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EFFECT OF RATE AND METHOD OF PLACEMENT OF $CuSO_4$ AND $ZnSO_4$ ON DRY MATTER YIELD AND NUTRIENT UPTAKE OF BARLEY (*HORDEUM VULGARE* L. VAR CONQUEST)

by

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ABSTRACT

Copper sulphate ($CuSO_4 \cdot 5H_2O$) at rates varying from 0 to 1000 ppm Cu and $ZnSO_4 \cdot 7H_2O$ at rates varying from 0 to 2000 ppm Zn were incubated for 7 days with Pine Ridge sand (Degraded Eutric Brunisol) and Lakeland clay loam (Gleyed Carbonated Rego Black), respectively. The higher rates were to simulate band application, whereas the lower rates were to simulate thorough mixing with the soil. The proportions of applied Cu and Zn extracted with water were very small (0.22-5.0%), were not appreciably affected by time of incubation, and decreased with increasing concentration of applied Cu or Zn. The proportions of applied Cu and Zn extracted with DTPA were considerably more (50 - 95%) than the proportions extracted with water, were not appreciably affected by rate and decreased slightly with time. The high proportions of applied Cu and Zn which were DTPA extractable suggested that much of the Cu and Zn which was not H_2O soluble was absorbed or complexed and therefore potentially plant available. Since the porportions of applied Cu and Zn which were H_2O or DTPA extractable, did not increase with increasing Cu and Zn, there was no evidence that banding Cu and Zn sulphates would increase their chemical availabilities.

The effect of rate and method of placement of $CuSO_4$ and $ZnSO_4$ into Pine Ridge sand and Lakeland clay loam, respectively, upon the growth and nutrient content of barley (<u>Hordeum vulgare</u> L.) were investigated in growth chamber studies. Concentration and total uptake of Cu and Zn into six week old barley shoots indicated that the most effective method of application of both $CuSO_4$ and $ZnSO_4$ was mixing throughout the soil, followed by banding with the seed which was more effective than banding below the seed. Applying $CuSO_4$ or $ZnSO_4$ in a point below the seed was not effective in increasing Cu or Zn uptake. Plant Cu and Zn concentrations increased more than dry matter yield as rates of Cu and Zn sulphates were increased. In addition, mixing $CuSO_4$ or $ZnSO_4$ with the soil was not more effective than banding with the seed in increasing dry matter yield. The failure of dry matter yield to respond to micronutrient fertilization as much as Cu and Zn uptake resulted at least partially from Zn deficiency in the Cu experiment and Fe deficiency in the Zn experiment. Application of $CuSO_4$ decreased total Zn uptake from Pine Ridge sand to the extent that most plants did not contain enough Zn for their nutritional needs. Pine Ridge sand was not only deficient in Cu but also marginal in its ability to supply Zn to barley. Application of $ZnSO_4$ to Lakeland clay loam decreased total Fe to the extent that most plants were Fe deficient. Lakeland clay loam was therefore marginally deficient in Fe in addition to being deficient in Zn. Those additional Zn and Fe deficiencies made it impossible to determine optimal application rates for $CuSO_4$ and $ZnSO_4$ or to accurately determine plant Cu and Zn critical levels. Nevertheless, the critical Cu concentration in six week old barley shoots was estimated at 5.2 ppm and the critical Zn concentration at 12.5 ppm.

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I. PREFACE

Micronutrients are just as essential for plant growth as macroelements. Notwithstanding the fact that micronutrients are required for plant growth in amounts considerably lower than that of macronutrients required, the metabolism of plants is still strongly affected by the nutritional levels of microelements. Copper and Zn have been established as two micronutrients which constitute potential nutrient deficiency problems in Manitoba soils. Previous field and greenhouse research workers have established that Cu deficiencies occur mainly on acidic, leached, sandy Podzolic and Gray Luvisolic mineral soils as well as organic soils of south-eastern Manitoba, whereas Zn deficiencies are prevalent on soils with high carbonate contents.

Previous researchers have also diagnosed soil environmental factors, such as the levels of micronutrients and/or macronutrients, carbonates, organic matter, hydrous oxides of Al, Fe and Mn, etc., which are capable of accentuating Cu and Zn deficiencies. Apart from delving extensively into chemistry of Cu and Zn in soils, these workers also investigated with useful results, appropriate diagnostic extraction methods for assessing the levels of plant available soil Cu and Zn. They recommended suitable organic and inorganic Cu and Zn fertilizer carriers which, when properly applied to soils, can supply adequate Cu and Zn to plants. However, most of these previous investigations left some questions unanswered in that they did not delve deeply enough into which methods or rates of application of their recommended Cu and Zn fertilizer carriers would be most appropriate. Moreover, investigations were concentrated on crops such as corn or field beans which are particularly susceptible to micronutrient deficiencies, thus neglecting important cereal crops such as barley and oats probably because of general fear that these crops might not bring about fruitful and conclusive research findings. This fear probably arose from the general assumption that these cereal crops are not as sensitive to micronutrient deficiencies.

Experiments were conducted, therefore, to:

(1) Assess the effect of time and method of placement of $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$ on their chemical availabilities;

(2) Evaluate the influence of rates and methods of placement of $CuSO_4$ and $ZnSO_4$ on the yield and nutrient uptake of barley plants;

(3) Establish the critical levels of Cu and Zn in barley plants below which deficiencies of these micronutrients become inevitable.

II. REVIEW OF THE LITERATURE

A. IMPORTANCE OF Cu AND Zn IN HIGHER PLANTS

Copper is very much involved in the metabolism of higher plants and is therefore essential for normal plant growth. Copper is an essential constituent of several important enzymes (113). In addition, enzymes such as phenolases (121), cytochrome oxidase and probably poly-phenol oxidase (142) are strongly affected by the nutritional levels of Cu. Copper is also a metal activator for several other enzymes, including tyrosinase, laccase, ascorbic acid oxidase and butyryl-A -dehydrogenase (76, 177, 187). Copper is essential in photosynthesis and chlorophyll formation (46, 113). Copper deficiency in tungtrees, for example, resulted in decreased CO_2 absorption (96).

Zinc is also essential in plant metabolism. It is a component of several metallo enzymes, including a variety of dehydrogenases, proteinases and peptidases (142, 187). Zinc is also a metal activator for several plant enzymes, such as carbonic anhydrase which catalyses decomposition of H_2CO_3 to CO_2 and H_2O (46). The activity of tryptophan synthetase in Neurospora is decreased by Zn deficiency (122). In higher plants, tryptophan is a precursor for the plant growth substance indole-3 -acetic acid which is also known as auxin (46). Considering that biosynthesis of IAA in higher plants is enhanced by Zn (142), and that Zn deficiency in potato decreases the level of IAA (162), Zn is likely necessary for the activity of tryptophan synthetase in higher plants.

Zinc deficiency also results in low RNA (ribo-nucleic acid) and ribosome levels in a number of plant species (140). Ribosome stability in cytoplasm of Euglena gracilis is decreased by Zn insufficiency (141). In addition, soluble nitrogen components such as amino acids and amides accumulated in Zn deficient potato plants (140). This implies that Zn is involved in protein synthesis in higher plants.

B. COPPER AND Zn DEFICIENCY SYMPTOMS IN HIGHER PLANTS

Copper deficiency symptoms have been observed in many crops and vary considerably among those crops (54, 177). In corn, the younger leaves become yellow and stunted. As Cu deficiency becomes more severe, the older leaves become pale and the younger leaves die, with dead tissues appearing first along the leaftips and edges (177). Cu deficient cereal plants lose colour in the younger leaves. Eventually, leaf midribs break and leaftips become necrotic (177). Severely Cu deficient cereal plants fail to develop heads (33). Severely Cu deficient vegetable crops often fail to flower. The leaves of many Cu deficient vegetables lack turgor and develop a bluish greenish cast leading to chlorosis and curling (177).

Zinc deficiency symptoms have been observed in a number of crops including corn, sorghum, deciduous and citrus fruits, nut trees, tung trees, legumes, cotton, and several vegetable crops (3, 6, 12, 20, 22, 36, 54, 72, 136, 160, 168). Early Zn deficiency symptoms usually involve interveinal chlorosis of the older leaves (6, 177), appearing first at the tips and margins (46). In cotton, interveinal chlorosis is followed quickly by necrotic spotting (34, 113). In corn, chlorosis is followed by bleached tissue on each side of midrib and at the base of the leaf (177). Severe Zn deficiency often results in smaller leaves, shortened internodes and stunted growth. Seed production in beans (Phaseolus vulgaris) and peas and fruit development in citrus are adversely affected by severe Zn deficiency (46). Zinc deficiency also causes defoliation, loss or absence of flowers (113) and increases in the period required for beans to reach maturity (22).

C. COPPER AND ZN DEFICIENT SOILS

Copper deficiencies were reported in numerous crops on organic (peat and muck) soils (8, 37, 66, 69, 72, 73, 104, 165, 177, 186). Highly weathered coarse-textured sandy mineral soils (8, 9, 66, 67, 72, 73, 137, 186, 187) often did not have sufficient exchangeable Cu for optimum growth of many crops. Examples of such soils are sandy soils of western U.S.A. (73), the sandy soils of Florida (137), podsols in eastern Canada receiving high annual precipitation (mean of 115 cm) (67), and several sandy soils on Prince Edward Island (66).

Manitoba has approximately 15 million hectares of organic soils of which approximately 100,000 hectares are suitable for agricultural development (165). In addition, there are many hectares of acidic sandy soils which one might expect to be Cu deficient. In fact, Cu deficiencies have been reported on some of these soils (37, 104, 144, 163, 166). Campbell and Gusta (37) reported that in field trials, peat deposits near Vivian could not supply sufficient Cu for the optimum growth of carrots (Daucus carota var sativa) and onions (Allium apa). Addition of Cu to carrots increased yield by 5.6 metric tons per hectare and improved the quality of onions. Racz (144) applied Cu in four corn and two sunflower trials on Almasippi sandy loams. In one corn trial on Almasippi sandy loam, there was a trend towards increased yields but this was not statistically significant. Soper (166) obtained small statistically insignificant responses to Cu in alfalfa on Miniota sand and Pelon loamy fine sand. Greenhouse experiments conducted by McGregor (104) confirmed that Pine Ridge sand contained inadequate Cu for the growth of flax and that Stockton sand contained barely adequate quantities of Cu for the growth of flax.

Zinc deficiencies have been observed under widely varying environmental conditions. Low levels of available Zn have been found in humic gleysols, regosols and organic soils (7, 184). Zinc deficiencies are most common on calcareous soils (19, 48, 73, 83, 84, 97, 107, 120, 136, 146, 154, 177, 186) but also occur on highly leached soils (97, 107, 120) and on soils containing little organic matter (97, 120). Alteration of soil by man can also lead to Zn deficiencies. For example, soils under corrals, barnyards and orchards (97), intensively cropped soils (112), and calcareous subsoils exposed by levelling and furrowing for irrigation (54, 86, 97, 120, 177) sometimes contain low levels of available Zn. High levels of soluble silica in acid soils (152) and excesses of other micronutrients (177) may result in Zn deficiency. Zinc deficiency is often more pronounced when spring weather is particularly wet and cool (7, 42, 48).

Most of the cultivated soils in Manitoba are calcareous and therefore may be deficient in Zn (37, 84, 104, 144, 163, 165). In addition, environmental conditions such as cool, wet springs which may accentuate Zn deficiency are quite common. Racz (144) found that application of Zn to corn and sunflower on Almasippi loamy fine sand resulted in small statistically insignificant yield increases. McGregor (104) also noted in a greenhouse experiment that a Plum Ridge calcareous soil was moderately Zn deficient while an Almasippi calcareous soil supplied barely adequate quantities of Zn for flax plants.

D. FORMS OF Cu AND Zn IN THE SOIL

The total amount of Cu in soil is often dependent upon the amount in the parent material. The average Cu content of the lithosphere is about 100 ppm whereas that of soil is reported to range between 2 - 100 ppm. The total amount of Cu in the soil, however, is not an indication of its biological availability (177).

The principal Cu containing minerals in the lithosphere are CuS, $Cu_2(OH)_2CO_3$ and $CuSiO_3$ (89). These weather to release Cu^{+2} into soil solution (183). Under slightly acid and oxidizing conditions, Cu combines with common anions in the soil solution to form compounds or complex ions which are water soluble (89). However, under alkaline or reducing conditions, insoluble compounds such as CuS, $Cu(OH)_2$, $CuCl_2$ and Cu_2O are precipitated (89).

Most of the Cu released into solution during weathering or decomposition of organic matter is adsorbed by soil particles (89) because Cu forms strong covalent bonds (129). The Cu^{+2} form is not only adsorbed strongly by clay but also adsorbed appreciably by quartz (11, 89). The Cu adsorptive capacity of clay minerals usually increases with pH (89, 147, 157).

Copper is also adsorbed readily by $Fe(OH)_3$, $Fe_2O_3.3H_2O$ and organic matter fractions (50, 73). Some Cu is also lost in drainage waters (132). Lindsay and Norvell (95) gave the equilibrium reaction in a Cu-soil system as

$$Cu^{+2} + soil \rightleftarrows Cu\text{-}soil + 2H$$

with solubility relationship

$$Cu^{+2} = 10^{3.2}(H^{+})^{2}$$

The level of Cu^{+2} in soil solution as predicted by this equation is far below that expected if complex ions, oxides, and carbonates of Cu were controlling the solubility of Cu (93). The level of Cu^{+2} in the soil solution decreases with increasing pH. However, Cu forms soluble and mobile complexes with organic matter more readily than does Zn. Up to 99% of Cu in the soil solution can be complexed with organic matter (73, 93). Even various copper hydroxide compounds and complex ions formed at pH values greater than 7.3 are strongly complexed by organic matter. Consequently, Cu deficiencies are not as prevalent as Zn deficiencies on calcareous soils even though the concentration of Cu^{+2} is related to soil pH (73,95).

The Zn concentration in the lithosphere is approximately 80 ppm. The total Zn concentration in the soil varies from 10 - 300 ppm but, like Cu, that range is not an indication of its availability to plants (177). Zinc in the lithosphere occurs in shales primarily as ZnS (Sphalerite) (183). Zinc containing minerals weather to release Zn^{+2} into soil solution (167, 183). Unlike Cu^{+2}, Zn^{+2} does not readily form soluble complexes with organic matter and Zn^{+2} remains dominant up to approximately pH 9.0 (183). Under alkaline conditions, Zn^{+2} may react with common anions in the soil solution to form compounds such as $Zn(OH)_2$ and $ZnCO_3$ (41, 120), particularly if the Zn^{+2} concentration is greater than 10^{-4} moles per litre (89). Zinc ions released into solution may also be adsorbed and/or fixed by clay minerals (12, 147, 157), hydrous oxides of Fe, Al, and Mn (93), carbonates (183), and organic matter (89).

Norvell (95) gave the equilibrium reaction in a Zn-soil system as

$$Zn^{+2} + soil \rightleftharpoons Zn\text{-}soil + 2H^{+}$$

with the solubility relationship

$$(Zn^{+2}) = 10^{6}(H^{+})^{2}.$$

His equation not only suggests that the solubility of Zn^{+2} is highly pH dependent, but also that compounds such as ZnS, $Zn(OH)_2$, $ZnCO_3$ and complex ions are far too soluble to account for the small concentrations of Zn found in most soil solutions. In fact, $Zn(OH)_2$ and ZnS might very well be good fertilizers (18). Although ZnS is the principal Zn containing mineral in the lithosphere, it is likely that in soils the solubility of Zn is controlled by clay minerals, hydrous oxides, carbonates and organic matter.

Interaction of Cu and Zn with organic matter is very important in the chemistry of soil Cu and Zn. Organic matter can interact with Cu and Zn in many ways. Organically bound Cu and Zn can be mineralized and be made available to plants (89). Consequently, soils low in organic matter may be low in available Cu and Zn (97, 120). Conversely, Cu and Zn can be bound into metallo-organic complexes which are immobile and unavailable to plants (50, 73, 89, 93, 129). Consequently, the addition of organic matter may actually aggravate Cu and Zn deficiencies. Lastly, organic (matter) constituents can form mobile and labile complexes with Cu and Zn (43, 104, 130). In general, the formation of soluble Cu and Zn organic complexes is directly related to the soluble organic fractions and not to total organic matter content of the soil (73).

Indigenous or applied Cu and Zn can form insoluble complexes with humic acids which are unavailable to plants (171). However, numerous other metallo-organic complexes are soluble and available to plants (130, 165, 171). These include individual biochemical molecules, such as organic acids, amino acids and fulvic acids. These constituents can convert insoluble metal complex ions and compounds which had precipitated at high pH (11, 89) into soluble and available metal complexes (71, 73, 93, 113, 165). Carboxyl groups and amides are ligands particularly involved in formation of complexes with metals by ion exchange, surface adsorption, chelation complex, coagulation and peptization (111).

Some natural chelating agents are produced by micro-organisms or excreted by plants and function in transporting Cu^{+2} and Zn^{+2} to plants' roots (49), or to lower soil horizons (71, 171). Biochemically synthesized chelating compounds include organic acids, peptides, protein molecules, amino acids, aliphatic acids and polysaccharides (60). Up to 99% of soluble Cu and 75% of soluble Zn occurs in soil as metallo-organic complexes (73, 93).

CONCLUSION

It may be summarized that Cu and Zn occur in the soil in at least five forms (183). These are: (a) water soluble Cu and Zn, the levels of which are usually very small; (b) exchangeable Cu and Zn which are also small except in soils very well supplied with these elements; (c) adsorbed, complexed or chelated forms of Cu or Zn, which make up a far greater proportion than the above two forms because of high affinity with which clay, hydrous oxide and organic materials adsorb Cu and Zn; (d) Cu and Zn occluded in the secondary clay minerals and insoluble metal oxides; (e) Cu and Zn cations in primary minerals. It is thought that the water soluble, exchangeable and adsorbed, complexed or chelated forms of Cu and Zn are the most important pools supplying these metals to plants. The three forms are also thought to be in equilibrium (45), and consequently any change in one of them would result in changes in the other two forms. It is important, therefore, that soil tests for plant available Cu and Zn should extract a portion or all of the three forms.

E. METHODS OF ASSESSING PLANT AVAILABLE Cu AND Zn AND THEIR RESPECTIVE CRITICAL LEVELS

1. SOIL ANALYSIS

Micronutrient soil tests entail many problems which sometimes render the results inevitably questionable: (a) Plant requirements are so small that the prevention of possible contamination, even in the face of the most adequate precautions is often impossible. (b) Environmental conditions such as soil pH, carbonate content, soil texture, water content, soil colloids, temperature, and activities of other micronutrient metals can sometimes correct or induce deficiencies in soils with borderline deficiencies. (c) Errors can be caused by improper sampling and by soil variability. (d) Plants differ in micronutrient requirements and in their susceptibility to micronutrient deficiencies so that the test crop or variety might influence the interpretation of the results (60, 183).

In soil testing, attempt is made to correlate the amounts of micronutrients extracted from the soil with plant micronutrient levels and/or with deficiency symptoms and yield responses of the crops (24, 53, 184). Extractants used to assess the availability of soil Cu and Zn can be placed into six categories (104): (a) extractants which extract total amount of Cu and Zn from the soil; (b) water; (c) biological extractants; (d) salt extractants; (e) acid extractants; and (f) chelating agents.

The various methods for estimating available soil Cu and Zn are summarized in Tables 1 and 2. A good micronutrient soil extractant should extract all or a proportional part of the available forms of the micronutrient such that the amount extracted can be correlated with crop growth and micronutrient uptake. In other words, a good extractant should extract a portion or all of (a) water soluble, (b) exchangeable, and (c) adsorbed, chelated or complexed forms of Cu and Zn, the three pools which are very important in supplying plants with micronutrients.

1. Total Cu and Zn concentrations in soils

Total Cu concentration in soil has been studied as a possible guide for assessing the availability of Cu to plants (40, 76, 104, 123, 135, 169). Neelakantan and Mehta (123) found a positive correlation between carbamate extractable total Cu and neutral 1*N* NH_4OAc extractable Cu on Western Indian soils. However, total soil Cu content is usually poorly correlated with plant growth, and therefore, is of limited value for predicting availability of Cu to plants (183) except where the total Cu content in soil is low.