ha) significantly reduced the N concentration in grain. The N uptake into grain was not influenced by either copper or P fertilization (Table 21). This indicates that the reduction in N content as a result of Cu fertilization was partially due to dilution effect. The high rate of P (87 kg P/ha) significantly reduced the N concentration in the grain when applied on plots receiving 15 kg Cu/ha. There was only a slight increase in grain yield when the high rate of P was applied together with 15 kg Cu/ha but this may have diluted the N content significantly.

In summary, it was demonstrated that wheat growing on this soil did not respond to applied copper under field conditions, yet it had been found to be copper deficient in the growth chamber experiment (growth chamber experiment I). Grain yield of wheat at this site was also not influenced by high phosphate application. However, 15 kg Cu/ha and high levels of P (87 kg P/ha) when applied together significantly increased grain yield.

The demonstration of a response in the growth chamber and not in the field may be due to the larger volume of the soil that plants can exploit in the field as opposed to the growth chamber. The plants growing in the growth chamber have only a small amount of soil to exploit and copper may therefore become limiting under these conditions.

Plants harvested at the boot stage were low in P, K, and Zn. All treatments except the one receiving 0.5 kg Cu/ha foliar had a copper concentration equal to or less than the critical level of 5.0 ppm determined by Melsted _et al_ (1969). P and K were applied at high application rates, and hence this should have been sufficient. Late planting and a hot dry spell at heading may have contributed to low grain yields observed at this site.

D. GROWTH CHAMBER EXPERIMENT II - The effect of different rates of Cu fertilization on yield and chemical composition of wheat and methods of evaluating availability of soil copper to wheat

1. INTRODUCTION

Field experiment I and II and growth chamber experiment I were all conducted at one level of applied copper (15 kg Cu/ha for the field experiments and 38 mg Cu/pot for the growth chamber experiment I). Responses to copper were obtained on six out of ten soils used in the growth chamber experiment. It was therefore felt necessary to have a rate trial in order to determine the response curve to applied copper more closely. A growth chamber experiment was therefore initiated in 1983 with the following objectives:

(a) to determine the response of wheat to various levels of copper under controlled environmental conditions;

(b) to determine the ability of each of five chemical extractants to reflect amounts of applied copper;

(c) to determine the ability of five chemical extractants as methods for assessing availability of soil copper to wheat.

2. EXPERIMENTAL PROCEDURES

This experiment was conducted on an Almasippi loamy fine sand obtained from the Ap horizon of a field adjacent to the Haywood field site. This soil had been found to be marginal in available soil Cu (growth chamber experiment I) but did not respond to applied copper in field experiment II. The characteristics for this soil are shown in Table 22. The soil was air-dried and sieved to remove debris. An equivalent of 5.25 kg of

Table 22: Characteristics of soil used in growth chamber Experiment II

Soil Characteristic	
Texture	loamy fine sand
Soil pH	7.3
Carbonate content (%)	0.2
Organic matter (%)	1.9
Salinity dSm^{-1}	0.17
NO_3-N (ppm)	2.7
Extractable P (ppm)	10.1
Extractable K (ppm)	10.2
Sulfate-Sulfur (ppm)	5.3
*1 Zn (ppm)	0.44
Cu (ppm)	0.24
Mn (ppm)	1.36
Fe (ppm)	3.42

*1 Zn, Cu, Mn and Fe were determined using the DTPA method of Lindsay and Norvell (1978).

oven dry soil was weighed into 6 litre plastic pots lined with polythene material.

The experiment was laid out as a completely randomized design with six treatments and three replicates. The treatments chosen were 0, 2.5, 5.0, 10.0, 15.0, and 25 kg Cu/ha applied as $CuCl_2.2H_2O$. The copper to be applied was dissolved in water, sprayed on thinly spread soil and mixed thoroughly with the soil. Each pot also received basal applications of N, P, K and Zn. Phosphorus was applied as $NH_4H_2PO_4$ at the rate of 50 ppm, N as NH_4NO_3 at 100 ppm and K applied at 100 ppm as K_2SO_4. Sulfur was applied at 41 ppm as K_2SO_4. To ensure that Zn was adequate, 38 mg Zn/pot was applied to all pots as ZnEDTA. All these nutrients were supplied in solution form on thinly spread soil and then thoroughly mixed with the soil. A soil sample equivalent to about 250 gm oven-dry soil was drawn from each pot for copper analysis and prepared as described in the general materials and methods section.

The field capacity of the soil was determined by equilibrating about 400 gm of soil with a small amount of water. The watered soil was covered with polythene material and allowed to equilibrate for 24 hours. A sample of soil was taken above the water-front and moisture determined by drying the sample at 105 degrees C to constant weight.

Wheat (*Triticum aestivum* Var Benito) was seeded at the rate of nine seeds per pot. The soils were watered to about half field capacity to ensure adequate moisture yet prevent seed rot due to high moisture. After emergence, the plants were thinned to six plants/pot and watered every two days to near field capacity. The plants were grown in a 16/8 hour light to dark cycle at a relative humidity of 70% and 40% respectively. The temperature regime was 21 degrees C and 17 degrees C for day and night, respectively.

At the boot stage of growth, one of the plants was harvested for copper analysis. The remaining five plants were grown to maturity at which time they were harvested for dry matter and grain determination. The straw and grain were analysed for both micronutrient and macronutrient content. The soil from each pot was dried and a sample for extractable copper analysis drawn and prepared as described in the General Methods and Materials section. Extractable soil copper was determined by each of the five chemical extractants described previously.

3. RESULTS AND DISCUSSION

(a) Soil Characteristics

The soil had a Cu content higher than the critical level (0.2 ppm) of Lindsay and Norvell (1978) (Table 22). However, this soil was used in this study because a yield response was demonstrated with the same soil in growth chamber experiment I even though there was no response to copper in the field (field experiment II). The soil was also low in Zn, NO_3-N and extractable phosphorus.

(b) Yield and Chemical Composition of Wheat Harvested at the Boot Stage

Dry matter yields at the boot stage were estimated from one plant per pot and ranged from 4.2 g to 4.5 g. No yield responses resulting from Cu application were apparent at this time (Table 23).

The Cu concentrations of whole plants at the boot stage ranged from 6.3 ppm to 9.9 ppm (Table 23). Copper concentrations were only increased significantly by application of 25 kg Cu/ha. The copper concentration of wheat at the boot stage was higher than the critical value of 4.9 ppm determined by McAndrew (1980) and was therefore adequate even on control pots.

The Cu uptake of wheat harvested at the boot stage ranged from 27.6 ug/pot to 42.7 ug/pot (Table 23). The copper uptake of wheat was significantly increased only when 25 kg Cu/ha was applied. However there were increasing trends in copper concentration and uptake at application rates lower than 25 kg Cu/ha.

(c) Yield and Chemical Composition of Wheat Harvested at Maturity

Grain yields of wheat varied from 22.7 g/pot to 24.2 g/pot (Table 24). Highest yields were obtained when 5, 10, 15 and 25 kg Cu/ha were applied. Significant yield increases occurred only when 10 and 15 kg Cu/ha were applied. Although there was a slight yield reduction when 25 kg Cu/ha was applied as compared to other Cu application rates, it was unlikely that Cu toxicity occurred.

Copper concentration of grain ranged from 2.4 ppm to 6.5 ppm (Table 24). Copper fertilization increased copper concentration in grain significantly when 5 kg Cu/ha or more were applied. There was no significant difference in copper concentration in grain at all levels of applied copper except at the rate of 25 kg Cu/ha which had a significantly higher concentration of Cu than

Table 23: The influence of copper fertilization on dry matter yield and copper concentration of wheat harvested at the boot stage---growth chamber experiment II

Treatment	Dry Matter g/pot	Copper Concentration (ppm)	Total Copper Uptake ug/pot
0 kg Cu/ha	4.4 a	6.3 b	27.6 b
2.5 kg Cu/ha	4.5 a	6.3 b	28.7 b
5.0 kg Cu/ha	4.2 a	6.8 b	28.8 b
10.0 kg Cu/ha	4.2 a	7.5 b	31.5 b
15.0 kg Cu/ha	4.5 a	7.5 b	33.9 b
25 kg Cu/ha	4.5 a	9.9 a	42.7 a

[1]Duncans Multiple Range Test. Values followed by the same letter within columns are not significantly different at $P = 0.05$.

Table 24: The influence of copper fertilization on grain yields, grain copper concentration and total copper uptake of wheat at maturity---growth chamber experiment II

Treatment	Grain Yield g/pot	Copper Concentration (ppm)	Total Copper Uptake ug/pot
0 kg Cu/ha	22.9 cb[1]	2.4 c	94.0 c
2.5 kg Cu/ha	22.7 c	3.7 cb	123.1 c
5.0 kg Cu/ha	24.0 ab	5.0 b	162.5 b
10.0 kg Cu/ha	24.2 a	4.7 b	173.6 ab
15.0 kg Cu/ha	24.2 a	4.8 b	170.0 ab
25 kg Cu/ha	23.6 abc	6.5 a	203.1 a

[1]Duncans Multiple Range Test. Values followed by the same letter within columns are not significantly different at $P = 0.05$.

all other treatments. All treatments had grain copper concentrations higher than the critical level of 2.0 ppm suggested by Davies et al (1971). Despite this, there was a significant increase in grain yield when 10 kg Cu/ha was applied, and thus supporting the finding from growth chamber experiment I that grain copper concentration and grain yield are not closely related.

The range in copper uptake of whole wheat plants at maturity was 94.0 to 203.1 ug/pot (Table 24). Copper fertilization increased copper uptake significantly only when 5 kg Cu/ha or more was applied. Grain yield was also significantly increased only when 10 kg Cu/ha or more was applied except at the rate of 25 kg Cu/ha. This would suggest that availability of copper was limiting grain yield at 0 and 2.5 kg Cu/ha application rates even though grain copper concentrations were above critical values of copper in wheat grain.

Copper fertilization reduced zinc concentration of grain in all cases (Table 25). The zinc uptake into grain was only significantly decreased when 2.5 kg Cu/ha was applied (Table 26). The Zn uptake at all other levels of applied Cu was not influenced by copper fertilizer. It can therefore be possible that the decrease in Zn concentration arising from copper application was mainly due to a dilution effect. Some researchers have demonstrated that high copper application rates inhibited absorption of Zn (Bowen 1969; Schmid et al 1965). This interaction could only be observed when 2.5 kg Cu/ha was applied but not in the other treatments where dilution was probably more important in

Table 25: The influence of copper fertilization on the chemical composition of grain---growth chamber experiment II

Treatment	Concentration (ppm)				Total Copper Uptake ug/pot
	Cu	Zn	Mn	Fe	
0 kg Cu/ha	2.4c[1]	38.7a	23.9a	25.5a	94.0c
2.5 kg Cu/ha	3.7cb	32.9b	22.5a	25.1a	123.1c
5.0 kg Cu/ha	5.0b	33.4b	21.4a	25.3a	162.5b
10 kg Cu/ha	4.7b	33.5b	22.5a	24.3a	173.6ab
15 kg Cu/ha	4.8b	32.9b	21.6a	26.1a	170.0ab
25 kg Cu/ha	6.5a	34.9b	23.6a	25.0a	203.1a

[1]Duncans Multiple Range Test. Values followed by the same letter within columns are not significantly different at (P = 0.05).

Table 26: The effect of copper fertilization on the micronutrient uptake into wheat grain---growth chamber experiment II

Treatment	Micronutrient Uptake ug/pot			
	Cu	Zn	Mn	Fe
0 kg Cu/ha	55.8 d[1]	887.8 a	548.6 a	585.3 a
2.5 kg Cu/ha	85.3 cd	749.5 b	512.4 a	572.1 a
5.0 kg Cu/ha	120.4 b	800.4 ab	513.6 a	606.3 a
10.0 kg Cu/ha	114.6 bc	812.7 ab	546.0 a	588.1 a
15 kg Cu/ha	115.9 bc	795.8 ab	521.2 a	631.2 a
25 kg Cu/ha	152.6 a	823.8 ab	557.7 a	590.3 a

[1]Duncans Multiple Range Test. Values followed by the same letter are not significantly different at (P = 0.05).

determining the Zn concentration of grain. These findings are consistent to findings of growth chamber experiment I and field experiment II.

The manganese concentration of grain ranged from 21.5 ppm to 23.9 ppm (Table 25). Copper fertilization did not have any significant effect on the concentration of Mn in grain. The Mn uptake into grain was also not influenced by copper fertilization (Table 26). These observations are inconsistent with results from field experiment II in which application of 15 kg Cu/ha with high rates of P (87 kg P/ha) reduced the concentration of Mn in grain significantly. This finding is also in contrast to the finding in growth chamber experiment I in which application of 38 mg Cu/pot containing 5 kg soil decreased the Mn concentration in grain significantly. Reduction in the Mn concentration of grain would be expected due to competition of Mn^{2+} and Cu^{2+} during uptake. The results of this experiment regarding Mn concentration would not have been expected and no literature supporting it was available.

The Fe concentration in grain ranged from 24.3 ppm to 26.1 ppm (Table 25). There was no significant effect on the Fe concentration of grain as a result of copper fertilization. In an earlier study, (growth chamber experiment I), the Fe concentration of grain was found to decrease significantly as a result of copper fertilization. Akinyende (1978) also found the decrease in Fe concentration as a result of copper fertilization. The data from this experiment is consistent with the finding in an earlier study (field experiment II) in which application of 15 kg Cu/ha did not have any influence on the Fe concentration of grain. The Fe uptake into wheat grain was also not affected by copper fertilization (Table 26). Fe is mainly taken up as Fe^{2+} and due to its similarity to the Cu^{2+} ion would be expected to compete with copper during uptake. It would therefore be expected to decrease in plant tissues or grain when large amounts of copper are applied to soils.

(d) Relationships between Extractable Soil Copper and Amounts of Copper Fertilizer Applied and Plant Performance---Growth Chamber Experiment II

Data from growth chamber experiment II was used to evaluate five chemical extractants for their ability to assess plant available soil copper. Soil samples were taken at seeding time and at final harvest time and analysed for extractable soil copper by five chemical extractants as described previously. The amounts of extractable copper determined by the five extractants were related mathematically to amounts of applied copper, and copper uptake of wheat at the boot stage. The extractable soil copper was also related to grain yield, grain copper concentration and total copper uptake of whole wheat plants at final harvest. The form of the mathematical relationships are indicated in the relevant sections.

(i) Relationships Between Extractable Soil Copper before Seeding and Amounts of Applied Fertilizer Copper

The amount of soil copper extracted by the five extracting solutions from soil samples taken at seeding was related to the amount of copper applied to each pot. The ability of each extractant to reflect the amount of copper applied was assessed on the basis of the r^2 values obtained. An equation of the form

Y = a + bX was used where Y was the amount of copper extracted in ppm and X was the amount of applied copper in kg/ha.

All extractants extracted copper in direct proportion to the amount of copper fertilizer applied (Table 27 and Appendix 6). The relationships between amounts of copper applied and amounts of copper extracted were all high with the exception of 0.1 M HCl (r^2 = 0.57). One molar HCl was only slightly poorer than the extractant with the highest r^2 value (Na_2DP). The three chelates used (DTPA, Na_2EDTA, and Na_2DP) had the highest r^2 values and therefore best reflected the amounts of applied copper. The form of the relationship between extractable copper and amount of applied copper is similar for these extractants and is shown for Na_2DP (Figure 2).

(ii) Relationships Between Extractable Soil Copper at Seeding Determined by Five Chemical Extractants and Copper Uptake by Wheat at the Boot Stage

The relationships between copper uptake of wheat at the boot stage and extractable soil copper at seeding were calculated, using measurements from all pots. A linear relationship of the form Y = a + bX was chosen where Y = copper uptake of wheat in ug/pot and X = extractable soil copper in ppm. The relationships between copper uptake of wheat and extractable copper by all methods were poor (Table 28). The highest r^2 value was obtained when 0.1 M HCl extractable copper was used as the independent variable.

The relationship between copper uptake of wheat and extractable soil copper determined by the other four extractants were significant but poorer than that of 0.1 M HCl with their r^2

Table 27: Relationship between the amount of copper fertilizer applied and the amount of extractable soil copper as determined by five chemical extractants---growth chamber experiment II

Extractant	Equation	F	r^2
1.0 M HCl	Y = 2.58 + 0.35X	61.7	0.79 **
0.1 M HCl	Y = 0.55 + 0.02X	21.3	0.57 **
DTPA	Y = 1.53 + 0.29X	60.1	0.79 **
Na_2EDTA	Y = 2.17 + 0.36X	74.8	0.82 **
Na_2DP	Y = 2.07 + 0.36X	77.3	0.83 **

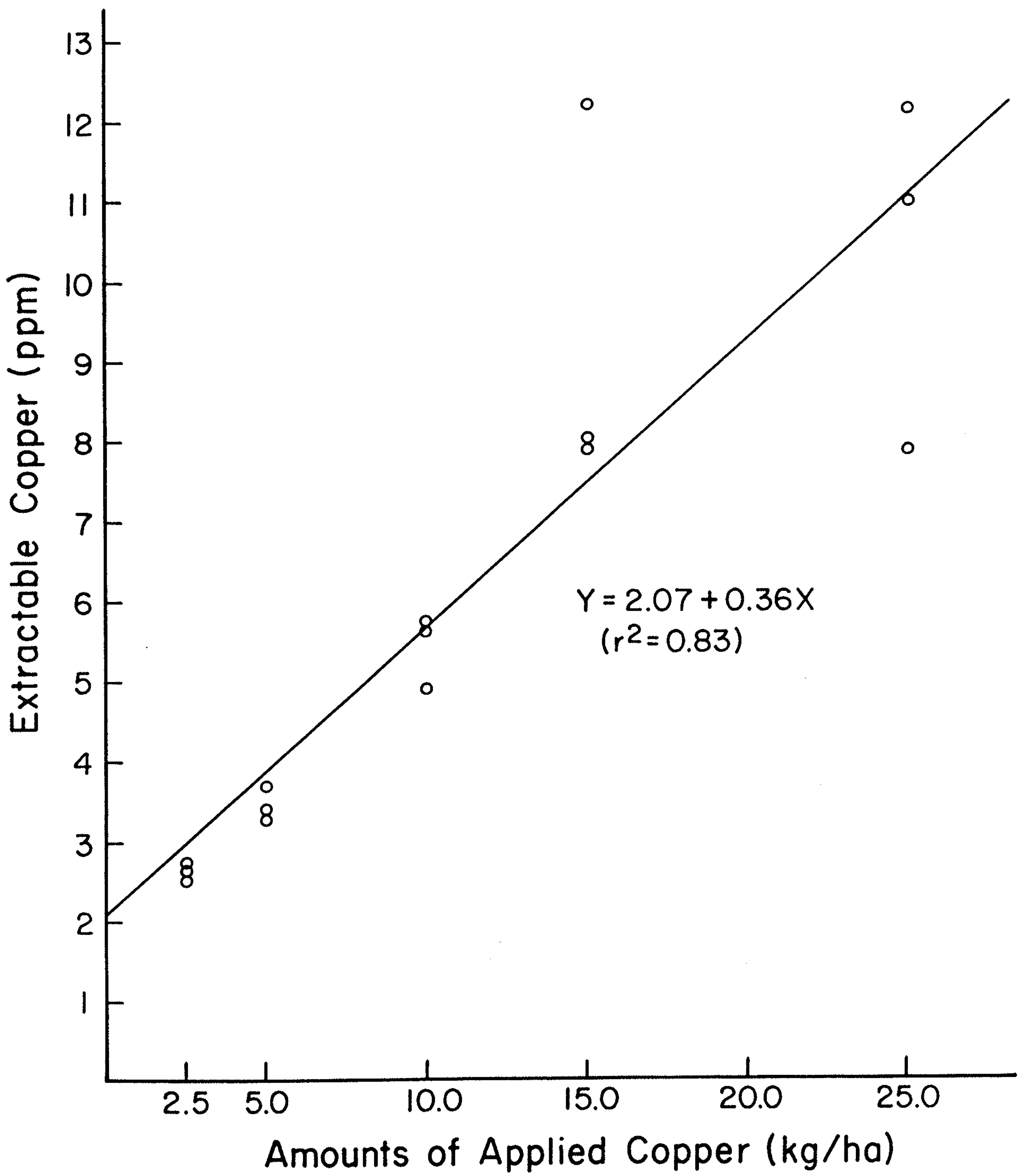
Y = extractable copper
X = applied copper in kg/ha
** significant at 1% level
* significant at 5% level

Table 28: Relationships between copper uptake at midseason and extractable soil copper at seeding as determined by five chemical extractants---growth chamber experiment II

Extractant	Equation	F	r^2
1.0 M HCl	Y = 25.02 + 1.21X	11.9	0.43 **
0.1 M HCl	Y = 14.49 + 24.91X	20.8	0.56 **
DTPA	Y = 25.93 + 1.50X	14.3	0.47 **
Na_2EDTA	Y = 25.37 + 1.22X	12.5	0.43 **
Na_2DP	Y = 25.44 + 1.22X	13.1	0.45 **

Y = copper uptake at midseason
X = extractable soil copper at seeding
* significant at 5% level of probability
** significant at 1% level of probability

Figure 2: The relationship between extractable copper by Na_2DP and amounts of applied copper---growth chamber experiment II



values ranging from 0.43 to 0.47. These other extractants had been shown in growth chamber experiment I to be better in predicting percent grain yield of wheat than 0.1 M HCl which was the poorest. Many other researchers have found poor correlations between 0.1 M HCl extractable copper and plant performance (Nelson et al 1956; McGregor 1972). Though the relationship between 0.1 M HCl extractable copper and Cu uptake of wheat had the highest r^2 value, it was not considered good enough to be of any value for diagnostic purposes. The relationship between 0.1 M HCl extractable Cu and Cu uptake of wheat at the boot stage is shown in Figure 3.

(iii) Relationships Between Grain Yield and Amounts of Extractable Soil Copper Determined at Seeding and at Final Harvest by Five Chemical Extractants

The amount of grain harvested was related to extractable copper at seeding and at final harvest. An equation of the form $Y = a + bX + cX^2$ was chosen on the basis of the higher r^2 values that were obtained against those that were obtained when a linear relationship was used. An example of the linear and quadratic plots are shown in Figure 4.

A significant relationship between grain yield and extractable soil copper was only obtained when 1 M HCl extractable soil copper was used as the independent variable (Table 29). The r^2 value obtained between 1.0 M HCl extractable soil copper and grain yield was however low ($r^2 = 0.35$) and not appreciably higher than that for the other extractants. The extractable soil copper determined by 1.0 M HCl, DTPA and Na_2EDTA from the soil samples taken at final harvest were significantly related to

Figure 3: The relationship between copper uptake at boot stage and 0.1 M HCl extractable copper at seeding---growth chamber experiment II

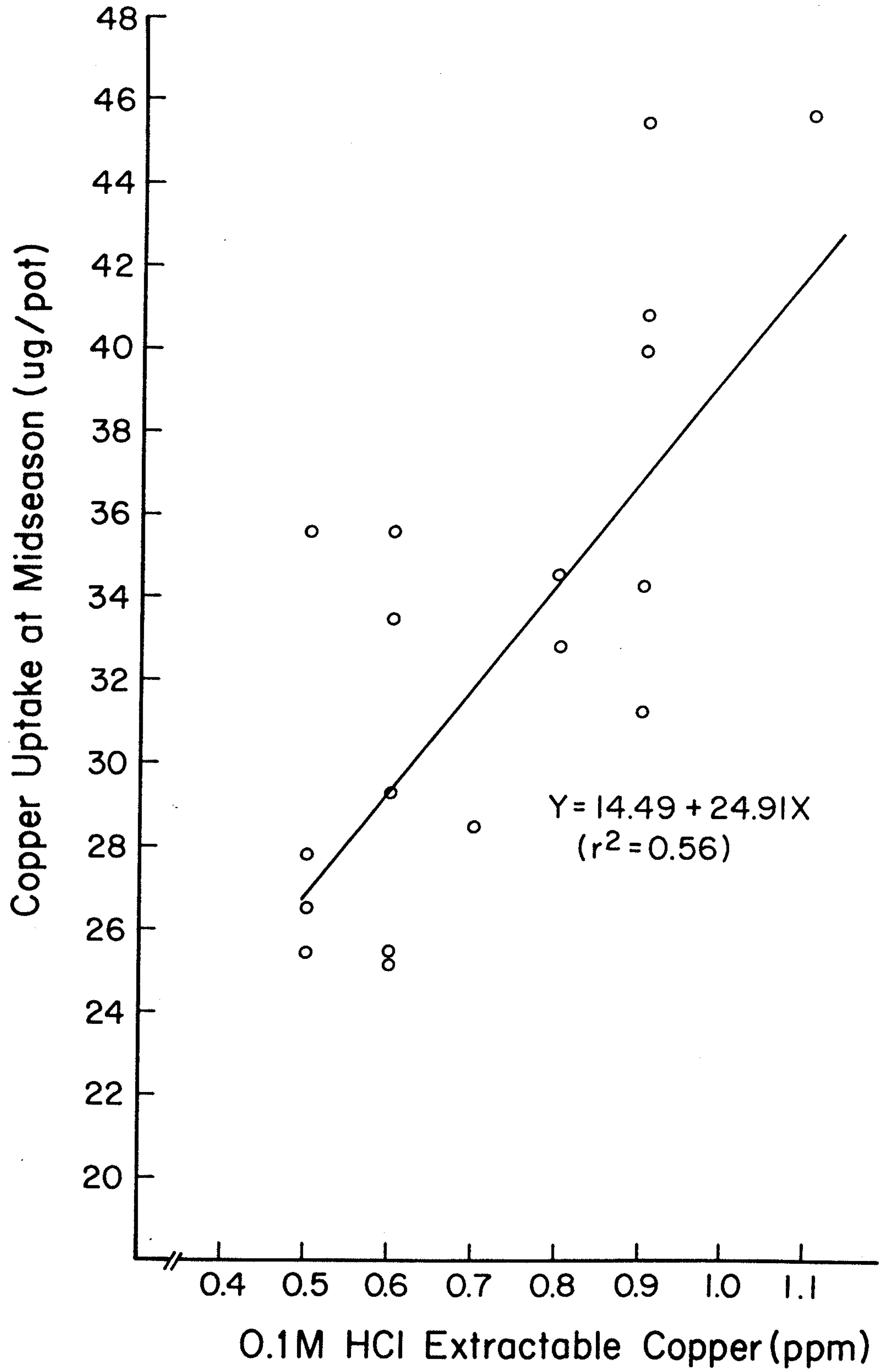
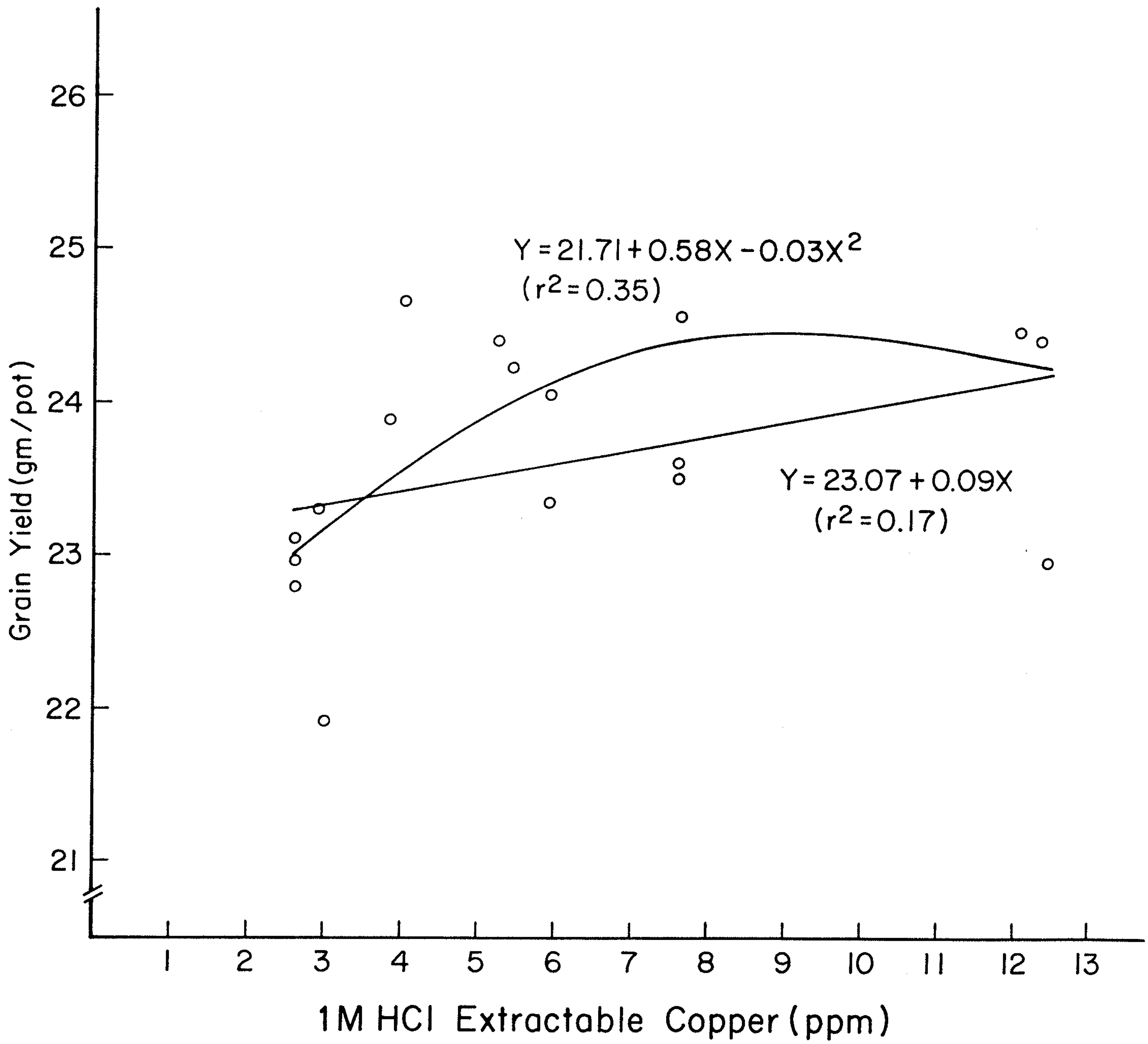


Figure 4: The relationship between grain yield and amounts of copper extracted by 1.0 M HCl at seeding---growth chamber experiment II



the grain yield of wheat (Table 30). Na_2EDTA was the best extractant with an r^2 value of 0.47 while the poorest at both sampling times was 0.1 M HCl. For each extractant, sampling at final harvest gave the best relationships between grain yield and extractable soil copper (Tables 29 and 30). This would suggest that sampling at end of the growing period is better than sampling before seeding. This however is not practical if the idea is to determine whether a soil has adequate levels of copper to sustain plant growth. It can however be used to determine whether a soil would have had adequate amounts of copper to sustain a crop.

(iv) Relationship Between Grain Copper Concentration and Extractable Soil Copper Determined by Five Chemical Extractants on Soil Samples taken before Seeding and After Final Harvest

An equation of the form $Y = a + bX + cX^2$ was used to relate grain copper concentration (Y) and extractable soil copper (X) determined by five chemical extractants. The extractable copper determined by all the extractants on the samples taken at seeding was significantly related to grain copper concentration (Table 31). One-tenth molar HCl extractable soil copper gave the poorest relationship with an r^2 value of 0.42 while the others ranged from 0.55 to 0.61. This indicates that the four other extractants are approximately equally able to predict grain copper concentration from extractable soil copper determined at seeding.

The relationships for the final harvest sampling time were generally much better than those for the seeding time sampling (Tables 31 and 32) except for Na_2DP which had a lower r^2

Table 29: Relationships between grain yield and extractable copper at seeding as determined by several extractants ---growth chamber experiment II

Extractant	Equation	F	r^2
1.0 M HCl	$Y = 21.71 + 0.58X - 0.03X^2$	4.06	0.35 *
0.1 M HCl	$Y = 18.75 + 13.26X - 8.45X^2$	1.27	0.14
Na_2EDTA	$Y = 22.29 + 0.40X - 0.02X^2$	3.01	0.28
Na_2DP	$Y = 22.17 + 0.46X - 0.03X^2$	3.47	0.31
DTPA	$Y = 22.33 + 0.55X - 0.04X^2$	3.50	0.31

Y = grain yield in gm/pot
X = extractable copper in ppm
* significant at 5% level of probability

Table 30: Relationship between grain yield and extractable copper at final harvest as determined by several extractants---growth chamber experiment II

Extractant	Equation	F	r^2
1.0 M HCl	$Y = 21.70 + 0.62X - 0.04X^2$	5.1	0.40 *
0.1 M HCl	$Y = 21.07 + 9.21X - 6.89X^2$	2.0	0.21
DTPA	$Y = 22.39 + 0.77X - 0.08X^2$	3.9	0.34 *
Na_2EDTA	$Y = 21.79 + 0.69X - 0.05X^2$	6.7	0.47 **
Na_2DP	$Y = 22.17 + 0.63X - 0.05X^2$	3.5	0.32

Y = grain yield in gm/pot
X = extractable copper in ppm
** significant at (P = 0.01)
* significant at (P = 0.05)

Table 31: Relationships between grain copper concentration and extractable copper at seeding determined by several extractants---growth chamber experiment II

Extractant	Equation	F	r^2
1.0 M HCl	$Y = 0.58 + 1.11X - 0.06X^2$	10.4	0.58 **
0.1 M HCl	$Y = 3.99 - 3.43X + 5.52X^2$	5.6	0.42 *
Na_2EDTA	$Y = 1.16 + 1.07X - 0.06X^2$	9.3	0.55 **
Na_2DP	$Y = 1.06 + 1.08X - 0.06X^2$	9.7	0.56 **
DTPA	$Y = 1.47 + 1.34X - 0.10X^2$	11.8	0.61 **

Y = grain copper concentration (ppm)
X = extractable copper in ppm
** significant at 1% level of probability
* significant at 5% level of probability

Table 32: Relationship between grain copper concentration and extractable soil copper at final harvest as determined by several extractants---growth chamber experiment II

Extractant	Equation	F	r^2
1.0 M HCl	$Y = 0.94 + 1.02X - 0.05X^2$	12.0	0.62 **
0.1 M HCl	$Y = -3.92 + 30.59X - 23.61X^2$	8.4	0.53 **
Na_2EDTA	$Y = 2.15 + 0.64X - 0.02X^2$	11.9	0.61 **
Na_2DP	$Y = 2.21 + 0.82X - 0.04X^2$	6.8	0.48 **
DTPA	$Y = 1.33 + 2.09X - 0.21X^2$	19.8	0.73 **

Y = grain copper concentration in ppm
X = extractable copper content in ppm
** significant at 1% level of probability
* significant at 5% level of probability

value for the final harvest sampling time. This same trend was observed for the relationship between extractable soil copper and grain yield. DTPA had the best r^2 value for the final harvest sampling time while Na_2DP had the poorest r^2 value. Generally however, sampling at final harvest appears to be more promising in terms of ability to predict grain copper concentration (Tables 31 and 32). However, sampling at final harvest is not practical for determining whether a soil has adequate levels of extractable soil copper. DTPA gave the best r^2 value for samples taken at seeding and would therefore be chosen as the best extractant for predicting grain copper concentration. The form of relationship between extractable Cu and grain copper concentration is shown in Figure 5.

(v) Relationship Between Total Copper Uptake of Whole Wheat Plants at Final Harvest and Extractable Soil Copper at Seeding and at Final Harvest as Determined by Five Chemical Extractants

A quadratic relationship of the form $Y = a + bX + cX^2$ was used to assess the ability of the five chemical extractants to predict total copper uptake at final harvest. One-tenth molar HCl was the poorest predictor of total copper uptake at final harvest when extractable soil copper at seeding time was used as the independent variable (Table 33). The other four extractants had almost equal r^2 values ranging from 0.67 to 0.69 which indicates that they are equally good in predicting total copper uptake of wheat at final harvest. Using final harvest extractable soil copper as the independent variable, DTPA had the highest r^2 value while 0.1 M HCl had the lowest but still significant r^2 value (Table 34). The form of the relationship between extract-

Figure 5: The relationship between grain copper concentration and extractable soil copper at final harvest---growth chamber experiment II

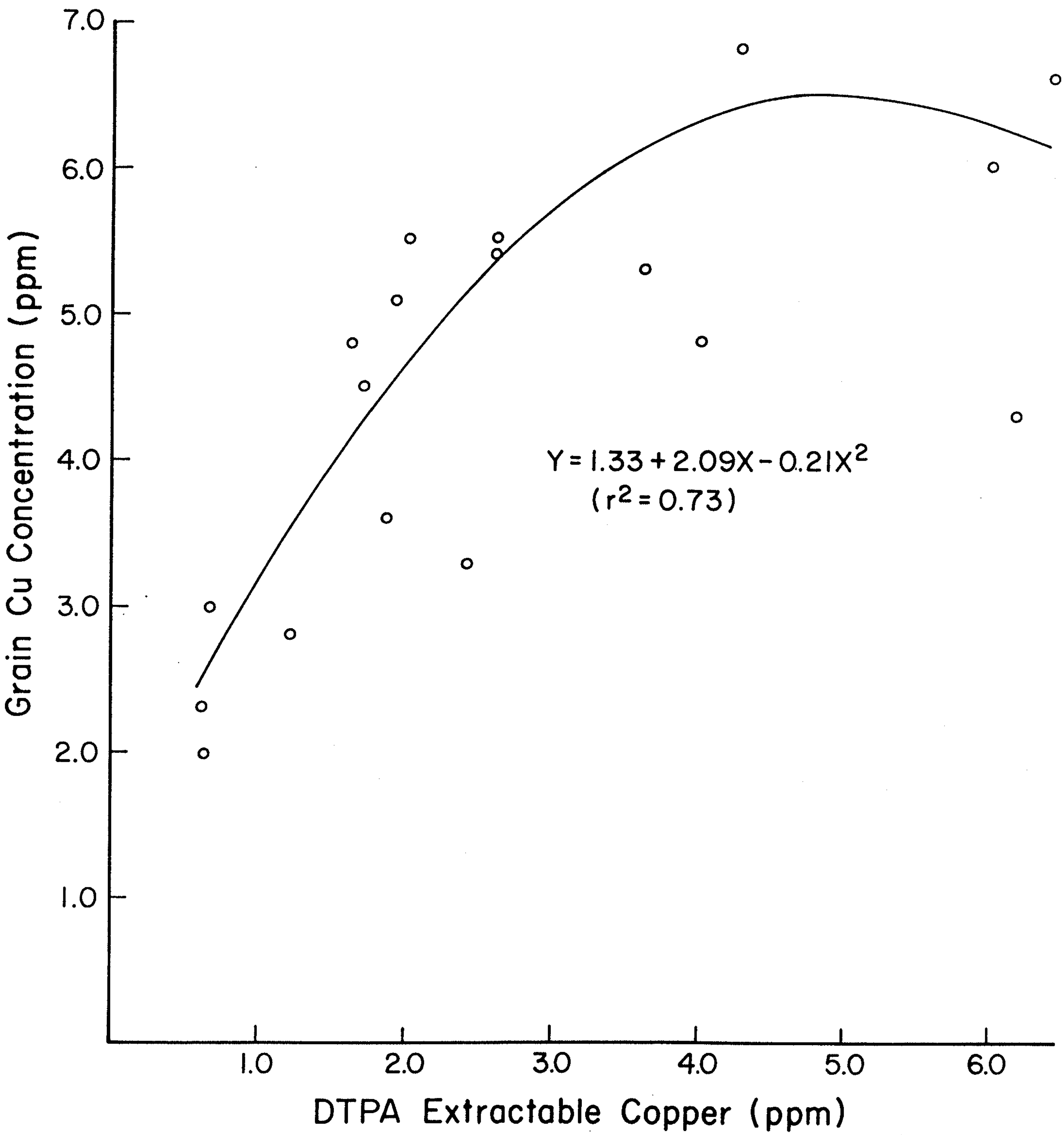


Table 33: Relationships between total copper uptake at final harvest and extractable soil copper at seeding as determined by several extractants---growth chamber experiment II

Extractant	Equation	F	r^2
1.0 M HCl	$Y = 33.29 + 33.74X - 1.71X^2$	16.9	0.69 **
0.1 M HCl	$Y = 27.07 + 210.80X - 41.84X^2$	7.3	0.49 **
Na_2EDTA	$Y = 48.35 + 32.13X - 1.74X^2$	15.2	0.67 **
Na_2DP	$Y = 46.44 + 33.24X - 1.81X^2$	18.9	0.69 **
DTPA	$Y = 59.65 + 40.66X - 2.87X^2$	15.4	0.67 **

Y = total copper uptake at harvest time in mg
X = extractable soil copper in ppm
** significant at 1% level of probability
* significant at 5% level of probability

Table 34: Relationship between total copper uptake at final harvest and extractable soil copper at final harvest as determined by several extractants---growth chamber experiment II

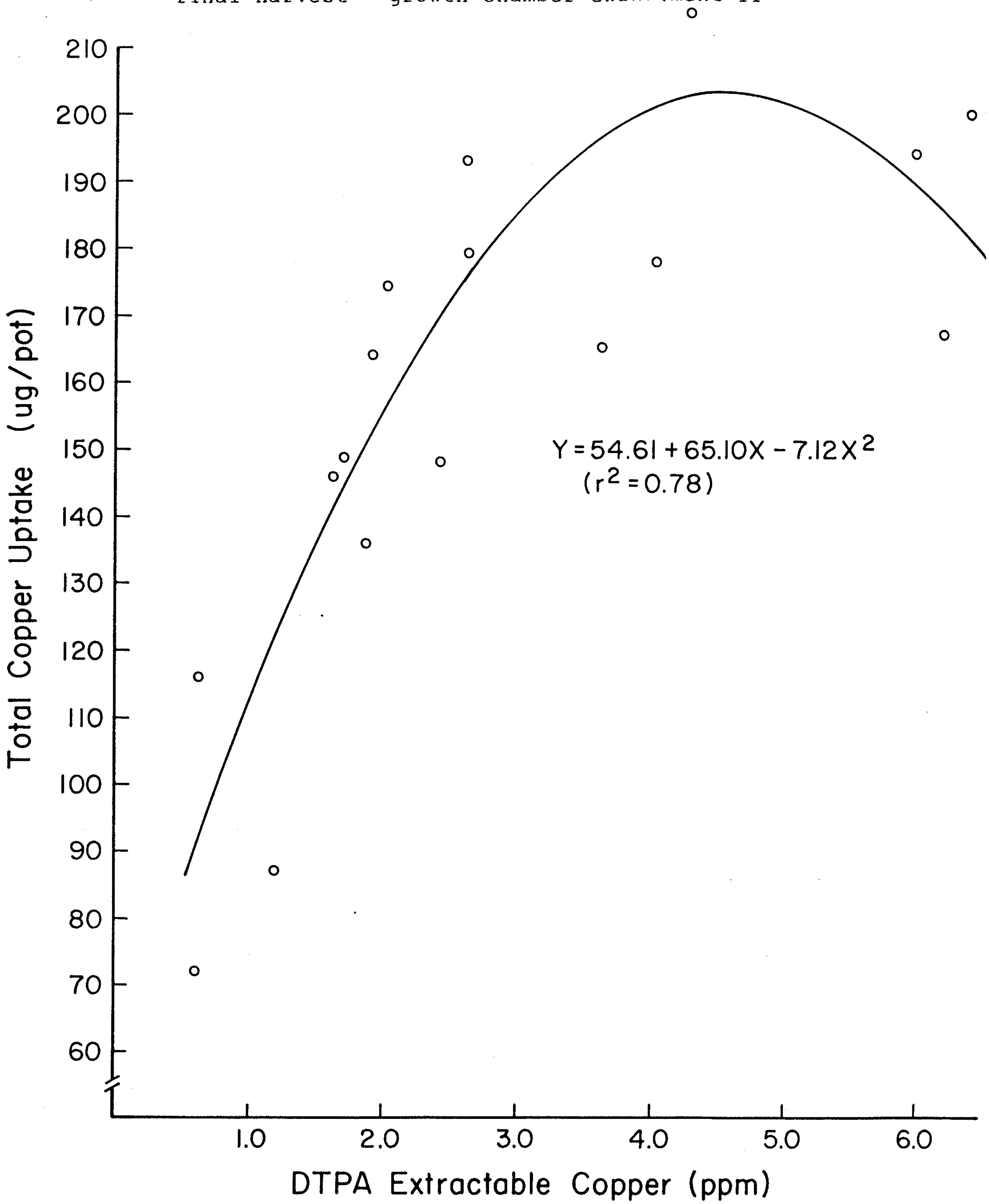
Extractant	Equation	F	r^2
1.0 M HCl	$Y = 38.50 + 33.40X - 1.74X^2$	22.3	0.74 **
0.1 M HCl	$Y = -83.42 + 849.21X - 642.54X^2$	10.2	0.57 **
Na_2EDTA	$Y = 65.19 + 27.27X - 1.36X^2$	18.8	0.71 **
Na_2DP	$Y = 63.02 + 36.13X - 2.38X^2$	12.7	0.63 **
DTPA	$Y = 63.02 + 36.13X - 2.38X^2$	26.6	0.78 **

Y = total copper uptake at final harvest mg
X = extractable soil copper in ppm
** significant at 1% level of probability
* significant at 5% level of probability

able soil Cu and copper uptake of wheat at final harvest is shown in Figure 6. Soil sampling at final harvest generally had higher r^2 values than sampling at seeding (Tables 33 and 34.)

In summary, copper fertilization increased grain yield in the growth chamber on a soil in which wheat did not respond to applied copper under field conditions. The Na_2DP extraction procedure best reflected applied copper while 0.1 M HCl was the poorest extractant in terms of ability to reflect amounts of copper applied. One molar HCl was the best extractant in terms of being able to predict grain yield from estimates of available soil copper determined at seeding. At final harvest Na_2EDTA best predicted grain yield from extractable soil copper from soil samples collected at maturity. Generally, sampling at final harvest gave the best relationships compared to sampling at seeding time. The choice of the extractant was shown to depend on the sampling period.

Figure 6: Relationship between the amounts of Cu extracted by DTPA at final harvest and total Cu uptake of wheat at final harvest---growth chamber experiment II



V SUMMARY AND CONCLUSIONS

Few field studies have been done on the effects of copper fertilization on yield and chemical composition of wheat growing on mineral soils of Manitoba. Some mineral soils have been shown to be marginal in available soil copper needed for the proper growth of plants. The extent and severity of copper deficiency on mineral soils in Manitoba is unknown. Both field and growth chamber experiments were conducted to determine the copper status of some Manitoba soils and to study the effects of copper fertilization on yield and chemical composition of wheat growing on these soils. These experiments were also intended to evaluate five chemical extractants for soil copper as means of assessing availability of soil copper to wheat.

A field experiment was set up to determine the effect of applying 15 kg Cu/ha on yield and chemical composition of wheat growing at two field sites. Dry matter yield of wheat was determined at the boot stage of growth and grain yield was determined at maturity. There was no yield response to applied copper at either of the two sites and at either of the two harvesting times. Though there was no response to applied copper, copper concentration of tissue from one site was believed to be marginal. Concentrations of copper in the grain harvested from the two sites was adequate. These soils were therefore not considered deficient in available soil copper. Due to a narrow range in the copper content of the soils used, extraction methods for assessing availability of soil copper to wheat were not evaluated.

To evaluate the copper status of more mineral soils with a wide range in extractable copper, ten mineral soils were collected from various locations in Manitoba. The effect of applying 38 mg Cu per pot containing 5.0 kg soil was studied with these ten soils. Out of the ten mineral soils, grain yield was significantly increased by application of copper on six soils. One of these soils (Sifton) had not responded to applied copper in the earlier field study. It was therefore demonstrated that copper deficiency may occur under growth chamber conditions and yet not occur under field conditions. This may be due to the smaller volume of soil that plants can exploit in the growth chamber as compared to field experiments. It may also be due to a higher demand for soil copper due to better growing conditions in the growth chamber. The suitability of five chemical extractants for soil copper as methods for assessing the soil copper status of mineral soils was investigated. One molar HCl extractable soil copper was found to correlate best with grain yield response resulting from application of copper. A critical level of 2.0 ppm was determined for 1.0 M HCl extractable soil copper. Relationships between extractable copper at seeding as determined by the other extractants and percent grain yield were not significant and soil copper critical levels for these extractants could therefore, not be determined. Soil sampling at seeding gave better relationships between soil extractable copper and grain yield response than sampling at maturity.

The effect of copper and P fertilization on yield and chemical composition of wheat was studied in a field experiment in 1983. Dry matter yields at the boot stage of wheat were not significantly influenced by application of 15 kg Cu/ha. Responses to high rates of P (87 kg P/ha) were obtained when no copper was applied but not when 15 kg Cu/ha was applied. Plants harvested at the boot stage of wheat were low in P, K and Zn. Copper concentrations were also lower than the critical level of 5.0 ppm determined by Melsted et al (1969) for all treatments except the one receiving a foliar application of 0.5 kg Cu/ha. Since P and K were supplied in adequate amounts, it is unlikely that they were limiting yield at this site. Zinc was not supplied and could have limited yields at this site. There was no dry matter response to 15 kg Cu/ha at any of the two levels of applied P.

Grain yield was not influenced by application of 15 kg Cu/ha at any of the two levels of applied P. High levels of applied P (87 kg P/ha) also did not influence grain yield at this site. However 87 kg P and 15 kg Cu/ha increased grain yield significantly indicating that both the two nutrients have to be applied together to achieve a significant grain yield increase. In this experiment, application of 15 kg Cu/ha increased copper concentration in the grain significantly. The foliar application of 0.5 kg Cu/ha together with 15 kg Cu/ha was found to be very effective in increasing the copper concentration of grain. The application of 87 kg P/ha did not have any significant influence on the copper concentration in the grain. The total copper uptake of wheat was also not influenced by application of high rates of P (87 kg P/ha).

Three of the four experiments reported in this thesis were conducted using only one level of applied copper. Growth chamber experiment II was conducted at five copper application rates so as to study the copper response curve more closely on a soil suspected to be copper deficient. Grain yield responses were obtained when 10 kg Cu/ha or more was applied except at the rate of 25 kg Cu/ha. Five kg Cu/ha supplied the copper requirements of wheat sufficiently.

This growth chamber experiment was also intended to study methods for assessing availability of soil copper to wheat. Out of five extraction procedures for soil copper, Na_2DP was found to reflect amounts of applied copper best. The 0.1 M HCl extraction procedure was poorest in terms of ability to reflect amounts of applied copper.

The 1.0 M HCl extraction procedure was best able to predict grain yield from estimates of available soil copper determined at seeding. The Na_2EDTA extraction procedure best predicted grain yield from extractable soil copper determined from soil samples taken at maturity. Both relationships were poor but significant and this indicates that the choice of the extraction procedure may depend on the sampling time.

The extractable soil copper content from initial soil samples (samples taken at seeding) determined by the various chemical extractants was more closely related to copper concentration in grain than to grain yield. The relationships were further improved when soil sampling was done at final harvest.

Copper uptake of wheat was the plant parameter that was best related to extractable soil copper content. As with the copper concentration in grain, copper uptake of wheat was best related to extractable soil copper determined from soil samples taken at maturity. Generally, it was found that sampling at final harvest gave better relationships than sampling at seeding time.

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VII APPENDIX

APPENDIX 1: YIELD, COPPER CONCENTRATION AND UPTAKE OF WHEAT AT MIDSEASON AND AT MATURITY AT HAYWOOD---FIELD EXPERIMENT 1

TREATMENT	REPLICATE	MIDSEASON HARVEST		FINAL HARVEST			
		Yield gms/plot	Copper Concentration ppm	Total Dry Matter gms/plot	Grain Yield gms/plot	Copper Concentration	
						Grain ppm	Straw ppm
0 kg Cu/ha	1	354.12	2.20	542	164	3.25	1.0
	2	388.52	2.10	592	218	3.0	1.50
	3	219.86	3.00	631	245	4.50	1.25
	4	312.87	2.50	610	249	3.25	1.25
	5	282.76	2.00	487	178	5.75	1.75
	6	218.87	2.25	606	194	8.00	1.25
	7	224.27	3.10	290	111	5.25	1.25
	8	128.45	2.50	210	76	3.75	2.25
	9	287.00	3.35	466	176	5.25	2.50
	10	249.90	3.35	344	125	5.25	3.00
	11	381.82	3.05	458	164	3.75	2.25
	12	162.27	2.90	333	133	4.00	1.75
15kg Cu/ha	1	352.78	4.15	494	168	4.00	2.25
	2	330.74	3.55	549	188	4.50	2.00
	3	458.61	4.15	474	193	4.75	2.00
	4	250.68	5.45	595	234	4.75	2.25
	5	313.81	4.10	535	215	4.50	2.25
	6	247.36	3.25	366	140	5.50	1.75
	7	268.82	4.25	447	173	6.00	2.00
	8	175.29	4.55	210	75	6.50	3.25
	9	174.33	4.90	433	160	6.75	2.25
	10	254.34	4.55	447	118	5.50	3.25
	11	256.78	4.50	387	146	4.50	3.75
	12	335.10	3.90	283	103	4.25	2.75

APPENDIX 2: YIELD, COPPER CONCENTRATION AND CU UPTAKE OF WHEAT AT MIDSEASON AND AT MATURITY AT SIFTON---FIELD EXPERIMENT 1

TREATMENT	REPLICATE	MIDSEASON HARVEST		FINAL HARVEST			
		Yield gms/plot	Copper Concentration ppm	Total Dry Matter gms/plot	Grain Yield gms/plot	Copper Concentration	
						Grain ppm	Straw ppm
0 kg Cu/ha	1	158	6.00	211	63	2.75	2.00
	2	188	6.85	332	96	2.00	1.75
	3	148	3.60	334	108	2.75	2.00
	4	177	3.10	333	100	2.25	2.00
	5	72	3.05	239	78	3.25	2.25
	6	100	7.70	384	196	5.00	2.50
	7	170	3.60	202	68	2.75	1.75
	8	88	4.55	267	87	3.50	2.50
	9	96	4.25	317	108	3.50	2.00
	10	163	4.10	373	123	3.50	2.25
	11	100	3.05	361	120	4.25	2.00
	12	187	3.65	339	105	4.4	2.00
15kg Cu/ha	1	169	6.95	297	88	5.00	3.75
	2	141	4.70	268	81	6.25	3.00
	3	164	5.60	369	102	6.00	3.00
	4	160	4.00	293	80	5.25	3.00
	5	137	8.40	277	74	5.75	3.25
	6	121	6.50	274	93	2.25	2.75
	7	118	4.60	288	91	5.75	3.25
	8	134	7.90	203	66	6.75	3.25
	9	127	6.55	381	118	5.50	3.00
	10	149	6.20	340	107	5.00	3.00
	11	136	6.05	286	89	5.75	3.00
	12	136	6.10	423	146	6.75	2.75

APPENDIX 3: CHEMICAL COMPOSITION OF GRAIN AS INFLUENCED BY COPPER FERTILIZATION---GROWTH CHAMBER EXPERIMENT I

Soil Locations	Cu ppm		Zn ppm		Mn ppm		Fe ppm		Ca %		Mg %		K %		P %		S %		N %	
	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu	-Cu	+Cu
1. Poppleton	1.36	5.6	27.53	20.37	28.20	18.26	41.0	32.86	0.11	0.11	0.18	0.14	0.42	0.35	0.57	0.37	0.24	0.16	4.03	2.83
2. Sandilands	-	6.53	-	27.37	-	54.2	-	34.47	-	0.10	-	0.14	-	0.41	-	0.45	-	0.16	-	2.84
3. Haywood A	0.97	4.67	48.43	21.37	50.03	28.93	68.63	38.97	0.12	0.10	0.20	0.14	0.48	0.35	0.66	0.40	0.25	0.17	4.00	3.28
4. Graysville	0.5	3.8	58.43	31.60	52.97	24.43	63.47	37.60	0.12	0.11	0.22	0.14	0.48	0.39	0.66	0.43	0.24	0.19	3.16	3.51
5. Sifton	1.5	5.53	19.27	15.27	14.53	8.63	34.93	10.20	0.11	0.11	0.15	0.14	0.41	0.39	0.47	0.43	0.21	0.18	3.51	2.94
6. Dauphin	3.1	3.2	53.70	39.33	27.53	19.60	42.33	37.20	0.09	0.10	0.12	0.12	0.39	0.36	0.36	0.38	0.18	0.20	3.53	2.98
7. Treherne	1.2	2.53	21.10	17.47	28.93	20.60	40.37	40.33	0.13	0.12	0.15	0.15	0.43	0.34	0.50	0.45	0.21	0.23	3.46	3.23
8. Haywood B	1.37	4.43	14.77	12.93	28.33	24.53	40.86	34.77	0.11	0.10	0.12	0.14	0.29	0.33	0.31	0.31	0.19	0.20	3.23	3.21
9. Steinbach	1.27	5.70	19.27	11.93	16.0	16.6	39.53	34.03	0.10	0.12	0.11	0.14	0.34	0.40	0.36	0.42	0.19	0.21	3.06	3.02
10. Cowan	-	5.1	-	21.2	-	37.53	-	33.93	-	0.12	-	0.14	-	0.37	-	0.45	-	0.19	-	2.53

- Cu No copper added
+ Cu 38 mg Cu added/pot
Mean of 3 replicates

APPENDIX 4: AMOUNTS OF COPPER EXTRACTED BY FIVE CHEMICAL EXTRACTANTS FROM INITIAL[1] SAMPLES OF THE UNFERTILIZED POTS---GROWTH CHAMBER EXPERIMENT I

Soil Location	CONCENTRATION (ppm)				
	DTPA	Na_2DP	Na_2EDTA	1 M HCl	0.1 M HCl
Poppleton	0.34	1.5	2.5	2.4	0.2
Sandilands	0.10	0.6	1.0	0.3	0.2
Haywood A	0.44	0.9	1.2	0.9	0.3
Graysville	0.30	1.1	2.1	1.1	0.1
Sifton	0.28	0.6	1.3	1.1	0.1
Dauphin	2.62	5.4	7.2	12.9	1.6
Treherne	0.28	1.0	1.2	1.7	0.1
Haywood B	0.44	1.6	1.7	2.4	0.1
Steinbach	0.86	1.5	1.9	2.2	0.5
Cowan	0.08	0.7	0.8	0.6	0.1

[1]Soil samples taken before seeding.

APPENDIX 5: AMOUNTS OF COPPER EXTRACTED BY FIVE CHEMICAL EXTRACTANTS FROM FINAL[1] SAMPLES OF THE UNFERTILIZED POTS--GROWTH CHAMBER EXPERIMENT I

Soil Location	CONCENTRATION (ppm)				
	DTPA	Na_2DP	Na_2EDTA	1 M HCl	0.1 M HCl
Poppleton	0.28	0.7	2.0	1.6	0.2
Sandilands	0.21	0.4	1.0	0.4	0.2
Haywood A	0.19	0.6	1.2	1.5	0.2
Graysville	0.15	1.3	1.5	1.5	0.0
Sifton	0.22	0.9	0.7	1.5	0.0
Dauphin	2.07	5.4	6.6	11.5	1.4
Treherne	0.22	0.6	0.6	1.4	0.0
Haywood B	0.26	0.5	0.7	6.1	0.0
Steinbach	0.23	0.3	1.0	1.5	0.2
Cowan	0.13	0.0	0.1	0.4	0.0

[1]Soil samples taken after maturity.

APPENDIX 6: THE INFLUENCE OF COPPER AND PHOSPHORUS FERTILIZATION ON THE YIELD AND CHEMICAL COMPOSITION OF WHEAT TISSUE HARVESTED AT THE BOOT STAGE---FIELD EXPERIMENT II

Treatment	Yield kg/ha	CONCENTRATION										Total Copper Uptake g/ha
		PPM				PERCENT						
		Cu	Zn	Mn	Fe	Ca	Mg	K	P	S	N	
1. 0 kg Cu/ha + 22 kg P/ha	1410c[1]	2.7b	12.2a	29.5cb	86.4a	0.51a	0.23a	1.02a	0.17a	0.16a	2.47a	3.7b
2. 15 kg Cu/ha + 22 kg P/ha	1475bc	5.0b	12.3a	26.8c	80.4a	0.45a	0.22a	0.75a	0.19a	0.17a	2.11b	7.4b
3. 15 kg Cu/ha + 87 kg P/ha	1592ab	4.7b	9.8b	30.9ab	83.2a	0.45a	0.23a	0.80a	0.18a	0.17a	2.15b	7.3b
4. 15 kg Cu/ha + 87 kg P/ha + 0.5 kg Cu foliar	1493bc	23.8a	10.4b	30.4b	79.1a	0.46a	0.23a	0.85a	0.19a	0.18a	2.18b	35.0a
5. 0 kg Cu/ha + 87 kg P/ha	1707a	3.4b	10.8b	33.5a	86.9a	0.50a	0.24a	0.85a	0.19a	0.17a	2.11b	5.7b

[1]Duncans Multiple Range Test: Values followed by same letter are not significantly different at (P = 0.05)

APPENDIX 7: THE INFLUENCE OF COPPER AND PHOSPHORUS FERTILIZATION ON GRAIN YIELD AND CHEMICAL COMPOSITION OF GRAIN AT FINAL HARVEST---FIELD EXPERIMENT II

Treatment	Grain Yield kg/ha	CONCENTRATION PPM Cu	Zn	Mn	Fe	PERCENT Ca	Mg	K	P	S	N	Total Copper Uptake gm/ha
1. 0 kg Cu/ha + 22 kg P/ha	1380b[1]	3.2c	26.0a	33.2b	40.6b	0.07a	0.15b	0.44a	0.29a	0.24a	3.45a	8.8c
2. 15 kg Cu/ha + 22 kg P/ha	1437ab	5.2ab	25.2a	32.7b	41.1b	0.07a	0.15b	0.39a	0.29a	0.23a	3.41ab	14.3b
3. 15 kg Cu/ha + 87 kg P/ha	1563a	4.6b	20.2c	34.8b	40.1b	0.07a	0.15b	0.42a	0.30a	0.23a	3.30c	12.8b
4. 15 kg Cu/ha + 87 kg P/ha + 0.5 kg Cu/ha foliar	1504ab	5.9a	20.4c	35.3b	39.8b	0.07a	0.15b	0.41a	0.31a	0.23a	3.33bc	18.0a
5. 0 kg Cu/ha + 87 kg P/ha	1459ab	2.5c	22.6b	38.9a	45.6a	0.07a	0.16a	0.43a	0.30a	0.24a	3.44a	9.3c

[1]Duncans Multiple Range Test: Values followed by the same letter are not significantly different at (P = 0.05).

APPENDIX 8: AMOUNTS OF COPPER EXTRACTED USING FIVE CHEMICAL EXTRACTANTS FROM POT SOILS AT SEEDING---GROWTH CHAMBER EXPERIMENT II

TREATMENT	REPLICATE	1.0 M HCl (ppm)	Na_2EDTA (ppm)	Na_2DP (ppm)	DTPA (ppm)	0.1 M HCl (ppm)
CONTROL	1	2.6	2.1	2.2	1.44	0.7
	2	2.6	2.1	2.2	1.44	0.5
	3	2.6	2.1	2.2	1.44	0.5
2.5 kg Cu/ha	1	3.0	3.2	2.6	1.90	0.5
	2	3.0	2.7	2.5	1.88	0.9
	3	2.9	2.6	2.7	1.72	0.6
5.0 kg Cu/ha	1	4.0	3.4	3.3	2.30	0.5
	2	3.8	3.5	3.4	2.46	0.5
	3	5.9	3.6	3.7	2.44	0.6
10 kg Cu/ha	1	5.9	5.6	5.7	4.50	0.6
	2	5.2	5.2	4.9	3.74	0.6
	3	5.4	5.9	5.6	4.08	0.9
15 kg Cu/ha	1	7.6	8.1	8.0	5.94	0.8
	2	12.3	12.1	12.2	9.48	0.9
	3	7.6	8.0	7.9	6.24	0.8
25 kg Cu/ha	1	12.4	10.8	11.0	9.16	1.1
	2	12.0	12.0	12.1	9.78	0.9
	3	7.6	7.7	7.9	6.04	0.9

APPENDIX 9: AMOUNTS OF COPPER EXTRACTED BY FIVE CHEMICAL EXTRACTANTS FROM POT SOILS AT FINAL HARVEST---GROWTH CHAMBER EXPERIMENT II

TREATMENT	POT NUMBER	1.0 M HCl (ppm)	Na_2EDTA (ppm)	Na_2DP (ppm)	DTPA (ppm)	0.1 M HCl (ppm)
CONTROL	1	2.3	1.9	1.5	0.62	0.3
	2	1.9	2.0	1.8	0.66	0.3
	3	2.3	1.7	1.7	0.62	0.3
2.5 kg Cu/ha	1	2.9	2.2	1.8	1.22	0.4
	2	3.0	2.4	1.9	1.60	0.3
	3	3.0	2.3	2.3	1.84	0.3
5.0 kg Cu/ha	1	3.9	3.1	1.8	1.72	0.4
	2	4.0	3.4	2.6	1.98	0.5
	3	4.4	3.2	2.7	1.90	0.4
10 kg Cu/ha	1	5.8	5.2	3.2	2.62	0.4
	2	5.4	4.6	4.6	2.42	0.4
	3	6.1	5.0	4.2	2.62	0.4
15 kg Cu/ha	1	8.4	6.9	4.2	3.62	0.6
	2	12.7	7.5	5.8	6.16	0.8
	3	7.9	7.0	6.1	3.98	0.6
25 kg Cu/ha	1	11.6	11.3	6.1	4.26	0.7
	2	11.5	11.7	8.9	5.96	0.8
	3	11.6	12.0	9.5	6.36	0.7

APPENDIX 10: EFFECT OF COPPER FERTILIZATION ON YIELD AND COPPER CONCENTRATION OF WHEAT---GROWTH CHAMBER EXPERIMENT II

TREATMENT	POT NUMBER	BOOT STAGE HARVEST		FINAL HARVEST			
		Dry Matter (g/pot)	Copper (ppm) Concentration	Total Dry Matter g/pot	Grain Yield g/pot	Straw Copper Concent. (ppm)	Grain Cu Concent. (ppm)
CONTROL	1	3.79	7.5	48.34	22.92	2.5	2.3
	2	4.81	5.5	45.91	22.79	1.1	3.0
	3	4.63	6.0	48.75	23.08	1.0	2.0
2.5 kg Cu/ha	1	4.19	6.3	47.81	21.91	1.0	2.8
	2	5.08	6.8	46.86	23.01	1.5	4.8
	3	4.34	5.8	49.31	23.32	2.0	3.6
5.0 kg Cu/ha	1	3.39	7.5	51.61	24.66	1.4	4.5
	2	5.09	7.0	47.89	23.86	1.8	5.5
	3	4.24	6.0	48.48	23.33	1.8	5.1
10 kg Cu/ha	1	4.18	8.0	50.09	24.09	1.9	5.4
	2	4.06	7.3	51.36	24.39	2.5	3.3
	3	4.29	7.3	49.28	24.23	2.4	5.5
15 kg Cu/ha	1	4.60	7.5	50.22	23.57	1.5	5.3
	2	4.57	7.5	48.45	24.39	2.6	4.3
	3	4.39	7.5	50.62	24.54	2.3	4.8
25 kg Cu/ha	1	3.71	12.3	47.62	22.96	2.4	6.8
	2	3.87	9.3	49.37	24.43	1.9	6.0
	3	5.82	8.0	47.08	23.51	1.9	6.6