AGRONOMIC EVALUATION OF LEONARDITE ON YIELD AND CHEMICAL COMPOSITION OF CANOLA AND WHEAT

BY

SEAN B. DILK

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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ABSTRACT

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Studies were conducted on the agronomic potential of leonardite affecting the emergence, chemical composition, and yield of canola and wheat. Fertilizer use efficiency was also evaluated. Field studies assessed the soil application of leonardite as a broadcast pre-plant or post-plant application. Leonardite did not affect the emergence, chemical composition, or yield of wheat or canola. A trend was noted in which the low rates of leonardite tended to increase the emergence and yield; however, this trend was not significant. The efficiency of phosphorus (P) fertilizer was studied with and without humic acid, derived from leonardite. Application of leonardite in a P fertilizer band significantly increased the P concentration of canola tissue in the early stages of development. However, the increase in P concentration did not result in an increase in yield. A growth chamber study was performed to determine whether humic and fulvic acids prepared from leonardite could increase plant absorption of copper (Cu) and zinc (Zn). Treatments were applied as a broadcast, band, or foliar application and were compared to Zn-or Cu-EDTA and an inorganic form of fertilizer. Fulvic and humic acid were ineffective when soil applied; however, Cu- or Zn-fulvic acid was superior to an inorganic source or EDTA as a foliar application. A field study was then conducted on the use of fulvic acids as a foliar carrier for Cu. Foliar application of Cu-fulvic acid was compared to an inorganic and organic source of Cu. Treatments were applied at 0.2, 0.4,

and 0.6 kg ha^{-1} Cu. Cu-fulvic acid improved the Cu concentration of wheat tissue versus Cu-EDTA but was inferior to $CuCl_2$. Final yield and biomass of the $CuCl_2$ treatment was superior to all treatments while the Cu-fulvic acids were similar to Cu-EDTA. A final growth chamber study was conducted on the effectiveness of leonardite in reducing cadmium (Cd) concentration of durum wheat tissue. Nitrogen (N) fertilizer was applied as urea and the leonardite was applied to the surface of the urea granule. Treatments were applied as a broadcast or band application. The Cd tissue concentrations were elevated with application of N fertilizer as a broadcast and band treatment. Broadcast application of leonardite significantly increased the Cd concentration of durum wheat tissue. However, the broadcast applications of leonardite in the previously mentioned studies did not increase the tissue concentrations of Cd. This suggests that leonardite will elevate Cd concentration only when applied to the surface of the N fertilizer.

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FOREWARD

This thesis was written in manuscript style outlined in the Department of Soil Science *Guide to Thesis Preparation for Graduate Students.* Publication of results as notes and/or full papers is planned.

LIST OF TABLES

TABLE OF CONTENTS

1. INTRODUCTION

Leonardite is a naturally occurring material enriched in humic and other organic acids and is defined as oxidized lignite produced through the weathering of subbituminous coals and carbonaceous shales (Hoffman et al. 1993). In Canada, leonardite deposits have been discovered in Alberta and Saskatchewan.

Leonardite has been shown to have variable effects on plant growth. Preliminary research trials by Akinremi (1999) showed that application of leonardite increased root growth of canola. In other trials, humic acid, derived from leonardite, improved the fresh and dry weight of tomato roots (Adani et al.1998). The increase in root growth due to the application of leonardite suggests that the leonardite material may contain compounds that elicit hormone-like responses. O'Donnell (1972) discovered that leonardite contained polyphenolic compounds, some of which behaved similarly to auxin.

The growth and dry matter production of crops have been enhanced with the addition of leonardite. Corn dry matter increased with the application of leonardite (Duplessis and MacKenzie 1983, Tan and Nopamornbodi 1979). Zaifnejad et al. (1996) showed that lignite products enhanced cereal crop growth. Duplessis and MacKenzie (1983) found that corn grain yield was significantly increased with application of leonardite while charcoal and coal products improved the yield of legumes (Iswaran et al. 1979). In other studies, the application of lignite was found to be ineffective in increasing the yield of corn and bean (Adriano et al. 1978).

Application of leonardite also affects the chemical composition of crop species. Nitrogen and P concentration of tomato and corn crops were elevated in the presence of leonardite (Adani et al. 1998, Lee and Bartlett 1976). The application of leonardite on a loamy sand increased the P concentration of corn plants (Duplessis and Mackenzie 1983). Micronutrient concentration of crops has also been enhanced with the application of leonardite. Iron (Fe) concentration in tomato was elevated with leonardite application (Adani et al. 1998) and lignite improved the concentration of Fe in mustard (DeKock 1960). Application of organic amendments, also containing humic acids, enhanced the Cu, Zn, Mn, and Fe concentrations of wheat seedlings (Moris 1985).

Generally, the studies conducted to date showed highly variable effects of leonardite. In some studies, leonardite increased yields and/or altered the chemical composition of crops. In other studies, leonardite and/or its products had virtually no effect on yield and/or chemical composition. Our study was conducted to evaluate the agronomic potential of leonardite to improve crop production in Manitoba. Yield, chemical composition of grain, phytoavailability of micronutrients, and efficiency of P fertilizer was assessed.

The studies reported in this manuscript included several individual studies to examine the agronomic potential of leonardite and/or products made from leonardite. The objectives of this study were to:

1) the effect of leonardite on the yield and chemical composition of canola and wheat.
2) the effect of leonardite on the emergence of canola on poorly structured soils.
3) whether or not addition of humic acid to a P fertilizer band can improve P fertilizer use efficiency.

4) whether or not humic or fulvic acid can improve chelation and plant availability of Cu and Zn.

5) whether or not application of leonardite can reduce the concentration and accumulation of Cd in durum wheat.

Literature pertaining to the five areas listed above was reviewed and discussed.

2. LITERATURE REVIEW

2.1 HUMIC SUBSTANCES AND CROP GROWTH

Definition of Leonardite

Leonardite is defined as a naturally occurring oxidized form of lignite coal rich in humic and other organic acids (Hoffman et al 1993). Leonardite is formed through weathering of subbituminous coals and carbonaceous shales (Hoffman et al. 1993). Although leonardite is a heterogeneous mixture of various compounds, it is primarily the humic substances in leonardite that are of interest.

Humic Substances

Humic substances are important components of soil that have been synthesized, do not have a defined composition (Sanchez-Andreu et al. 1994), and are believed to be highly degradation-resistant materials formed during decomposition of organic matter (Ashley 1996). Humic substances are described as amorphous, dark colored, hydrophilic, acidic, chemically complex organic materials of varying degrees of aromaticity that range in molecular weight from a few hundred to several thousand daltons (Ashley 1996). Humic substances may exist in either a dissolved or suspended form. When present as an organic solid phase, humic substances provide a surface for metal adsorption. The adsorption of a soluble free metal ion by an available humic substance solid phase can be viewed as a reversible equilibrium reaction that is dependent on the bulk concentration of both metal and humic substance.

Humic substances can be separated into three fractions: humic acids, fulvic acids, and humin. Humic acids are defined as the component of organic matter that is soluble in alkaline solutions and is subsequently precipitated through the process of acidification (Duval et al. 1998). Fulvic acids is the fraction of humic substances that is water soluble at all pH values (Sanchez-Andreu et al. 1994), while humin is the fraction of humic substances that is not soluble at any pH (Duval et al. 1998). Although the structures of humic and fulvic acids have not been entirely defined, there are some differences in properties that have been identified. Humic acids have a higher molecular weight and greater C and N concentration than fulvic acids, while fulvic acids have higher concentrations of O, are more acidic, and have greater cation exchange capacity (Duval et al. 1998).

Humic Substances and Germination

Humic substances are capable of increasing the rate of germination (Petrovic et al. 1982) as well as percent germination (Vaughan and Malcolm 1985, Petrovic et al.1982). Vaughan and Malcolm (1985) postulated that humic substances influence biochemical and physiological processes within the plant and soil, increasing rate and percent germination. For example, Smidova (1962) found that humic acids increase imbibition by seeds, which results in greater enzyme synthesis. Addition of potassium humates and fulvic acids to tobacco seeds stimulated respiration (Csicsor et al. 1994), which enhanced germination.

However, some other studies have shown that humic substances did not affect germination. Petrovic et al. (1982) discovered that high concentrations of humic extracts inhibited germination. Piccolo et al. (1992) found that humic acid derived from oxidized coal had no effect on germination of lettuce (*Lactuga sativa*) or tomato (*Lycopersicum esculentum*). Vaughan and Malcolm (1985) concluded that fulvic acid, at a concentration of 100 mg L^{-1}, had no effect on germination of wheat (*Triticum aestivum*) or barley (*Hordeum vulgare*).

Humic substances originate from a number of sources and vary in properties of pH, molecular weight, cation exchange capacity, and chemical composition. All of the previously mentioned properties influence germination. Seed species also vary in optimal conditions for germination such as temperature, pH, moisture, and nutrients. Therefore, the reason for variation in germination due to application of humic substances is believed to be due to the large variation of humic substance characteristics and the conditions required for seed germination.

Humic Substances and Emergence

Nuttal (1970) studied the effect of organic amendments on the emergence of rapeseed (*Brassica napus*) and found that organic amendments improved plant emergence by as much as threefold. The enhanced emergence was shown to be due to improved soil physical properties indicated by a reduced modulus of rupture.

Humic Substances and Root Growth

Several studies have shown that humic substances influence root growth. Humic acids (derived from leonardite) increased root growth of tomato (Adani et al. 1998), corn (*Zea mays*) (Tan and Nopamornbodi 1979), bean (Schnitzer and Poapst 1967), and wheat (*Triticum aestivum*) (Malik and Azam 1985). Tan and Tantiwiramanond (1983) found that there was a positive correlation between dry weight of roots and application of humic acid for soybean (*Glycine max*), peanut (*Arachis hypogaea*), and clover (*Trifolium vesiculosum*).

Humic materials affect the morphology of roots. Application of humic acid caused roots to be highly branched with proliferant root hair development (Lee and Bartlett 1976). Linehan (1976) found that the main axis extension and the development of laterals, as well as the fresh weight had all been elevated with application of fulvic acid. The increased root growth resulted in greater absorption of water and nutrients resulting in increased root area and biomass (Lee and Bartlett 1976).

Application of humic substances to above-ground plant tissue has been shown to increase root growth. Xudan (1986) found that foliar application of fulvic acid enhanced root activity in wheat, while Sladky (1959) discovered that foliar application of fulvic acid significantly increased the fresh and dry weight of roots in Begonias (*Begonia semperflorens*).

Researchers have suggested reasons for increased root growth in the presence of humic substances. Some researchers suggest that humic substances increase root growth via the polyphenolic compounds, some of which provide hormonal activity similar to auxin (O'Donnell 1972), a hormone known to increase the growth of plants. Other researchers have reported that humic substances increase the absorption capacity of roots, increasing the moisture and nutrient uptake by the roots resulting in greater growth (Vaughan and MacDonald 1976). Researchers have also suggested that humic substances may increase the permeability of the cell membrane of the root, resulting in greater absorption of water and nutrients (Cheng 1977).

Application of humic acid has caused cells to extend their growth for a greater length of time delaying the maturity of the cells and improving growth of the root (Vaughan 1974). In the same study, Vaughan noted that the fresh weight of the roots had increased despite a reduction in root cell numbers. In a separate study, Linehan (1977) concluded that cell expansion increased to an extent that more than compensated for the depressed cell numbers. Linehan (1977) also suggested that the growth of roots was due to the direct effect of polycarboxylic acid (PCA), a component of fulvic acid.

Humic Substances and Shoot Growth

Humic substances have positive effects on plant shoot growth (Moris 1985, Elgala et al. 1978). Corn dry matter was significantly increased with addition of leonardite (Duplessis and Mackenzie 1983, Tan and Nopamornbodi 1979). Humic acid, derived from leonardite, significantly increased the fresh and dry weight yields of tomato by 8% and 9%, respectively (Adani et al. 1998). Tan and Binger (1986) discovered that dry matter production of corn plants increased by 32.5 to 42.5% with addition of humic acids. Wheat growth has been reported to increase in the presence of organic amendments (Ishac 1986). Shoot dry matter of wheat increased with addition of coal combustion by-products (Zaifnejad et al. 1996) while humic acid increased seedling growth of wheat, by 30% and 33%, on a fresh and dry weight basis, respectively (Malik and Azam 1985).

The addition of lignite has occasionally hindered or not influenced plant growth. Schisler and Linderman (1989) discovered that application of high rates of leonardite reduced the growth of coniferous trees. Wallace and Romney (1980) found that application of coal reduced vegetative yields of bush bean plants (*Phaseolus vulgaris*) when applied to a noncalcareous soil. When the coal was applied to a calcareous soil, no effect on bush bean growth was observed. Coal and sulphomethylated coal (coal that has been processed for greater N content and solubility) had no effect on growth of barley (Cairns and Moschopedis 1970) while sulphonated coal was found to be toxic to barley plants. The toxic effect was believed to be due to a sulphur (S) compound produced during the sulphonation process that may have been toxic to crops (Cairns and Moschopedis 1970). Lee and Bartlett (1976) discovered that application of humic acid to a soil high in organic matter gave a slightly negative response in corn growth. Piccolo et al. (1992) found that dry weight of lettuce (*Lactuga sativa*) did not change with the application of humic acids.

Humic Substances and Yield

Humic substances and organic amendments increased yields of field crops in several studies. Duplessis and Mackenzie (1983) found that addition of leonardite to clay and loamy sand soils increased corn yields. Application of charcoal, coal, and peat improved the grain yields of legumes such as moong (*Vigna radiata*), soybean (*Glycine max*), and pea (*Pisum sativum*) (Iswaran et al. 1979). The increase in grain yield was believed to be due to phenolic compounds that were toxic to soil bacteria and protozoa that are antagonistic towards *Rhizobium* species. Xudan (1986) discovered that foliar application of fulvic acid increased the yields of wheat. This was believed to be due to the reduction in water stress on the wheat plants.

In other studies, organic amendments such as described above, have been ineffective in enhancing crop yields. Coal by-product application was ineffective in improving corn and bean yields (Adriano et al. 1978). Rowberry and Collin (1977) found that addition of humic acid did not improve yield or quality of potatoes. However, this study was conducted in Ontario on soils that typically have been shown to have high soil concentrations of Fe and Al. Iron and Al may inactivate humic acids by forming water-stable cements, which may reduce soil permeability (Martin 1960, Martin and Reeve 1960).

Humic Substances and Macronutrient Composition of Plants

Organic amendments containing humic substances may improve macronutrient composition of plant species (Moris 1985). Humic acid, derived from leonardite, increased the N and P concentrations of tomato (Adani et al. 1998) and corn plants (Lee and Bartlett 1976, Duplessis and MacKenzie 1983). Wallace and Romney (1980) found that powdered coal elevated the P and K concentration of bush bean on a calcareous soil. Coal products increased the N content of barley (Cairns and Moschopedis 1970). The application of coal combustion by-products (CCBP) remedied Al toxicity on acid soils (Zaifnejad et al. 1996). The CCBP consequently created an increase in the uptake of magnesium (Mg) by wheat plants (Zaifnejad et al. 1996). CCBP reduced Al toxicity because of the high pH and high concentration of calcium (Ca) in the CCBP. Calcium displaces Al from the exchange sites and allows for precipitation at the elevated pH. High concentrations of Al have been shown to interfere with Mg uptake.

The increase in metallic macronutrients concentrated in plants with humic or fulvic acids may be a result of water-soluble complexes. The organic acids chelate the metal so that the metal remains water-soluble and is less likely to participate in precipitation or adsorption reactions.

Humic or fulvic acids have also been shown to improve the root's ability to absorb nutrients. Cheng (1977) concluded that the application of humic or fulvic acids increased the permeability of the cell membrane of the root resulting in greater uptake of nutrients. Vaughan and MacDonald (1976) discovered that humic substances increased the nutrient uptake by roots, which resulted in greater growth. This increase in absorption capacity of the roots generated an increase in nutrient concentrations within the plant. Increases in nutrient uptake may also improve root growth, which will increase root exploration and consequently nutrient uptake by plants.

Humic acids improved solubilization of precipitated nutrients (Sinha 1972), increased the soil solution concentration and phytoavailability of nutrients and consequently the chemical composition of crops.

Organic amendments and humic substances, in contrast to the studies previously cited, can also have an adverse effect on macronutrient composition of plants. Application of coal ash created a P deficiency in corn and bean (Adriano et al. 1978). Sulphonated coal suppressed the uptake of Na and K in barley seedlings (Cairns and Moschopedis 1970). Powdered coal has been shown to reduce the P and K concentrations of bush bean plants on a calcareous soil (Wallace and Romney 1980). Vaughan and Macdonald (1971) found that the addition of humic acids to a soil decreased the uptake of Cl in beet (*Beta vulgaris*).

Tan and Nopamornbodi (1979) found that humic acid decreased the P concentration in corn plants. They suggested that the reduction in P concentration was due to the reaction of P with the phenolic functional groups on the humic acid ion, altering the ionic behavior of the phosphate anion. Studies have shown that humic substances are capable of forming complexes with P that are unavailable to the plant. Saeed (1978) discovered that P content of thickspike wheatgrass was significantly reduced in the presence of leonardite, due to adsorption of P by the leonardite. The subsequent desorption of P from leonardite was low resulting in reduction in P availability to the wheatgrass.

Humic and fulvic acid functional groups may also bind to cations found at the surface of soil minerals. The humic or fulvic acid may then form complexes with metals that are in soil solution, which may reduce availability of the metal to the plant (Paul and Clark 1996).

Humic Substances and Micronutrient Composition of Plants

Organic amendments and humic substances can increase the concentration of micronutrients in plants. Humic acid derived from leonardite increased the Fe concentration in tomato (Adani et al. 1998) while humates and fulvates were shown to increase the concentration of Fe in the shoots of wheat seedlings (Linehan and Shepherd 1979). The addition of lignite increased the concentration of Fe in mustard plants (DeKock 1960) and humic acid significantly increased the Fe uptake by barley (Elgala et al. 1978).

Copper concentration in barley increased with soil application of humic acids (Elgala et al. 1978, Metwally et al. 1976). Gupta and Deb (1985) found that the uptake of Zn by rice and wheat significantly increased with the addition of fulvic acid. Manganese concentration of bush bean plants was enhanced when powdered coal was added to an acidified soil (Wallace and Romney 1980). The increase in Mn uptake was believed to be due to the acidification of microregions due to the low pH of the powdered coal. Increases in acidity will increase the solubility of Mn precipitates and increase the bioavailability of Mn.

Soil application of either humic or fulvic acid elevated the Cu, Zn, Mn, and Fe content of wheat seedlings (Moris 1985). Zinc and Fe concentrations in barley were increased when humic acids were added to a soil high in calcium carbonate (Metwally et al. 1976). Addition of humic material increased the uptake of Fe and Cu in teak seedlings (Fagbenro and Agboola 1993) and coal ash significantly increased the concentration of Cu, Zn, and Mn in corn and bush beans. The same study showed that the coal ash had increased the concentration of B in plants to toxic levels (Adriano et al. 1978).

The increase in micronutrient composition of plants with humic acids and other organic amendments may be due to an increase in rooting area arising from improved permeability of the root cells (Cheng 1977) or a hormonal effect (O'Donnell 1972). Formation of water-soluble metal complexes or dissolution of precipitates may also be responsible for increased micronutrient concentration of plants.

Humic substances and organic amendments can also have adverse effects on the micronutrient composition of plant species. Linehan (1978) found that humic acids depressed the absorption of Fe by wheat roots. In other studies, Fagbenro and Agboola (1993) found that Fe concentration in barley decreased in the presence of humic acids at low and high concentrations of soil solution Fe, and that increasing concentration of humic acid reduced the concentration of Mn in teak seedlings. Copper and Mn concentration in bean and corn plants decreased when coal by-products were added (Adriano et al. 1978). Elgala et al. (1978) found that the addition of humic acid reduced the Zn concentration and uptake at low soil solution concentrations of Zn, and at high levels of Zn the humic acid protected the barley plants from Zn toxicity (Elgala et al. 1978). In the same study, humic acid protected the barley plants from Fe toxicity (Elgala et al. 1978). Addition of humic acid decreased the activity of Cd near the corn root but did not affect the translocation of Cd within the corn plant (Tyler and M^{c}Bride 1982).

The reduction in micronutrient composition of plant species may be due to the formation of stable complexes that are unavailable to the plant. The large size of the humic or fulvic acid complex may not allow absorption by the plant root.

2.2 HUMIC SUBSTANCES AND PHOSPHORUS AVAILABILITY

Phosphorus and Crop Growth

Phosphorus is an essential element for the growth and reproduction of plants (Tisdale et al. 1993). Phosphorus is a component of intermediate metabolites, cell membranes, and has numerous roles in transfer of energy and regulation of plant growth (Salisbury and Ross 1992). There are three main pools of soil P; the P in soil solution, the labile P, and the non-labile P. Soil solution P is considered available to plant roots at normal soil pH and primarily consists of orthophosphates ($H_2PO_4^-$ and HPO_4^{2-}). The labile P exists in solid form, but exhibits a high dissociation rate or exchange rate between soil solution and soil solid phases and thus readily replenishes soil solution P (Tisdale et al. 1993). The non-labile P pool has the ability to replenish the labile pool when depleted, but this occurs at a very slow rate. The labile and the non-labile pools contain inorganic and organic P.

Phosphorus fertilizers are numerous and include phosphates such as rock phosphate, calcium orthophosphates, ammonium phosphates, ammonium polyphosphate, nitric phosphate, and phosphoric acid. Numerous studies have documented instances in which application of P fertilizer increased crop growth and yields (Duplessis and MacKenzie 1983). However, the efficiency of P fertilizer is low with approximately 20% of P applied being used by crops in the first year (Hammond 1997). The reasons for poor P efficiency in base saturated soils include the formation of highly insoluble phosphates, particularly at high soil pH (Tisdale et al. 1993, Srivastava and Gupta 1996).

Humic Substances and Phosphorus Use Efficiency

Researchers have suggested that humic substances may improve the P use efficiency of P fertilizers. Duplessis and Mackenzie (1983) found that the addition of leonardite, an oxidized lignite, increased corn yields due to an increase in P levels in the plants which was directly associated with leonardite application. Yang et al. (1985) discovered that humic acids significantly increased plant total P and P uptake, as well as the yield of wheat. The humic acids increased P fertilizer use efficiency by 40%. Li and Wang (1988) discovered that addition of humic substances to monoammonium phosphate ($NH_4H_2PO_4$) significantly reduced fixation of the added P by an alkaline soil. The reduction in fixation created an increase in the total amount of P available to the plant. Wang et al. (1995) found that P fertilizer with humic acids provided more water-soluble P to the plant than P fertilizer without humic acid; yield of wheat and P uptake with humic acids was greater than without humic acid.

Vaughan and MacDonald (1971) concluded that addition of humic acids to P fertilizer increased the concentration of plant P. Improved uptake of P was due to competition between the negatively charged functional groups in the humic acid and the phosphate anion for adsorption sites as well as complex formations with cations.

Organic matter has been shown to increase the availability of soil P and rock phosphate (Dalton et al. 1952) due to increased microbial activity when organic matter is added to soils. Certain metabolic products of microbial decomposition form stable complexes with Fe and Al that are responsible for P precipitation in acid soils (Dalton et al. 1952). Phosphorus concentration of corn plants was elevated with humic acid application on an acidic soil (Tan and Binger 1986). The increase in P concentration was due to a stimulation of root growth. Foliar application of humic substances have been shown to affect the P content of plants. Phosphorus concentrations in wheat plants were increased with the application of a foliar fulvic acid (Xudan 1986). The author attributed the increase in P uptake to a greater root mass with fulvic acid.

Leonardite has also been shown to reduce P phytoavailability (Saeed 1978). Leonardite application significantly reduced P content and uptake of thickspike wheatgrass (*Agropyron dasystachum*) at all rates of P application. The reduction in P content was attributed to a decrease in desorption of P from the sorbed phase in the presence of leonardite. The high sorption of P by leonardite and the subsequent low desorption to replenish the depleted P from soil solution resulted in reduced plant growth and yields (Saeed 1978).

Humic Substances and Soil P Solubility

Humic substances dissolve precipitated phosphates (Sinha 1972) and thereby enhance P availability (Bermudez et al. 1993). The increase in P availability is thought to be a modification of the crystalline form of the P precipitates and varying the particle size (Bermudez et al. 1993). Humic acid, derived from lignite, increased solubilization of P fertilizer at both acidic and alkaline pH (Martinez et al. 1984) and thereby contributed to the available P in soil. Oxidized and ammoniated oxidized coals have increased the solubility of P added as monocalcium, dicalcium, and tricalcium phosphates (Rubinchik et al. 1985).

Humic Substances and P Precipitation

The main cause of phosphate retention in neutral to alkaline soils is through reaction of P with adsorbed Ca and free calcium carbonate in soil (Deb and Datta 1967). Humic and fulvic acids are capable of reducing P fixation in the presence of calcium (Wang et al. 1995, Grossl and Inskeep 1991). The formation constant of dicalcium phosphate dihydrate (DCPD) crystals without humic or fulvic acids was 9.7 L^2 mol^{-1} m^{-1} s^{-1}. When humic acid was added the formation constant was reduced to 1.1 L^2 mol^{-1} m^{-1} s^{-1} and when fulvic acid was added the formation constant was further reduced to 0.1 L^2 mol^{-1} m^{-1} s^{-1}. The precipitation of the DCPD crystals was inhibited by the adsorption of the humic and fulvic acids onto the DCPD surfaces, which blocked sites acting as nuclei for new crystal growth (Grossl and Inskeep 1991).

Humic substances can reduce P precipitation through complexation of cations that normally precipitate P. Moreno et al. (1960) found that organic matter interfered with precipitation of phosphate as calcium phosphates through the formation of a complex between organic matter functional groups and Ca. Complexation of Ca by organic matter reduced the concentration of Ca in solution that was available for reaction with phosphate. Leaver and Russell (1957) also reported that fulvic acid blocked phosphate fixation sites in soils.

The primary agents of chemical fixation for phosphates in acid soils are active Fe and Al (Struthers and Sieling 1950). Reaction of Fe and Al with phosphorus produces phosphate precipitates that are only sparingly soluble and available to plant species. The most important compounds formed are ferric dihydroxyl dihydrogen phosphate (strengite), Al dihydroxyl dihydrogen phosphate (variscite), and isomorphous combinations of the two (barrandite). Organic anions form stable complexes with active Fe and Al, which suppresses phosphate fixation by reducing the concentration of free Fe and Al in soil solution (Struthers and Sieling 1950). Swenson et al. (1949) concluded that certain organic anions greatly reduce the amount of phosphate precipitated by Fe and Al at acidic pH.

The efficiency of the organic anion to reduce phosphate precipitation at acidic pH is affected by anion structure (Struthers and Sieling 1950). Amino acid functional groups are ineffective at suppressing phosphate precipitation. Hydroxyl groups are effective in reducing phosphate precipitation and the effect is enhanced as the number of hydroxyl groups increases. As the number of carboxylic functional groups increases, the effectiveness of the organic anion in reducing phosphate precipitation is enhanced. The size of the carbon chain (skeleton) is also important. The shorter the carbon chain, the greater the effectiveness of the organic anion.

Humic Substances and P Adsorption

Humic substances (humic and fulvic acids) diminish the adsorption of P (Sibanda and Young 1986) by competing strongly with the phosphate ion for soil adsorption sites (Sibanda and Young 1986). The reduction in adsorption of P increased with increases in humic acid and was evident throughout a wide range in soil pH.

Sibanda and Young (1986) postulated that humic substances reduce P adsorption reactions as follows: Humic substances contain a number of carboxylic and phenolic functional groups that are dissociated at alkaline pH and therefore become negatively charged. These negatively charged functional groups compete with the phosphate anion for adsorption sites on the soil surfaces, reducing the adsorption and fixation of P to the soil surface, thereby increasing the soil solution P concentration and the phytoavailability of P to the plant. A second postulate is that an unfavorable electrostatic field may be generated around an adsorbed humic acid molecule that will reduce phosphate adsorption to the soil adsorption sites (Sibanda and Young 1986). A third theory is that humic and fulvic acids may have a proton buffer-power effect on the phosphate ion (Sibanda and Young 1986). The humic or fulvic acid is able to donate a proton to the orthophosphate anion resulting in a neutral phosphate molecule. The neutral molecule (H_3PO_4) will participate in fewer fixation reactions within soil.

In particular instances, P adsorption has been enhanced with application of humic acids (Wild 1950). Three reasons have been postulated for the increased adsorption of P in the presence of organic acids. First, organic acids may associate with cations that may then bind to the phosphate anion. Second, a complex formed between humic acids and Al may be active in P sorption. Third, basal spacing contraction of montmorillonite layers would decrease electrostatic repulsion between negative charges on borders. Phosphorus may then interact with the active sites in the clay (Bermudez et al. 1993).

The effect of organic acids on phosphate adsorption is related to pH. Oxalic acid was found to reduce phosphate adsorption at pH of 5.7 to 5.8. However, at pH of 7.9 to 8.0, oxalic acid was only as effective as Cl^- in reducing phosphate adsorption (Kafkafi et al. 1988). Possible explanations of the above results are: First, at pH 7.9 all of the oxalate ions are completely dissociated (Lopez-Hernandez et al. 1979) while the phosphate prevailed as $H_2PO_4^-$. At alkaline pH, OH^- can exchange for $H_2PO_4^-$ which will reduce the amount of phophate adsorbed. Second, the charge on the clay edges becomes more negative as the pH increases (Kafkafi et al 1988), decreasing P adsorption.

Lopez-Hernandez et al. (1979) also found the effect of humic substances on P desorption to vary with pH. At low pH, the decrease in P desorption was attributed to three factors: (1) a direct exchange between the dissociated organic anion and the phosphate; (2) dissolution and chelation of "active" forms of Fe and Al and; (3) partial hydrolysis of organic compounds. Lopez-Hernandez et al. (1979) found the desorption of phosphate from an acid soil was minimal at neutral pH. At high pH, the desorption of phosphate from the acid soil was due to chelation and exchange of hydroxyl ions and phosphate.

The amount of phosphate that desorbs from a calcareous soil is related to pH. The greater the hydrogen ion concentration, the more phosphate that was dissolved from the relatively insoluble calcium phosphates (Lopez-Hernandez et al. 1979). Organic anions generally are dissociated and negatively charged at pH greater than 6.5, therefore, the ability of the organic anions to release adsorbed phosphate may be negligible in soils with neutral to alkaline pH (Lopez-Hernandez et al. 1979).

Arsenate has been shown to increase desorption of adsorbed phosphate (Barrow 1972). Authors have shown that desorption of previously adsorbed phosphate increases with the concentration of arsenate and with increases in pH. Arsenate is believed to compete with the specifically adsorbed phosphate. However, the effectiveness of anion competition is also related to the accompanying cations (Barrow 1972). As the cation distance from the soil surface increases, the effectiveness of competition by the anion also increases. Barrow (1972) found that the effectiveness of arsenate decreased in presence of cations in the following sequence: Li, Na, K. Therefore, the amount of potassium arsenate required to displace adsorbed phosphate was greater than the amount of sodium arsenate required to displace the same amount of phosphate.

The concentration of the competitive anions also affects the P retention in soils (Deb and Datta 1967) and should be at least of the same order of magnitude as that of solution P to be effective in counteracting P retention.

2.3 HUMIC SUBSTANCES AND MICRONUTRIENT COMPLEXATION

Micronutrients and Plant Growth

Micronutrients are defined as essential nutrients required in low concentrations by plants (Tisdale et al. 1993) and include nutrients such as Cu, Zn, Fe, Mn, and B. Phytoavailability of micronutrient fertilizer can be limiting to plant growth due to the formation of very stable surface complexes or formation of precipitates that are not readily plant available. Stable metal complexes can include complexes with organic matter, sesquioxides, clay silicates (Bibak 1994), formation of porphyrin complexes (Goodman and Chesire 1976) and formation of highly insoluble phosphates. The formation and stability of these complexes results in a low fertilizer use efficiency of micronutrient fertilizers. Plant recovery of micronutrient fertilizers is typically between 5 and 10% (Mortvedt 1994).

Humic Substances and Metal Bonding

Humic and fulvic acids are adsorbed by clays and the adsorption is due to the association between the organic acids and polyvalent cations at the clay surface (Greenland 1971). Humic acids adsorb metal ions, producing a water-soluble complex. The complexation of metals with humic acid is affected by pH. Humic acids compete more strongly for metal ions at acidic pH than at alkaline pH, whereas the clay particles of soil are competitive for metal adsorption at alkaline pH (Hatton and Pickering 1980). Humic acid adsorption of metals varies with the type of metal and metal concentration within the humic acid. The general selective adsorption of metals (determined by the distribution coefficients) for humic acid is $Cu^{2+} > Cd^{2+} > Zn^{2+} > Ca^{2+}$. The half lives of the sorption process change very little with increasing concentration for Cu^{2+} and Cd^{2+}. However, as the concentration of Zn^{2+} and Ca^{2+} increase, the half lives for the sorption process decrease significantly as metal ions are adsorbed (Bunzl et al. 1976).

There is an interaction between the absorption of Cu and Zn metal ions through competing ions. The uptake of one of these metals is inversely proportional to the other. That is, the uptake of one metal causes a decrease in uptake of the other metal because both metals have the same mode of uptake by the plant roots. Vaughan and MacDonald (1976) discovered that humic acid forms complexes with both Zn and Cu. In this study, humic acid complexed Zn and rendered the Zn unavailable to the plant. However, when both Zn and Cu were in solution, humic acid had a preference for Cu. Humic acid formed a complex with Cu resulting in an increase in Zn uptake.

Humic Substances and Micronutrient Availability

Humic substances have been shown to improve the bioavailability of micronutrients (Metwally et al. 1976). Humic and fulvic acids added to micronutrient fertilizers increased the uptake of Cu, Zn, Fe, and Mn in tropical kudzu (*Pueraria phaseoloides*) (Moris 1985). In this particular study, fulvic acids were superior to humic acids in increasing micronutrient uptake. This is due to the smaller size and less complex nature of the fulvic acid resulting in greater absorption of the metal.

Metwally et al. (1976) showed that humic acid counteracted the adverse effect of calcium carbonate in soils. Calcium carbonate was added to soil to decrease micronutrient availability through precipitation reactions. However, the addition of humic acid negated the effects of calcium carbonate addition and increased the uptake and concentration of Cu, Zn, and Fe in plants (Metwally et al. 1976).

Humic Acid and Stability of Metal Complexes

Micronutrient fertilizers have very low efficiencies (Moris 1985) and poor plant growth and production may occur even when micronutrient fertilizers are added. Any increase in the stability of a micronutrient complex would reduce fertilizer precipitation and adsorption losses to the soil increasing micronutrient fertilizer use efficiency.

One of the most important properties of soil humic acids is their ability to interact with metal ions to form metal-inorganic complexes of differing stability and structural characteristics (Senesi et al. 1986). The stability of metal complexes with humic substances depends on the pH of the soil. Generally, the stability of a metal complex with humic substances will increase as the pH of the soil increases (Cheam and Gamble 1974). Humic acids can reduce adsorption of metals at a low pH; however, as the pH of soil increases, the complexation of metals by humic substances generally decreases (Hatton and Pickering 1980). This is attributed to the clay fraction which can strongly retain the soluble metal hydroxy species formed at pH>6. Humic and fulvic acids can be adsorbed by the clay fraction of the soil; however, the extent of adsorption depends on the concentration and molecular weight of the organic polymer, clay mineral type, and the nature of the clay counter ions (Hatton and Pickering 1980).

The Irving-Williams series describes the order of stability of metal complexes (Cotton and Wilkinson 1962). For a given ligand such as humic acid, the stability of complexes with dipositive metal ions follows the order: Cu>Zn>Cd (Ashley 1996) which is due partially from ligand field effects and from a decrease in the ionic radius. Metals such as Cu and Cd seem to have a preferred complexation with humic substances while the affinity for metals such as Zn and Ca seem to be less significant (Bunzl et al. 1976)

Fulvic acid forms stable complexes with metal ions. Cheam and Gamble (1974) found that the affinity for metals toward fulvic acid increased as follows: Mercury (Hg) > Cu > Cd. The stability of the metal complex was also influenced by pH. For Hg complexes the stability constants (log K) were 4.86 and 5.08 at pH 3 and 4, respectively. For Cd complexes the stability constants (log K) were 3.04 and 3.64 at pH 4.9 and 5.95, respectively. Stability complexes (log K) for the Cu complexes were 3.22, 3.72, and 4.35 at pH 3, 4, and 5, respectively.

Humic Substances and Fe Complexation

There are two principal classes of binding sites available on humic acids for complexation of Fe^{3+} ions (Senesi et al. 1986). The first class includes weak surface sites that bind metal ions in complexes that are water-stable but easily disrupted by proton exchange. The second class includes functional groups that form highly stable, inner-sphere complexes resistant to extensive proton exchange.

The degree of association between Fe and humic acid increases as pH decreases to a maximum at pH 1.5 (Bedrock et al. 1997, Goodman et al. 1991). At low Fe concentrations, Fe appears to be very strongly bound to the fulvic acid (Goodman et al. 1991). However, when the Fe content approaches 1-2%, some uncomplexed Fe^{3+} can be present (Goodman et al. 1991).

Concentration of the humic substance is also important. In solutions where the fulvic acid content is high relative to Fe (greater than 100 fold) the reactions with Fe are completely reversible. In solutions with a lower proportion of fulvic acid to Fe, where free ions are present, there is a lack of reversibility (Goodman et al. 1991).

Fulvic acid has also been shown to interfere with the crystallization of Fe^{3+} oxides (Kodama and Schnitzer 1977). However, this is dependent upon the form in which the Fe^{3+} exists initially, the quantity of fulvic acid present, and the pH. Three forms of Fe are produced in the presence of fulvic acid, a sextet from magnetically dilute Fe^{3+}, a doublet from Fe^{2+}, and a doublet from Fe^{3+} where the later may come from both organic complexes and poorly crystalline oxide species (Goodman et al. 1991). The addition of fulvic acid results in the reduction of the Fe from Fe^{3+} to Fe^{2+} (Goodman et al. 1991) increasing the availability of Fe to plants.

Humic Substances and Fe Phytoavailability

Addition of humates have improved the efficiency of Fe fertilizers. The increase in stability reduced Fe fixation and resulted in greater Fe uptake by mustard plants (*Brassica juncea*) (DeKock 1960). Researchers have discovered that at low concentrations of Fe, humic acid increased plant uptake and concentration of Fe, while at high concentrations of Fe the addition of humic acid reduced the uptake and concentration of Fe (DeKock 1960).

Addition of humate to a solution containing Fe-EDTA caused a decrease in Fe availability and uptake by wheat plants (Linehan et al. 1978) at a pH of 5 to 7. This suggested that humates would act as a carrier only when inorganic forms of Fe were present.

Humic Substances and Cu Complexation

Copper is held by more than one type of site within a soil system and the strength of the bonding between Cu and the soil site increases as the total Cu decreases (Davies et al. 1969). Copper fixation in the soil can be quite high which can result in nutrient deficiencies. The addition of humic acid to a soil resulted in an increase of available Cu by 50%. This increase was due to the action of the humic acids blocking carboxylic, phenolic or both functional groups (Davies et al. 1969) used to fix Cu^{2+}.

Humic substances form very stable complexes with Cu. The stability constant (log K) for a Cu-humic acid derived from leonardite was 8.9 (Stevenson 1976). Cheam and Gamble (1974) discovered that the log K values for Cu fulvate complexes were 3.22, 3.72, and 4.35 at a pH of 3, 4, and 5, respectively. Cu^{2+} has been shown to form a stable complex with porphyrin (Goodman and Chesire 1976) a component of soil humic acids (Goodman and Chesire 1976). Cu-porphyrin complexes have been suggested as the reason for the low availability of Cu on high organic matter soils (Goodman and Chesire 1976).

Adhikari and Ray (1975) discovered that modifications of humic substances affect the stability of Cu^{2+} complexes. Stability constants were higher for Cu complexed with the phosphorylated humic acid compared to Cu^{2+}-humic acid. This was due to bonding of Cu by both the phosphate ion and the oxygen donor groups from the humic acid (Adhikari and Ray 1975).

The stability of Cu-humic acid increases with pH (Adhikari and Ray 1975) due to two factors. At low pH the H^+ ions compete with the Cu^{2+} for adsorption sites while at high pH the H^+ are removed from the adsorption sites and the hydroxyl groups compete with donor groups for metal ions. Secondly, as pH increases hydroxylated type of bonds are formed (Adhikari and Ray 1975).

The bond between Cu and humic acid is either in simple exchangeable form or as complexes involving dissociated acidic groups (Davies et al. 1969). The bonding strength of the Cu^{2+} ion and the humic acid ligand is stronger than that of H_2O as a ligand (Boyd et al. 1983). The formation of the humic acid complex with Cu is primarily of two types, the polyvalent metal cation can be bound to two carboxylic (COOH) groups or the polyvalent cation is bound to a single COOH group and to a phenolic OH group (Schnitzer 1969).

Reaction between Cu and the oxygen functional groups of humic acid are primarily with oxygen of carboxylic or phenolic functional groups (Boyd et al. 1981). The bonding can take place with or without the formation of a chelate ring (Boyd et al. 1981). The bonding between Cu^{2+} and the humic acid oxygen donor groups is through a unidentate complex (Boyd et al. 1981) and forms the chelate ring.

Electron Spin Resonance (ESR) has shown that Cu^{2+} forms inner sphere complexes with humic acid ligands containing oxygen donors (Boyd et al. 1981). The Jahn Teller Effect (Cotton and Wilkinson 1995) results in Cu^{2+} binding axial ligands weakly. This may enable the humic acid to displace axial aqua (H_2O) groups from Cu^{2+} resulting in the formation of an inner sphere complex (McBride 1978). McBride (1978) also suggested that there was no direct evidence for the formation of more than a single bond between humic acid and Cu^{2+}. However, Boyd et al. (1981) discovered, through ESR, that the mechanism of Cu^{2+} bonding is through the formation of two equatorial bonds with oxygen donor atoms originating from the functional groups of humic acid. In addition, the Cu-humic acid oxygen bonds occupy *cis*-positions in the square plane of Cu^{2+} (Boyd et al. 1981). The authors suggested that the Cu^{2+} formed axial bonds with ligands of the humic acid.

The concentration of Cu does not have an effect on the sorption rate in the presence of humic acid (Bunzl et al. 1976). Adsorption constants (K_{ads}) for Cu adsorption onto humic acids are approximately 2×10^4 (Ashley 1996). Large K_{ads} are associated with humic acids that contain a greater abundance of aromatic carbon (lignin rich). Therefore, the aromatic carbon content influences the degree of metal adsorption on the humic acid surface. However, studies with lignin rich samples show distinct peaks in the 150-152 ppm region of the aromatic region suggesting that greater K_{ads} are associated with phenolic and methoxylated aromatic groups (Ashley 1996).

Fulvic acid also has a high binding capacity with Cu^{2+} (Senesi et al. 1986). The bond between fulvic acid and Cu^{2+} is derived from both oxygenated and nitrogenated functional groups. ESR spectra suggests that Cu^{2+} complexation by fulvic acid is through inner-sphere, polydentate complexes, with the Cu^{2+} coordinated equatorially by oxygenated and nitrogenated fulvic acid ligands (Senesi et al. 1985). As the amount of Cu^{2+} that is complexed increases, the greater is the formation of less reactive functional groups of fulvic acid with Cu^{2+}. This results in formation of weaker complexes (Senesi et al. 1985) between Cu and fulvic acid as the concentration of Cu increases. Gamble (1970) reported that there are two types of COOH groups in fulvic acids, one group with a K_a of 2.2×10^{-3} and the second group with a K_a of 2.5×10^{-5}. Gamble (1970) also reported that it is likely that a maximum of two acidic groups per metal may be involved in complex formation.

Humic Substances and Cu Phytoavailability

Addition of humic acid with Cu has been shown to increase Cu uptake, Cu concentration, and the dry weight of barley (Elgala et al. 1978). Conversely, humic acid has been shown to have little or no effect on the availability of Cu. Humic acid addition resulted in an increase in the adsorption of Cu (Mittal et al. 1984). However, this trend was very slight and the control of Cu availability was primarily due to pH, with humic acid having only a secondary effect. The soluble Cu participated immediately in precipitation reactions and did not allow for chelation of Cu by the humic acid (Mittal et al. 1984).

Humic Substances and Zn Complexation

Broadbent and Ott (1957) discovered that the stability of organic matter-Zn complexes, measured formation constant as log K values, varied from 3.4 to 5.6. As soil pH becomes more alkaline, Zn-organic acid complexes become more stable (Dkhar et al. 1985) due to enhanced ionization of functional groups and a greater number of active ions being involved in coordination to the metal ion (Dkhar et al. 1985). Humic acids form more stable complexes with Zn than fulvic acids (Dkhar et al. 1985).

The concentration of Zn in relation to humic acid has an influence on the sorption process (Bunzl et al. 1976). The half life for the Zn sorption process significantly decreases as the Zn concentration increases, at a constant level of humic acid. The adsorption constants (K_{ads}) for Zn are approximately 0.25×10^4 which is a magnitude lower than for Cu (Ashley 1996). However, the same functional groups are responsible for adsorption of Zn and Cu. Therefore, Zn adsorption will increase as the phenolic and methoxylated aromatic groups increase within the humic acids (Ashley 1996).

The free energy associated with formation of a Zn-humic acid complex is greater than for a Zn-fulvic acid complex suggesting that formation of Zn-humic acid is preferred. The formation constant for both humic and fulvic acids with Zn are greater than the formation constant of Zn with inorganic anions such as Cl, SO_4^-, or CO^{2-}, suggesting that the formation of humic and fulvic complexes is preferred over the formation of complexes with inorganic anions (Dkhar et al. 1985).

Humic substances affect diffusion of Zn in soil. Application of increasing amounts of fulvic acid resulted in greater diffusion of Zn (Gupta and Deb 1985).

However, addition of humic acid resulted in greater adsorption of Zn resulting in reduced mobility of the complexed Zn (Warwick et al. 1998).

Humic substances have affected the uptake of Zn by plants. Fulvic acid reduced the uptake of Zn by plant roots and resulted in an accumulation of Zn around the roots due to chelation of Zn (Gupta and Deb 1985). Zinc uptake in beet disks was unaffected in the presence of humic acids while the rate of uptake was negatively affected (Vaughan and MacDonald 1976). The reason for the reduced rate of uptake was due to the complexation between humic acid and Zn resulting in a larger molecule that interfered with adsorption.

Humic Substances and Zn Phytoavailability

Application of humic substances to soil has had variable effects on Zn availability. Zinc uptake in rice and wheat increased when fulvic acid was added (Gupta and Deb 1985); however, Zn uptake by barley was unaffected by application of humic acid (Elgala et al. 1978). Humic substances have been shown to protect plants from Zn toxicity. For example, Elgala et al. (1978) found that humic acid reduced Zn uptake and Zn concentration in barley plants at all Zn concentrations and protected the plant from toxic Zn levels.

Concentration of Zn in the soil solution was significantly increased (ten times greater) when fulvic acid was applied to soil (Gupta and Deb 1985); however, phytoavailability of Zn was reduced when the addition of humic acids increased adsorption of Zn to soil surfaces (Hanafi and Salwa 1998).

2.4 HUMIC SUBSTANCES AND Cd COMPLEXATION

Cadmium is a heavy metal that has been associated with a variety of health problems when ingested above particular limits. As a result, consumption of food containing high levels of Cd is undesirable. Several plant species have been shown to accumulate high concentrations of Cd; some of these plants are consumed by humans.

Application of humic substances can reduce the uptake of Cd by plants. According to Tyler and McBride (1982) the addition of humic acid reduces the activity of Cd in solution (Tyler and McBride 1982) reducing the Cd absorption by plant roots. They also noted that humic acid did not have an effect on the Cd translocation within the corn plants once the Cd was absorbed (Tyler and McBride 1982).

Stevenson (1976) determined the stability (log K) of a Cd-humic acid complex derived from leonardite to be 6.9. Cheam and Gamble (1974) found that the log K values for Cd-fulvate complexes were 3.04 and 3.64 at pH 4.9 and 5.95, respectively. Saha et al. (1979) had shown that the stability constant for a Cd-humic acid water-soluble complex was from 3.25 to 3.93 and that the stability constants vary little with the degree of saturation of the humic acid (Saha et al. 1979). Stability constants do not tend to vary much with the origin of the humic acid (Stevenson 1976). However, researchers have documented a direct correlation between stability of metal ions and degree of humification with the stability of the metal ion - humic acid increasing with degree of humification (Matsuda and Ito 1970).

The concentration of Cd does not have an effect on the sorption rate in the presence of humic acid (Bunzl et al. 1976).

The pH of the soil is an important factor in the fate of Cd in the environment (Bolton et al. 1996). As the pH of the soil increases, the formation and stability of Cd complexes becomes greater (Bolton 1996) reducing the availability of Cd to plants. The pH of the soil is also important because of the effect of pH on the dissociation of the carboxyl groups within the humic acid. At pH 4.2 about 8.5% of carboxyls are dissociated, while at pH 6.3, 80% of the carboxyls groups are ionized (Taylor and Theng 1995). Dissociation of humic acid also has an effect on the formation and stability of Cd species in the environment. For example, Bolton et al. (1996), using a nonelectrostatic model, showed that there are four Cd complexes formed in the presence of humic acid: $CdHA^+$, CdA^0, $CdH_{-1}A^-$, and $CdHB^+$. The complexation constants for the four species were $\log \beta = -1.29$, $\log \beta = -5.92$, $\log \beta =-14.39$, and $\log \beta = -3.72$, respectively (Bolton et al. 1996).

There are two possible complexation mechanisms for Cd. The first involves the formation of a 1:1 complex forming a hydroxo Cd complex (Saha et al. 1979). The second complex is a 2:1 complex formed through the binding of a Cd ion to two bidentate ligands when there is a high site to metal ratio (Stevenson 1977). Cadmium is known to form inner-sphere complexes with carboxyl groups (Taylor and Theng 1995).

The addition of humic acid causes increased adsorption of Cd and reduced mobility of the complexed Cd (Warwick et al. 1998). Campbell et al. (1987) found that addition of humic acid to soil results in enhanced adsorption of Cd relative to clay alone. However, when the Cd in soil solution became low, the addition of humic acid resulted in greater desorption. The concentration of humic acid also influences the adsorption of

Cd^{2+} (Davis and Bhatnagar 1994) with adsorption of Cd^{2+} increasing with increases in concentration of humic acid.

Brady and Pagenkopf (1978) found that as the degree of saturation of fulvic acid increased, the stability of the Cd complex increased. The stability constants (log K) were CdFA=5.3, Cd_2FA=9.8, and Cd_3FA=14.0 at pH 5.7. As the pH increased from 5.7 to 7.7 the stability of each of the three complexes increased but remained in the same order. Therefore, increasing the saturation of fulvic acid by transition metals resulting in the formation of more stable complexes (Brady and Pagenkopf 1978). However, when the fulvic acid becomes fully saturated with transition metals, the fulvic acid will precipitate (Brady and Pagenkopf 1978).

3. EFFECT OF PRE-PLANT APPLICATIONS OF LEONARDITE ON YIELD AND CHEMICAL COMPOSITION OF CANOLA AND WHEAT

3.1 Abstract

A two-year field study was performed to study the yield and chemical composition of wheat and canola as affected by rate and form of leonardite (oxidized lignite). Wheat *Triticum aestivum* (cv. AC Barrie) and Argentine canola *Brassica napus* (cv. LG 3310) were grown on two soil types, a Black Chernozem and a Dark Grey Luvisol. Three forms of leonardite were evaluated; a mine material, powder (colloidal mine material), and liquid (composed of humic extracts from the leonardite). In the first year, mine material and powder were applied at five rates, 50, 150, 500, 1000, and 5000 kg ha^{-1}, whereas the liquid leonardite was applied at three rates, 50, 100, and 300 L ha^{-1}. In the second year, the 50 and 150 kg ha^{-1} treatments of mine material and powder and the 50 and 100 L ha^{-1} treatments of the liquid leonardite were reapplied to the same plots. Leonardite treatments were incorporated prior to seeding. Emergence of canola and wheat, yield of canola and wheat, and chemical composition of wheat grain were determined. Results from this study were inconsistent. Emergence of both crops was not affected by the application of leonardite. Overall, yield was not significantly affected by the application of leonardite. However, highest yields were usually attained on plots with leonardite at low rates. A significant form effect of leonardite was observed where the powder form of leonardite significantly increased wheat yield compared to the mine

material. Canola yield was not affected by leonardite application. Chemical composition of wheat grain was not affected by the application of leonardite.

3.2 Introduction

Leonardite is oxidized lignite that contains humic substances (Hoffman et al. 1995) and is a precursor to coal. In some cases, the addition of leonardite has increased plant growth and, consequently, yield. In other instances, the application of leonardite has resulted in either no response or a hindering effect on plant growth. Addition of leonardite has increased shoot growth and biomass of corn (Duplessis and MacKenzie 1983, Tan and Nopamornbodi 1979). Coal combustion by-products have increased the shoot dry matter of wheat (Zaifnejad et al. 1996) while humic acid (derived from leonardite) significantly increased dry matter production of tomato by 8% and 9%, on a fresh and dry weight basis, respectively (Adani et al. 1998). Corn yields were significantly increased with the addition of leonardite (Duplessis and MacKenzie 1983) when applied to a loamy sand. Charcoal, coal, and peat were found to improve the grain yields of legumes (Iswaran et al. 1979).

Leonardite has also had detrimental effects on crops. Wallace and Romney (1980) found that application of coal reduced the vegetative growth of bush bean plants. Schisler and Linderman (1989) discovered that application of leonardite at high rates reduced the growth of coniferous trees. Additions of coal by-products to corn and bean did not yield differently than crops that had not received coal by-product application (Adriano et al. 1978). This suggests that the application of coal by-product had no effect on yield.

Leonardite may also increase the uptake of nutrients by plants. Humic acid, derived from leonardite, increased the N and P content of tomato (Adani et al. 1998) and corn plants (Duplessis and MacKenzie 1983). Wallace and Romney (1980) discovered that addition of powdered coal elevated the P and K concentration of bush bean plants in a calcareous soil. Iron concentration in tomato was enhanced with the addition of humic acid derived from leonardite (Adani et al. 1998) and Mn concentration in bush bean plants was increased with addition of coal (Wallace and Romney 1980). Adriano et al. (1978) found that coal ash enhanced the concentration of Cu, Zn, and Mn of corn and bush bean plants.

Conversely, some studies showed that lignite can decrease nutrient uptake. Application of coal ash resulted in a P deficiency in corn and bean (Adriano et al. 1978) and powdered coal reduced P and K concentrations of bush bean plants (Wallace and Romney 1980). Adriano et al. (1978) found the concentration of Cu and Mn of corn and bean decreased due to the addition of coal by-products.

The effects of leonardite on crop growth, yield, and chemical composition have been inconsistent. Therefore, the purpose of this study was to investigate the effects of leonardite on the growth, yield, and chemical composition of canola and wheat. These two crops have received little to no attention with respect to the effects of leonardite on crop productivity. Various rates of leonardite were used in this study. Low rates of leonardite were used to determine whether or not leonardite could elicit hormone-like responses to plant growth while high rates of leonardite were used to determine whether or not soil physical properties could be altered and crop growth enhanced.

3.3 Materials and Methods

Field studies were conducted in 1998 and 1999 determine whether the addition of leonardite could increase yield and/or alter the nutrient uptake of wheat and canola. The experiment was designed as a randomized complete block with 14 treatments and 4 replicates. Soil analysis was conducted by Norwest labs. Procedures for specific soil chemical properties are described in Appendix Xa. Chemical properties of the soils as well as extractable P, K, and S are listed below (Table 3.1).

Table 3.1 Characteristics of a Chernozem and Luvisol soil in 1998 and 1999.

	Soil Type and Year					
	Chernozem			Luvisol		
Soil Characteristic	**1998**	**1999-canola**	**1999-wheat**	**1998**	**1999-canola**	**1999-wheat**
pH	7.7	7.7	7.7	7.5	7.5	7.5
E.C. (dS m^{-2})	1.2	1.2	1.2	0.2	0.2	0.2
O.M. (%)	5.4	5.4	5.4	3.6	3.6	3.6
N (kg ha^{-1})	120	44	65	28	12	16
P (kg ha^{-1})	36	30	30	16	19	17
K (kg ha^{-1})	1200	1200	1200	347	340	340
S (kg ha^{-1})	94	95	95	14	20	18

E.C. = Electrical Conductivity
O.M. = Organic Matter

Three forms of leonardite: a mine material, a powder, and a liquid form of leonardite were studied. The mine material leonardite (L-14) was the original or undisturbed form of leonardite that was extracted directly from the mine and consisted of varying particle sizes. The powder form of leonardite (colloidal L-14) was prepared by finely grinding the mine material. The liquid leonardite (L-11) was an alkali-extract of leonardite. The liquid leonardite was characterized as being primarily humic and fulvic acids. The forms of leonardite used were identical for each site and the characterizations of the three forms of leonardite are presented in Appendix I. The laboratory preparing the various leonardite products used the terms L-14 and L-11 for identification.

The rates of leonardite used in this study varied with the year and form of leonardite. Various rates of leonardite were used to determine the effect of residual and annual applications on productivity of canola and wheat. Rates of leonardite by year are presented below (Table 3.2).

Table 3.2 Application rates of leonardite in 1998 and 1999 on a Chernozem and Dark Grey Luvisol.

	Year	
Form	**1998**	**1999**
Mine Material (kg ha^{-1})	50 150 500 1000 5000	50 150
Powder (kg ha^{-1})	50 150 500 1000 5000	50 150
Liquid (L ha^{-1})	50 100 300	50 100

The leonardite was spread uniformly on the soil surfaces as a pre-plant broadcast application and then incorporated using a roto-tiller to an approximate depth of 5 to 7 cm. Seeding of the plots occurred the day following leonardite application.

Fertility of the Sites

Fertility was maintained at high levels to eliminate nutrient deficiency as a factor in the study and soil tests were used as a guide for fertilizer application. Nitrogen on the Chernozem soil in 1998 was applied as a pre-plant band. For the 1999 field season, N was applied as a broadcast post-plant application. Nitrogen on the Luvisol in both years was applied as a broadcast post-plant treatment with split applications due to the high rates applied. Phosphorus was applied in the seed row. All other nutrients were applied as a broadcast post-plant treatment. Nitrogen was applied as urea, P as monoammonium phosphate, and S as ammonium sulphate. Rates of nutrients applied are summarized in the table below (Table 3.3).

Table 3.3 Rates of nutrients applied as fertilizer (kg ha^{-1}) for a Luvisol and Chernozem soil in 1998 and 1999.

	Soil Type and Year							
	Luvisol				Chernozem			
	Canola		Wheat		Canola		Wheat	
Nutrient	1998	1999	1998	1999	1998	1999	1998	1999
N	175	200	125	150	175	55	105	50
P	8	8	17	17	8	8	17	17
K	0	0	0	0	0	0	0	0
S	42	42	24	24	25	25	0	0

The crop variety of Canadian Western Red Spring wheat used for each site was identical, cv. AC Barrie while the variety of canola at each site was an argentine type *Brassica napus* cv. LG 3310. Wheat and canola plots were located adjacent to each other and received identical application of leonardite, as previously indicated. In 1999, the canola was planted on the wheat plot from the previous year and the wheat was grown on the plots previously seeded to canola. The plot seeder was a double disc press drill with 18 cm row spacing. In 1998, the wheat and canola on the Luvisol site was seeded on May 15 and in 1999, the plots were seeded on June 10. For the Chernozem site, the plot was seeded on May 22 in 1998 and on June 19 in 1999. The wheat was seeded at a depth of 4 to 5 cm and the canola at 2 to 3 cm. Wheat was seeded at a rate of 84 kg ha^{-1} and canola was seeded at 5.8 kg ha^{-1}. The canola and wheat plots were identical in size at 3.2 meters in width x 5 meters in length.

Crop emergence was determined by counting the number of plants that emerged in one meter of a single seed row, using the same seed row within each plot.

Grain yield at maturity was determined by hand harvesting 6 meter-rows (2 rows x 3 meter in length) of wheat or canola. The samples were dried, threshed, and weighed for grain and straw. Chemical analysis of plant material was conducted by digesting a one-gram sample with a nitric-perchloric acid mixture that was heated to a temperature of 230 degree Celsius for two hours and then diluted to a 1:25 solution with distilled water. Concentration of various metals was determined by Inductively Coupled Plasma (ICP) Spectrophotometry.

Statistical analyses of the data were completed using ANOVA and contrasts procedures in the Statistical Analysis System (SAS Institute (Canada) Inc., Version 8.0, BCE Place, Suite 2220, 181 Bay Street, P.O. Box 819, Toronto, ON, M5J 2T3). Treatments were considered significant if the Pr>F value was less than 0.05. Bartlett's test for homogeneity of variance was conducted for soil type and year combined analyses. Where possible, combined analysis was done by soil type and year. In cases where data from a location was unavailable, the data from soil type and year were combined into site years.

3.4 Results

Effect of Leonardite on Emergence

Leonardite application did not affect wheat emergence (Table 3.4 and 3.6). However, year and location effects were observed. Wheat emergence on the Chernozem soil was significantly greater than on the Luvisol soil and significantly greater in 1998 than in 1999.

Leonardite application did not affect canola emergence (Table 3.5 and 3.6). However, year and location effects were observed. Emergence of canola on the Chernozem soil was significantly greater than on the Luvisol soil. The emergence of canola was greater in 1998 than in 1999, primarily a result of the formation of a very hard surface crust that impeded canola emergence on the Luvisol soil in 1999. A significant year, location, and treatment interaction was also observed.

Table 3.4 Effect of pre-plant application of leonardite on wheat emergence (plants m^{-2}) for a Luvisol and Chernozem soil in 1998 and 1999.

	Soil Type and Year			
	Luvisol		Chernozem	
Treatment	1998	1999	1998	1999
Control	222	236	176	202
50kg ha^{-1} P	198	223	201	177
150kg ha^{-1} P	192	208	178	209
500kg ha^{-1} P	201	244	167	180
1000kg ha^{-1} P	223	225	155	177
5000kg ha^{-1} P	200	243	159	201
50kg ha^{-1} M	195	235	167	216
150kg ha^{-1} M	201	235	169	211
500kg ha^{-1} M	204	233	177	184
1000kg ha^{-1} M	173	229	181	205
5000kg ha^{-1} M	174	219	162	162
50 L ha^{-1}	205	229	185	188
100 L ha^{-1}	191	267	174	180
300 L ha^{-1}	184	226	164	197

P = Powder, M = Mine Material, L = Liquid

Table 3.5 Effect of pre-plant application of leonardite on canola emergence (plants m^{-2}) on a Luvisol and Chernozem soil in 1998 and 1999.

	Soil Type and Year			
	Luvisol		Chernozem	
Treatment	1998	1999	1998	1999
Control	117	72	98	97
50kg ha^{-1} P	126	66	89	117
150kg ha^{-1} P	128	74	76	94
500kg ha^{-1} P	119	72	98	111
1000kg ha^{-1} P	126	72	74	133
5000kg ha^{-1} P	115	67	96	115
50kg ha^{-1} M	135	66	87	155
150kg ha^{-1} M	119	60	90	118
500kg ha^{-1} M	122	66	90	131
1000kg ha^{-1} M	141	56	108	115
5000kg ha^{-1} M	124	74	103	90
50 L ha^{-1}	128	73	94	159
100 L ha^{-1}	111	74	103	131
300 L ha^{-1}	146	80	111	105

P = Powder, M = Mine Material, L = Liquid

Table 3.6 Combined statistical analysis of leonardite application on wheat and canola emergence on a Chernozem and Dark Grey Luvisol in 1998 and 1999.

		Crop			
		Wheat		Canola	
Source	df	MS	F-Value	MS	F-Value
Rep	3	182.66	7.27*	47.83	1.76
Year	1	1316.29	52.41**	409.86	15.08**
Location	1	1880.36	74.87**	143.04	5.26*
Treatment	13	24.34	0.97	19.06	0.7
Treatment x Year	13	23.31	0.93	22.36	0.82
Treatment x Location	13	30.05	1.2	17.21	0.63
Treatment x Year x Location	14	24.68	1.21	232	8.54**
Error	165	27.93		27.18	

* = significant at 0.05 level

** = significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

Table 3.7 Contrast analysis of leonardite application on wheat and canola emergence on a Dark Grey Luvisol and a Chernozem in 1998 and 1999.

	Crop	
Contrast	Wheat	Canola
Chernozem vs Luvisol	**	*
1998 vs 1999	**	*

* = significant at 0.05 level
** = significant at 0.0001 level

Effect of Leonardite on Yield

Significant differences in wheat yield did not exist between the control and any of the leonardite treatments. However, form of leonardite had a significant effect on wheat yield (Table 3.8 and 3.9). The powder form of leonardite significantly increased wheat yield compared to the mine material. There were some significant differences between specific treatments but no consistent effect by leonardite form or rate was evident. Significant year and location effects were also observed. Wheat yield on the Chernozem soil was significantly greater than on the Luvisol soil and wheat yield in 1998 was significantly greater than in 1999. A significant interaction between leonardite treatment and year and between leonardite treatment and location was also observed (Table 3.9).

Canola yield was not significantly affected by leonardite application (Table 3.12). However, a significant site year effect was observed. Canola yield significantly decreased in the order Luvisol 1999 > Chernozem 1998 > Luvisol 1999.

Yield data was unavailable for canola on the Chernozem soil in 1999 due to late seeding and a frost prior to maturity of the crop. In 1999, the wheat crop on the Chernozem was seeded in late June and yields were very low due to frost damage prior to grain filling.

Table 3.8 Effect of pre-plant applications of leonardite on yield of wheat (kg ha^{-1}) on a Luvisol and Chernozem soil in 1998 and 1999.

	Soil Type and Year			
	Luvisol		Chernozem	
Treatment	1998	1999	1998	1999
Control	3406	2418	1767	405
50kg ha^{-1} P	3264	2695	1483	490
150kg ha^{-1} P	3960	2334	1469	316
500kg ha^{-1} P	3621	2332	1741	344
1000kg ha^{-1} P	3609	2571	1513	473
5000kg ha^{-1} P	3536	2397	1563	431
50kg ha^{-1} M	2995	1856	1605	309
150kg ha^{-1} M	4017	2020	1472	356
500kg ha^{-1} M	2786	2299	1537	483
1000kg ha^{-1} M	3853	2475	1469	344
5000kg ha^{-1} M	2761	2462	1362	400
50 L ha^{-1}	3297	2163	1439	412
100 L ha^{-1}	3782	2332	1699	323
300 L ha^{-1}	3496	2435	1397	434

P = Powder, M = Mine Material, L = Liquid

Table 3.9 Combined statistical analysis of leonardite application on wheat yield on a Chernozem and a Dark Grey Luvisol in 1998 and 1999.

		Wheat	
Source	df	MS	F-Value
Rep	3	922	0.65
Year	1	818444	572.79**
Location	1	2369828	1658.54**
Treatment	13	2847	1.99*
Treatment x Year	13	2920	2.04*
Treatment x Location	13	2753	1.93*
Treatment x Year x Location	14	1599	1.12
Error	1428		

* = significant at 0.05 level

** = significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

Table 3.10 Contrast analysis of leonardite application on wheat yield on a Dark Grey Luvisol and a Chernozem in 1998 and 1999.

Contrast	Wheat Yield
Chernozem vs Luvisol	**
1998 vs 1999	**
Powder vs Mine Material	*

* = significant at 0.05 level

** = significant at 0.0001 level

Table 3.11 Effect of pre-plant leonardite application on canola yield (kg ha^{-1}) on a Luvisol and Chernozem soil.

	Soil Type and Year		
	Luvisol		Chernozem
Treatment	1998	1999	1998
Control	4078	2076	1776
50kg P	4694	1922	2086
150kg P	4952	1753	2074
500kg P	4310	1516	1659
1000kg P	4837	1432	1922
5000kg P	4490	1804	1741
50kg M	3571	1931	1680
150kg M	4099	1746	1774
500kg M	4228	1868	1823
1000kg M	4101	1476	1961
5000kg M	4064	1760	1926
50 L	4284	1734	1609
100L	4209	1183	1752
300L	4506	1879	2196

P = Powder, M = Mine Material, L = Liquid

Table 3.12 Combined statistical analysis of leonardite application on canola yield on a Chernozem in 1998 and a Dark Grey Luvisol in 1998 and 1999.

		Canola	
Source	df	MS	F-Value
Rep	3	3007	0.78
Site Year	2	1360408	353.29**
Treatment	13	4137	1.07
Treatment x Site Year	26	2868	0.74
Error	123	3850	

* = significant at 0.05 level

**= significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

Table 3.13 Contrast analysis of leonardite application on canola yield on a Dark Grey Luvisol and a Chernozem in 1998 and 1999.

Contrast	Canola Yield
Erickson 1998 vs Morris 1998	**
Erickson 1998 vs Erickson 1999	**
Erickson 1999 vs Morris 1998	**

* = significant at 0.05 level

** = significant at 0.0001 level

Effect of Leonardite on Chemical Composition of Grain

Analysis was conducted on wheat grain to determine whether or not the addition of leonardite had any effect on the chemical composition. Wheat grain was analyzed for N, P, K, S, Ca, Mg, Na, Cu, Zn, Fe, Mn, B, and Cd concentrations. In order to reduce cost of analysis, samples from the various replicates were composited by treatment prior to analysis. Thus, a statistical evaluation of the data could not be conducted. Leonardite did not appear to have any discernable effects on the nutritional quality of the wheat grain (Appendix II). In addition, the protein content (measured as %N) of the wheat grain was not affected by the addition of leonardite (Appendix II).

3.5 Discussion

The application of organic amendments has been shown to affect the germination (Vaughan and Malcolm 1985) and emergence of crop species (Nuttall 1970). However, in this study, leonardite did not affect the emergence of canola or wheat on the Luvisol or Chernozem soil in 1998 or 1999. Leonardite had no effect on the emergence of canola or wheat on the Luvisol soil in 1999 even though a heavy rainfall immediately after seeding resulted in crusting of the soil surface.

Although not significant, the low rates of leonardite seemed to be more effective in improving emergence than the high rates. This would also suggest that annual application of leonardite was more suitable at improving the emergence of canola than a single large application of leonardite.

In cases where leonardite has improved emergence of canola or wheat the application of the leonardite may have modified the soil physical properties to allow penetration of the plant (coleoptile or epicotyl/hypocotyl hook) through the soil crust with greater ease (Nuttall 1970). However, the greatest effects of leonardite were with low rates. If leonardite were to improve soil physical properties, the high rates of leonardite would be more likely to have the greatest effects on emergence.

Another possibility for improved emergence may be a hormonal effect due to polyphenolic compounds within the leonardite material. The hormonal effect would explain why low rates of leonardite resulted in the greatest emergence since high rates of hormones can reduce growth. Application of organic amendments has enhanced root growth (Vaughan and MacDonald 1976, Akinremi 1999) as well as percent seed germination (Petrovic et al. 1982). Increases in seed germination usually result in greater emergence. The increased root growth could cause improved absorption of nutrients, resulting in greater biomass and increased competition between plants, which may actually decrease the emergence of adjacent plants.

Research with leonardite has shown that application of leonardite can increase yields of crops. Duplessis and MacKenzie (1983) found that application of leonardite significantly increased the yield of corn while charcoal, coal, and peat have been shown to increase the yield of moong, soybean, and pea (Iswaran et al. 1979). In our study, the application of leonardite had no beneficial effect on wheat or canola yield for the Luvisol or Chernozem in either year of the study.

The reason for variation in yield response is possibly because of variation in the quality of the leonardite. Leonardite that is produced in different regions may vary in the concentration of humic substances, quality of humic substances, quality of organic carbon, and amounts of nitrogen. All of these factors contribute to the microbial activity and dynamics of the soil. Preliminary studies by Smirl (1999) and Akinremi (1999) showed that the leonardite material used in this study was inert or recalcitrant. Therefore, the carbon in this leonardite material may have had little or no effect on the energy dynamics of the soil system thus having little effect on nutrient cycling, production of organic acids, and organic matter content.

Alternately, the application rates of leonardite may not have been high enough to affect the soil dynamics. This seems quite possible since the high rate of leonardite was equivalent to 0.25% of the soil on a weight/weight basis.

Leonardite has been shown to have beneficial effects on the chemical composition of crop species (Adani et al. 1998, Wallace and Romney 1980). In contrast, other researchers have found that humic substances can have detrimental effects on chemical composition (Adriano et al. 1978, Linehan 1978). Analysis of the wheat grain showed that leonardite had no effect on the chemical composition of the wheat grain. This may suggest that the functional groups that are a component of humic substances within leonardite did not affect the chelation, diffusion, complexation or mobility of nutrients within the soil to a significant degree. Nutrient concentrations in the wheat grain also suggest that leonardite did not affect the turnover or mineralization / immobilization of nutrients within the soil.

3.6 Conclusion

The application of leonardite as a mine material, colloidal powder, or liquid formulation had no significant beneficial or detrimental effects on the yield of wheat or canola when applied at low (50 kg ha^{-1}) or very high rates (5,000 kg ha^{-1}). No significant hormone-like responses in plant growth were noted for the leonardite or extract of leonardite at low rates of application. Also, high rates of leonardite did not significantly increase yields indicating that the leonrdite did not affect soil physical properties or the plant did not respond to changes in physical properties if changes in properties had occurred.

The application of leonardite did not appear to affect the chemical composition of wheat grain and therefore did not appear to affect the phytoavailability of metals in the soil and translocation of nutrients or heavy metals within the plant.

4. EFFECT OF POST-PLANT APPLICATIONS OF LEONARDITE ON THE EMERGENCE OF CANOLA

4.1 Abstract

A field study was conducted to determine the effect of a post-plant broadcast application of leonardite on emergence and yield of canola. Argentine canola *Brassica napus* cv. LG 3310 was grown on two soil types, a Dark Grey Luvisol and a Black Chernozem. Three forms of leonardite were evaluated: a mine material, powder (colloidal mine material), and a liquid (composed of humic extracts from the mine material). Mine material and powder were applied at a single rate of 150 kg ha^{-1} on a dry weight basis. Liquid leonardite was applied at two rates, 100 and 300 L ha^{-1}. The leonardite products were uniformly applied immediately after seeding on the soil surface. Emergence and yield were not significantly affected by the post-plant broadcast application of leonardite and no significant effects on emergence and yield were obtained among the various forms and rates of leonardite.

4.2 Introduction

Applications of humic substances have been shown to have a beneficial effect on seed germination (Petrovic et al. 1982). Vaughan and Malcolm (1985) showed that the proportion of seeds that germinate increased with the application of humic substances and that the increase in germination may result in an increase in emergence. Reasons for improved germination have been attributed to biochemical and physiological processes such as increased water imbibition, enzyme synthesis (Smidova 1962), and respiration (Csicsor et al. 1994). However, humic acids can also have detrimental effects on germination. Piccolo et al. (1993) found that increasing application rates of humic acids from 40 to 5,000 ppm significantly reduced germination percentage of tomato seeds.

Organic amendments can have a favorable effect on the emergence of rapeseed (*Brassica napus*). Nuttall (1970) discovered that the emergence of rapeseed was increased by as much as threefold with the application of an organic amendment at a rate of 2.65% by soil weight. The reason for the improved emergence was due to improved soil physical properties, as indicated by a reduced modulus of rupture. However, studies conducted by Akinremi (1999) showed that the addition of leonardite had no effect on the germination of canola seed. The same study also showed that leonardite did not affect emergence of canola.

This study was conducted as a separate study but located on the same sites as the study described previously in section 3 of this manuscript to specifically determine the effect of post-plant applications of leonardite on emergence of canola. Low rates of leonardite were applied immediately after seeding to determine whether application of leonardite on the soil surface could enhance canola emergence through improved soil physical properties of the soil surface.

4.3 Materials and Methods

In 1998 and 1999, a field study was conducted to determine the effect of post-plant application of leonardite on emergence of argentine canola *Brassica napus* (cv. LG 3310). The experimental design was a randomized complete block with 5 treatments and 4 replicates. Soil analysis was conducted by Norwest labs. Procedures for specific for various soil chemical characteristics are described in Appendix Xa. Characteristics of the two sites used in the study are described below (Table 4.1).

Table 4.1 Characteristics of a Chernozem and Luvisol soil used in canola emergence study.

	Soil Type	
Characteristic	**Chernozem**	**Luvisol**
pH	7.7	7.5
E.C. (dS m^{-2})	1.2	0.2
O.M. (%)	5.4	3.6

E.C. = Electrical Conductivity
O.M. = Organic Matter

The forms of leonardite used were identical to the pre-plant application study and included the mine material (L-14), powder (colloidal L-14), and liquid form (L-11) of leonardite (Appendix I). The mine material and powder forms of leonardite were applied at a rate of 150 kg ha^{-1} (dry weight basis). Two rates of liquid leonardite, 100 L ha^{-1} and 300 L ha^{-1} were applied. All leonardite treatments were applied as a broadcast post-plant treatment to test whether or not the leonardite would be effective in reducing surface soil crusting thereby increasing the emergence of canola. The canola was seeded and followed by a uniform application of leonardite over the entire plot. The solid forms of leonardite were spread by hand whereas the liquid form of leonardite was sprayed on the soil surface using hand-pump sprayers.

Conventional fertilizers were applied as described for the pre-plant application study in section 3 (Table 4.2). On the Chernozem soil in the 1998 field season, N was applied as a pre-plant band, while in the 1999 field season, the N was applied as a broadcast post-plant treatment. On the Luvisol, N was applied as a broadcast post-plant treatment with split applications due to the high rates applied (50% of the N was broadcast applied immediately after seeding, 25% at the 3 leaf stage, and 25% just prior to bolting). Phosphorus was applied in the seed row. All other nutrients were applied as a broadcast post-plant treatment, after leonardite application.

Table 4.2 Rates of nutrients applied (kg ha^{-1}) for a Luvisol and Chernozem soil in 1998 and 1999.

	Soil Type and Year			
	Luvisol		Chernozem	
Nutrient	**1998**	**1999**	**1998**	**1999**
N	175	200	175	55
P	8	8	8	8
K	0	0	0	0
S	42	42	25	25

Nitrogen was applied as urea, P as monoammonium phosphate, and S as ammonium sulphate. *Brassica napus* cv. LG 3310 was seeded at a rate of 5.8 kg ha^{-1}. Seeding date for the Luvisol was May 15 in 1998 and June 10 in 1999. The seeding date for the Chernozem was May 22 in 1998 and June 19 in 1999. Seeding depth was 2 to 3 cm and plot size was 1.6 m in width and 5 m in length.

Emergence was determined by counting the number of plants that emerged in one meter of a single seed row using the same seed row within each plot.

Grain yields at maturity were determined by hand-harvesting 6 meter-rows (2 rows x 3 meter in length). The plant samples were dried, threshed and weighed for seed and straw yield. Statistical analyses of the data were conducted using ANOVA and contrast procedures using the Statistical Analysis System (SAS Institute (Canada) Inc., Version 8.0, BCE Place, Suite 2220, 181 Bay Street, P.O. Box 819, Toronto, ON, M5J 2T3). Treatments were considered significant if the Pr>F value was less than 0.05. Bartlett's test for homogeneity of variance was conducted for soil type and year combined analyses. Where possible, combined analysis was conducted. In cases where data was unavailable for a year or location, analysis of site years was performed.

4.4 Results

Effect of Leonardite on Canola Emergence

Canola emergence was significantly greater in 1998 than in 1999 due to a hard surface crust that formed after a large rainfall event, soon after seeding on the Luvisol soil (Table 4.3 and 4.4). Canola that emerged in 1999 on the Luvisol soil had emerged mainly through cracks formed in the crust after drying of the soil. Canola emergence on the Chernozem was significantly greater than on the Luvisol soil. Leonardite application did not affect canola emergence (Table 4.4).

Table 4.3 Effect of post-plant application of leonardite on canola emergence (plants m^{-2}).

	Soil Type and Year			
	Luvisol		Chernozem	
Treatment	1998	1999	1998	1999
Control	126	28	87	101
150kg ha^{-1} P	122	48	108	122
150kg ha^{-1} M	129	45	86	117
100 L ha^{-1}	128	44	81	100
300 L ha^{-1}	132	32	84	96

P = Powder, M = Mine Material, L = Liquid

Table 4.4 Combined statistical analysis of leonardite application on canola emergence on a Chernozem and a Dark Grey Luvisol in 1998 and 1999.

		Canola	
Source	df	MS	F-Value
Rep	3	0.44	0.02
Year	1	787.51	40.28**
Location	1	137.81	7.05*
Treatment	4	19.39	0.99
Treatment x Year	4	7.36	0.38
Treatment x Location	4	12.34	0.63
Treatment x Year x Location	5	358.28	18.33*
Error	57	19.55	

* = significant at 0.05 level

** = significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

Yield of canola on the Luvisol soil was significantly higher in 1998 than in 1999. Yields in 1998 on the Luvisol were extremely high due to favorable weather and low in 1999 due to poor emergence and late seeding (Table 4.5 and 4.6). Canola yield on the Chernozem was significantly greater than yields on the Luvisol in 1999 but significantly less than on the Luvisol in 1998. Leonardite application had no effect on the yield of canola.

Table 4.5 Effect of post-plant application of leonardite on canola yield (kg ha^{-1}).

	Soil Type and Year		
	Luvisol		Chernozem
Treatment	1998	1999	1998
Control	4333	1380	2118
150kg P	4084	1425	2297
150kg M	4393	1387	2189
100L	3761	1389	2229
300L	3641	796	2280

P = Powder, M = Mine Material, L = Liquid

Table 4.6 Combined statistical analysis of leonardite application on canola yield on a Chernozem and a Dark Grey Luvisol in 1998 and 1999.

		Canola	
Source	df	MS	F-Value
Rep	3	2799	0.4
Site Year	2	450130	64.85**
Treatment	4	3959	0.57
Treatment x Site Year	8	2339	0.34
Error	42	6941	

* = significant at 0.05 level

** = significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

4.5 Discussion

Post-plant application of leonardite did not affect the emergence of canola on either soil type in either year. These results are consistent with those found by Akinremi (1999). However, these results do not agree with those from Nuttall (1970) in which the application of an organic amendment improved emergence of rapeseed threefold with addition of organic amendments at 2.5% by soil weight. The highest rate of leonardite used in our study 0.25% by soil weight.

Preliminary research by Akinremi (1999) and Smirl (1999) showed that the leonardite material is recalcitrant and not actively used as an energy source by microorganisms. This may explain why the addition of leonardite had no effect on germination or emergence of canola. Microbial activity can result in the production of enzymes, hormones or organic acids which can have biochemical or physiological effects on seeds (Smidova 1962, Csicsor et al. 1994). Since microbial activity was not enhanced with the application of leonardite, microbial activity in the rhizosphere would probably be unaffected by leonardite.

Post-plant applications did not affect yield. This is consistent with the results from the study with pre-plant applications of leonardite.

4.6 Conclusions

The application of leonardite did not affect the emergence of canola when applied as a post-plant treatment. All forms of leonardite were ineffective in enhancing emergence even under conditions where emergence was reduced due to surface crusting.

5. EFFECT OF LEONARDITE ON P FERTILIZER USE EFFICIENCY

5.1 Abstract

A field study was conducted to determine the effect of humic acid on efficiency of P fertilizer. Argentine canola *Brassica napus* cv. 45A51, a herbicide tolerant canola (Roundup Ready^{TM}) was grown on two soil types, a Black Chernozem and a Dark Grey Luvisol. Phosphorus was applied as ammonium polyphosphate (APP) at four rates, 0, 6.5, 13, and 20 kg ha^{-1} P. Phosphorus was applied as a side-band 2.5 cm below and 2.5 cm beside the seed. Humic acid was applied with the P fertilizer. Two formulations of humic acid, designated as L-45 and L-52, were used at three rates, 0, 12 and 24 L ha^{-1}. Canola biomass and P concentration in plant tissues were measured at 20 and 35 days after emergence. Seed yield at maturity was also determined. Humic acid did not affect canola biomass on the Luvisol soil. Increasing rates of humic acid significantly increased canola biomass on the Chernozem. Canola biomass at 20 days after emergence tended to increase with humic acid application when no P fertilizer was applied. Humic acid significantly increased the P concentration of canola tissue at 20 and 35 d after emergence on the Chernozem. A significant formulation difference was evident where the L-52 increased P concentration compared to L-45 at both the 20 and 35 day sampling date. A humic acid rate effect was also evident where increasing rate of humic acid increased P concentration of canola. Humic acid did not affect P concentration on the Luvisol. Humic acid did not affect P accumulation at either location. Phosphorus fertilizer increased early season biomass as well as final seed yield on the Luvisol. Early

season biomass, P concentration, and P accumulation of canola on the Chernozem was increased with P fertilizer but final seed yields with and without P fertilizer were not significantly different. Boron (B) concentrations in plant tissues were significantly higher with humic acid most likely as a result of the B present in the humic acid. Humic acid had no significant effect on the final yield of canola on either site.

5.2 Introduction

The crop utilization of P fertilizer is relatively low by crops due to reactions with the soil. The efficiency of P fertilizer is approximately 20% for prairie crops in the year of application (Hammond 1997). The low efficiency of P fertilizer is especially evident on calcareous, high pH soils, such as those found in Manitoba. Reasons for the poor efficiency of P fertilizer include the formation of moderately insoluble phosphates with cations such as Ca and Mg.

Researchers have discovered that addition of leonardite can improve the efficiency of P fertilizers by plants (Duplessis and MacKenzie 1983). Researchers have also discovered that application of humic substances from other sources can improve P fertilizer use efficiency (Yang et al. 1985, Li and Wang 1988). However, leonardite application has also been shown to reduce the availability of P to plants (Saeed 1978).

This study was conducted to determine whether or not the addition of humic acid, derived from leonardite, would improve the efficiency of P fertilizer by canola on alkaline soils. Canola has a high demand for P and is very efficient at using P from soil and fertilizer through proliferation of roots into fertilizer reaction zones and by acidification of the rhizosphere to solubilize soil P. Canola has also received little attention in P fertilizer use efficiency studies conducted with humic acids. It was postulated that humic acids applied with P fertilizer may reduce reaction between soil constituents and the P fertilizer thereby enhancing P fertilizer phytoavailability.

5.3 Materials and Methods

The study was designed as a 4x2x2 (4 phosphorus rates, 2 forms of humic acid and 2 rates of humic acid) factorial with 4 replicates. Two soils in Manitoba known to be P deficient were selected, a Black Chernozem near Portage la Prairie and a Dark Grey Luvisol near Teulon. Soil analysis was conducted by Norwest Labs. Procedures specific for determination of the various soil chemical characteristics are described in Appendix Xa. Characteristics of the sites are presented below (Table 5.1).

Table 5.1 Soil characteristics of a Black Chernozem and Dark Grey Luvisol in 1999.

	Soil Type	
Characteristic	Black Chernozem	Dark Grey Luvisol
pH	7.4	6.8
texture	silt loam	sandy loam
O.M. (%)	4.5	2.6
E.C. (dS m^{-2})	1.2	1.4
soil test NO_3^- (kg ha^{-1})	140	10
soil test P (kg ha^{-1})	20	8
soil test K (kg ha^{-1})	260	90
soil test SO_4^{2-} (kg ha^{-1})	88	38

Argentine canola *Brassica napus* (cv. 45A51) was planted on June 1, 1999 on the Chernozem soil and June 7, 1999 on the Luvisol site using a double-disc press drill with on-row packers.

Phosphorus fertilizer was applied at four rates in order to produce a P response curve. Rates of P fertilizer were 0, 6.5, 13, and 20 kg ha^{-1} P. Form of P fertilizer was ammonium polyphosphate (10-34-0). The P fertilizer was placed as a side-band 2.5 cm beside and 2.5 cm below the seed at time of seeding.

Two formulations of humic acids, designated as either L-45 or L-52, were used in the study. Characterization of the humic acids are provided in the Appendix (I). Each of the humic acids was applied at three rates, 0, 12 L ha^{-1} and 24 L ha^{-1}, with each rate of P fertilizer. Treatments were composed of solutions that contained humic acid, P fertilizer, and water which were prepared to ensure that the same volume was used for each treatment.

A basal application of nutrients was applied as fertilizer on the Dark Grey Luvisol at the following rates: 90 kg ha^{-1} of N, 93 kg ha^{-1} of K, and 60 kg ha^{-1} of S. Nitrogen was applied as urea, K as KCl, and sulphur as ammonium sulphate. All fertilizer was applied as a post-plant broadcast application. Nitrogen, K, and S were not applied to the Chernozem soil as soil analysis indicated sufficient levels of N, K, and S for crop growth.

Concentrations of P in plant tissue were determined 20 and 35 days after emergence. Small areas of each plot (0.1 m^2) were harvested for biomass, dried, weighed and then analyzed for P. The concentration of N, K, S, Ca, Mg, Na, Fe, Cu, Zn, Mn, and B in plant samples from the Luvisol site collected 20 and 35 days after emergence was also determined. Only the P and B concentrations of samples from the Chernozem site were determined as these were the only nutrients that varied, as indicated by tissue analysis, on the Dark Grey Luvisol. Procedures specific for determination of the various tissue nutrient analysis are described in Appendix Xb. Seed yields at maturity were determined by harvesting 6 meter of crop row (2 rows x 3 meter in length). The plant samples were dried, threshed, and grain weight measured. Statistical analyses of the data were conducted using ANOVA and contrast procedures using the Statistical Analysis System (SAS Institute (Canada) Inc., Version 8.0, BCE Place, Suite 2220, 181 Bay Street, P.O. Box 819, Toronto, ON, M5J 2T3). Treatments were considered significant if the Pr>F value was less than 0.05.

5.4 Results

Effect of Humic Acid on Canola Biomass

Biomass of canola on the Chernozem 20 and 35 d after emergence was significantly increased by P fertilization (Table 5.2 and 5.3). Humic acid in either form or rate had no effect on biomass of canola at 20 d after emergence. There was a significant humic acid rate effect on canola biomass for 35 d after emergence where biomass with the 24 L ha^{-1} rate was significantly greater than with the 12 L ha^{-1}. A significant interaction between P and rate of humic acid was also noted. The major factor in this interaction was the response to P fertilizer. A humic acid form effect was not evident on the canola biomass at either sampling date. An interesting trend was noted; the application of humic acid consistently increased the biomass of canola on the Chernozemic soil at 20 d after emergence without P fertilizer. This trend, however, was not significant at the 0.05 level of probability.

A highly significant P fertilizer effect was evident for canola biomass for both sampling dates on the Luvisolic soil (Table 5.3). Biomass with P fertilizer was about 2 to 3 fold greater than without P (Table 5.2). Humic acid form or rate had no effect on biomass of canola 20 or 35 days after emergence

Humic acid tended to increase the biomass of canola plants in the early stages of crop growth without P fertilizer. However, when P fertilizer was applied, the application of humic acid with the P fertilizer had no effect on the biomass of canola plants.

Table 5.2 Effect of Humic Acid on Canola Biomass at 20 and 35 days after emergence (kg ha^{-1}) on a Chernozem and Dark Grey Luvisol in 1999.

	Soil and Sampling Date			
	Chernozem		Luvisol	
Treatment	20 days	35 days	20 days	35 days
0 P, No Humic acid	805	2930	210	856
6.5 P, No Humic acid	1310	3868	635	2451
13 P, No Humic acid	945	3208	505	2700
20 P, No Humic acid	1587	3766	627	2540
0 P, 12 L ha^{-1} L-45	990	2777	336	1163
6.5 P, 12 L ha^{-1} L-45	1293	3673	464	2757
13 P, 12 L ha^{-1} L-45	1296	4104	685	2474
20 P, 12 L ha^{-1} L-45	1605	4057	622	2817
0 P, 24 L ha^{-1} L-45	870	3531	297	1180
6.5 P, 24 L ha^{-1} L-45	1052	3361	469	1666
13 P, 24 L ha^{-1} L-45	1220	5036	490	2337
20 P, 24 L ha^{-1} L-45	1285	4073	699	3261
0 P, 12 L ha^{-1} L-52	925	2337	312	808
6.5 P, 12 L ha^{-1} L-52	1112	3286	468	2204
13 P, 12 L ha^{-1} L-52	1110	2905	479	1996
20 P, 12 L ha^{-1} L-52	1400	4682	617	2848
0 P, 24 L ha^{-1} L-52	890	3537	342	1049
6.5 P, 24 L ha^{-1} L-52	1325	3012	779	2426
13 P, 24 L ha^{-1} L-52	1060	4271	696	2856
20 P, 24 L ha^{-1} L-52	1257	3612	653	3084

Table 5.3 Statistical analysis of canola biomass at 20 and 35 day after emergence for a Chernozem and Dark Grey Luvisol in 1999.

		Soil							
		Chernozem				Luvisol			
		20d		35d		20d		35d	
Source	df	MS	F-Value	MS	F-Value	MS	F-Value	MS	F-Value
Rep	3	87.18	8.15**	261.25	4.82*	2.93	0.85	91.81	2.4
Prate	3	119.63	11.19**	355.39	6.56*	40.42	11.74**	1384.00	36.17**
Lrate	2	6.48	0.61	108.49	2.01*	3.11	0.90	2.13	0.06
Lform	1	4.08	0.38	180.61	3.33	0.23	0.07	8.69	0.23
Prate*Lform	3	2.15	0.20	100.24	1.85	2.17	0.63	5.11	0.13
Prate*Lrate	6	10.54	0.99	121.26	2.24*	3.46	1.00	51.32	1.34
Lform*Lrate	2	6.95	0.65	34.41	0.64	6.73	1.95	58.93	1.54
Prate*Lform*Lrate	6	2.09	0.18	83.77	1.62	2.32	0.65	22.33	0.56
Error	75	10.69		54.19		3.42		38.26	

*= significant at 0.05 level

** = significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

P = Phosphorous

L = Leonardite

Table 5.4 Contrast analysis of canola biomass at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

	Soil Type and Sampling Date			
	Chernozem		Luvisol	
Contrast	20 d	35 d	20 d	35 d
Phosphorus Rate				
0 vs 15	*	*	**	**
0 vs 30	*	*	**	**
0 vs 45	**	**	**	**
15 vs 30	ns	ns	ns	ns
15 vs 45	*	ns	ns	*
30 vs 45	*	ns	ns	ns
Leonardite rate				
Control vs Low	ns	ns	ns	ns
Control vs High	ns	*	ns	ns
Low vs High	ns	*	ns	ns

ns = Non-significant

* = significant at 0.05 level of probability

** = significant at 0.001 level of probability

Effect of Humic Acid on Canola Tissue P Concentrations

On the Chernozemic soil, a humic acid formulation effect was evident for P concentration of canola at 20 d after emergence (Table 5.5 and 5.6). The L-52 formulation significantly increased P concentration in canola versus the L-45 formulation at this site. The positive effect of the humic acids on P concentration in canola tissue was also observed at 35 d after emergence. In addition, a humic acid rate effect was also observed. The 12 L ha^{-1} rate of humic acid significantly increased P concentration of canola versus the control treatments. The P concentration in canola with 24 L ha^{-1} was significantly greater than with 12 L ha^{-1}. Highly significant P rate effects were observed at both sampling dates on the Chernozem soil. A P interaction with humic acid rate was also evident; the interaction between P fertilizer and rate of humic acid was not evident at the 35 day sampling date.

On the Luvisolic soil, phosphorus fertilization significantly increased P concentration in canola tissues at both sampling dates (Table 5.5 and 5.6). However, humic acid did not have an effect on P concentration of canola tissues as was evident on the Chernozemic soil and no form or rate effect was evident.

Phosphorus accumulation in plants grown on the Chernozem and Luvisolic soil was also determined (Table 5.8 and 5.9). Humic acid had no effect on P accumulation. No rate or formulation effects were evident on either soil or sampling date. However, as expected, P fertilization significantly increased P accumulation on both soils and sampling dates.

Table 5.5 Effect of humic acid on P concentration of canola (%) on a Chernozem and Dark Grey Luvisol in 1999.

	Soil and Sampling Date			
	Chernozem		Luvisol	
Treatment	20 days	35 days	20 days	35 days
0 P, No Humic acid	0.30	0.35	0.31	0.17
6.5 P, No Humic acid	0.39	0.30	0.60	0.31
13 P, No Humic acid	0.45	0.40	0.65	0.34
20 P, No Humic acid	0.41	0.46	0.63	0.37
0 P, 12 L ha^{-1} L-45	0.32	0.32	0.36	0.18
6.5 P, 12 L ha^{-1} L-45	0.41	0.29	0.61	0.29
13 P, 12 L ha^{-1} L-45	0.45	0.38	0.59	0.35
20 P, 12 L ha^{-1} L-45	0.43	0.40	0.70	0.37
0 P, 24 L ha^{-1} L-45	0.32	0.31	0.35	0.18
6.5 P, 24 L ha^{-1} L-45	0.44	0.37	0.55	0.29
13 P, 24 L ha^{-1} L-45	0.47	0.38	0.63	0.33
20 P, 24 L ha^{-1} L-45	0.46	0.45	0.63	0.37
0 P, 12 L ha^{-1} L-52	0.33	0.35	0.34	0.18
6.5 P, 12 L ha^{-1} L-52	0.48	0.38	0.58	0.28
13 P, 12 L ha^{-1} L-52	0.49	0.45	0.62	0.35
20 P, 12 L ha^{-1} L-52	0.45	0.39	0.62	0.36
0 P, 24 L ha^{-1} L-52	0.39	0.33	0.32	0.17
6.5 P, 24 L ha^{-1} L-52	0.47	0.39	0.62	0.30
13 P, 24 L ha^{-1} L-52	0.48	0.42	0.67	0.34
20 P, 24 L ha^{-1} L-52	0.52	0.46	0.63	0.36

Table 5.6 Statistical analysis of canola P concentration at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

		Soil Type							
		Chernozem				Luvisol			
		20d		35d		20d		35d	
Source	df	MS	F-Value	MS	F-Value	MS	F-Value	MS	F-Value
Rep	3	0.020	10.02**	0.069	28.62**	0.011	2.20	0.022	7.56*
Prate	3	0.060	26.26**	0.092	38.53**	0.516	103.48**	0.175	60.61**
Lrate	2	0.010	5.11	0.018	7.72**	0.001	0.02	0.001	0.09
Lform	1	0.002	1.14*	0.024	10.27*	0.001	0.09	0.001	0.04
Prate*Lform	3	0.001	0.77	0.001	0.18	0.004	0.72	0.001	0.06
Prate*Lrate	6	0.007	2.99*	0.001	0.76	0.004	0.76	0.001	0.19
Lform*Lrate	2	0.004	1.63	0.005	2.04	0.005	0.94	0.001	0.09
Prate*Lform*Lrate	6	0.001	0.56	0.001	0.53	0.002	0.47	0.001	0.10
Error	75	0.002		0.002		0.005		0.003	

* = significant at 0.05 level

** = significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

P = Phosphorous

L = Leonardite

Table 5.7 Contrast analysis of canola P concentration at 20 and 35 day after emergence for a Chernozem and Dark Grey Luvisol in 1999.

Contrast	Chernozem		Luvisol	
Phosphorus rate	20 d	35 d	20 d	35 d
0 vs 15	ns	**	**	**
0 vs 30	**	**	**	**
0 vs 45	**	**	**	**
15 vs 30	**	*	*	*
15 vs 45	**	ns	*	**
30 vs 45	*	ns	ns	ns
Leonardite rate				
Control vs Low	ns	*	ns	ns
Control vs High	ns	**	ns	ns
Low vs High	ns	*	ns	ns
L formulation				
L45 vs L52	ns	*	ns	ns

ns = non-significant

* = significant at 0.05 level of probability

** = significant at 0.001 level of probability

Table 5.8 Effect of humic acids on P accumulation in canola tissue (kg ha^{-1}) at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

	Soil Type and Sampling Date			
	Chernozem		Luvisol	
Treatment	20 days	35 days	20 days	35 days
0 P, No Humic acid	2.4	10.3	0.7	1.5
6.5 P, No Humic acid	5.1	11.6	3.8	7.6
13 P, No Humic acid	4.3	12.8	3.3	9.2
20 P, No Humic acid	6.5	17.3	4.0	9.4
0 P, 12 L ha^{-1} L-45	3.2	8.9	1.2	2.1
6.5 P, 12 L ha^{-1} L-45	5.3	10.7	2.8	8.0
13 P, 12 L ha^{-1} L-45	5.8	15.6	4.0	8.7
20 P, 12 L ha^{-1} L-45	6.9	16.2	4.4	10.4
0 P, 24 L ha^{-1} L-45	2.8	10.9	1.0	2.1
6.5 P, 24 L ha^{-1} L-45	4.6	12.4	2.6	4.8
13 P, 24 L ha^{-1} L-45	5.7	19.1	3.1	7.7
20 P, 24 L ha^{-1} L-45	5.9	18.3	4.4	12.1
0 P, 12 L ha^{-1} L-52	3.1	8.2	1.1	1.5
6.5 P, 12 L ha^{-1} L-52	5.3	12.5	2.7	6.2
13 P, 12 L ha^{-1} L-52	5.4	13.1	3.0	7.0
20 P, 12 L ha^{-1} L-52	6.3	18.3	3.8	10.3
0 P, 24 L ha^{-1} L-52	3.5	11.7	1.1	1.8
6.5 P, 24 L ha^{-1} L-52	6.2	11.7	4.8	7.3
13 P, 24 L ha^{-1} L-52	5.1	17.9	4.7	9.7
20 P, 24 L ha^{-1} L-52	6.5	16.6	4.1	11.1

Table 5.9 Statistical analysis of canola P accumulation at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

		Soil Type							
		Chernozem				Luvisol			
		20d		35d		20d		35d	
Source	df	MS	F-Value	MS	F-Value	MS	F-Value	MS	F-Value
Rep	3	25.81	12.92**	109.49	12.11**	2.9	1.53	21.89	2.96*
Prate	3	47.22	23.64**	234.37	25.92**	42.09	22.17**	298.53	40.41**
Lrate	2	0.55	0.28	0.77	0.09	0.26	0.14	0.55	0.07
Lform	1	2.77	1.39	26.12	2.89	1.06	0.56	0.37	0.05
Prate*Lform	3	0.61	0.3	4.04	0.45	0.93	0.49	0.62	0.08
Prate*Lrate	6	0.92	0.46	13.08	1.45	0.99	0.52	4.63	0.63
Lform*Lrate	2	1.71	0.85	1.24	0.14	3.29	1.73	7.99	1.08
Prate*Lform*Lrate	6	0.32	0.15	3.46	0.36	0.97	0.49	3.63	0.47
Error	75	1.99		9.04		1.9		7.39	

* = significant at 0.05 level
** = significant at 0.0001 level
df = degrees of freedom
MS = Means Squares
P = Phosphorous
L = Leonardite

Table 5.10 Contrast analysis of canola P accumulation at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

Contrast	Chernozem		Luvisol	
Phosphorus rate	20 d	35 d	20 d	35 d
0 vs 15	**	ns	**	**
0 vs 30	**	**	**	**
0 vs 45	**	**	**	**
15 vs 30	ns	*	ns	ns
15 vs 45	*	**	ns	**
30 vs 45	*	*	ns	*

ns = non-significant
* = significant at 0.05 level of probability
** = significant at 0.001 level of probability

Effect of Humic Acid on Nutrient Concentrations

The plant samples from the Luvisolic soil were also analyzed for N, K, S, Ca, Mg, Fe, Cu, Zn, Mn, and B concentrations (Appendix III). The plant samples from the Chernozemic site were analyzed only for B since data obtained for the Luvisolic site indicated that leonardite affected only B concentrations in plants. However, with the exception of B (Table 5.11 and 5.12), humic acid application had no effect on the concentration and accumulation of nutrients or other elements in canola tissue.

Table 5.11 Effect of humic acid on B concentration (mg kg^{-1}) of canola at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

	Soil Type and Sampling Date			
	Chernozem		Luvisol	
Treatment	20 days	35 days	20 days	35 days
0 P, No Humic acid	33.8	34.3	12.7	20.4
6.5 P, No Humic acid	32.1	35.4	16.0	20.3
13 P, No Humic acid	33.1	35.7	13.6	22.6
20 P, No Humic acid	30.2	38.3	13.8	21.8
0 P, 12 L ha^{-1} L-45	34.0	34.2	14.6	20.4
6.5 P, 12 L ha^{-1} L-45	32.5	32.2	15.8	20.0
13 P, 12 L ha^{-1} L-45	32.9	33.7	14.0	20.3
20 P, 12 L ha^{-1} L-45	32.1	36.4	15.2	20.3
0 P, 24 L ha^{-1} L-45	31.8	33.3	14.5	20.1
6.5 P, 24 L ha^{-1} L-45	34.6	34.5	14.9	20.1
13 P, 24 L ha^{-1} L-45	34.7	35.3	15.5	21.2
20 P, 24 L ha^{-1} L-45	36.0	37.3	14.2	22.1
0 P, 12 L ha^{-1} L-52	36.3	34.7	18.0	19.6
6.5 P, 12 L ha^{-1} L-52	34.5	35.9	16.0	20.7
13 P, 12 L ha^{-1} L-52	34.7	36.5	15.3	22.4
20 P, 12 L ha^{-1} L-52	35.6	37.4	14.4	23.7
0 P, 24 L ha^{-1} L-52	35.5	33.2	16.0	19.3
6.5 P, 24 L ha^{-1} L-52	35.6	35.0	15.7	19.6
13 P, 24 L ha^{-1} L-52	33.7	38.7	14.2	21.3
20 P, 24 L ha^{-1} L-52	34.9	35.8	16.1	26.4

Table 5.12 Statistical analysis of canola B concentration at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

		Soil Type							
		Chernozem				Luvisol			
		20d		35d		20d		35d	
Source	df	MS	F-Value	MS	F-Value	MS	F-Value	MS	F-Value
Rep	3	2.04	0.85	42.18	6.81*	70.15	13.03**	50.25	6.86*
Prate	3	4.33	1.8	46.06	7.43*	11.65	2.16	38.41	5.25*
Lrate	2	46.3	19.19**	1.81	0.29	18.52	3.44*	1.22	0.17
Lform	1	24.4	10.12*	22.65	3.65	7.19	1.34	11.54	1.58
Prate*Lform	3	3.24	1.34	8.59	1.39	3.38	0.63	10.65	1.45
Prate*Lrate	6	12.29	5.09*	7.05	1.14	5.15	0.96	6.48	0.88
Lform*Lrate	2	12.31	5.10*	5.14	0.83	2.47	0.46	3.6	0.49
Prate*Lform*Lrate	6	4.06	1.79	2.54	0.39	3.56	0.64	3.54	0.46
Error	75	2.41		6.2		5.38		7.32	

* = significant at 0.05 level
** = significant at 0.0001 level
df = degrees of freedom
MS = Means Squares
P = Phosphorous
L = Leonardite

Table 5.13 Contrast analysis of canola B concentration at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

Contrast	Chernozem		Luvisol	
Phosphorus rate	20 d	35 d	20 d	35 d
0 vs 15	ns	ns	ns	ns
0 vs 30	ns	ns	ns	*
0 vs 45	ns	**	ns	*
15 vs 30	ns	ns	ns	ns
15 vs 45	ns	*	ns	*
30 vs 45	ns	*	ns	ns
Leonardite rate				
Control vs Low	**	ns	*	ns
Control vs High	**	ns	*	ns
Low vs High	ns	ns	ns	ns
L formulation				
L45 vs L52	*	ns	ns	ns

ns = non-significant
* = significant at 0.05 level of probability
** = significant at 0.001 level of probability

On the Chernozemic soil, B concentration in canola tissue at the 20 d sampling date was significantly greater with humic acid than without (Table 5.11 and 5.12). A form effect of humic acid was evident in which the B concentration in canola tissue with the L-52 humic acid was significantly greater than with the L-45 humic acid. A humic acid rate effect was also observed. The 12 and 24 L ha^{-1} rates of humic acid both significantly increased B concentration of canola at the 20 day sampling date. However, the effects of the two rates on B concentration in canola were similar. Humic acid had no effect on the B concentration at 35 d after emergence on the Chernozemic soil. However, B concentration was increased by P fertilization.

On the Luvisolic soil, concentration of B in canola was affected by humic acid at the 20 day sampling date (Table 5.11 and 5.12). The 12 and 24 L ha^{-1} rates of humic acid increased the B concentration of canola tissue. However, there was no difference between the rates of humic acid applied. By the 35 day sampling date, the humic acid effect was no longer evident. However, P fertilization increased B concentration of canola at the 35 day sampling date on the Luvisolic soil.

Boron accumulation was affected by P fertilization on the Chernozem at both sampling dates (Table 5.14 and 5.15). A P fertilizer with humic acid rate interaction was evident at the 35 day sampling date on the Chernozem soil. However, P fertilizer was the main contributing factor. No other humic acid effect on B accumulation were evident on the Chernozem soil. Phosphorus fertilizer had a significant effect on B accumulation at both sampling dates on the Luvisolic soil, due to a combination of increased B concentration at the early sampling date and increased plant biomass. A humic acid rate effect was evident at the 20 day sampling date where the 24 L ha^{-1} of humic acid significantly increased the B accumulation of canola as compared to the 12 L ha^{-1} rate. On the 35 day sampling date, an interaction between P fertilizer and humic acid rate was observed.

Table 5.14 Effect of humic acid on B accumulation in canola (g ha^{-1}) at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

	Soil Type and Sampling date			
	Chernozem		Luvisol	
Treatment	20 days	35 days	20 days	35 days
0 P, No Humic acid	27	100	3	17
6.5 P, No Humic acid	42	137	10	50
13 P, No Humic acid	31	115	7	61
20 P, No Humic acid	48	144	9	55
0 P, 12 L ha^{-1} L-45	34	95	5	24
6.5 P, 12 L ha^{-1} L-45	42	118	7	55
13 P, 12 L ha^{-1} L-45	43	138	10	50
20 P, 12 L ha^{-1} L-45	52	148	9	57
0 P, 24 L ha^{-1} L-45	28	118	4	24
6.5 P, 24 L ha^{-1} L-45	36	116	7	33
13 P, 24 L ha^{-1} L-45	42	178	8	50
20 P, 24 L ha^{-1} L-45	46	152	10	72
0 P, 12 L ha^{-1} L-52	34	81	6	16
6.5 P, 12 L ha^{-1} L-52	38	118	7	46
13 P, 12 L ha^{-1} L-52	39	106	7	45
20 P, 12 L ha^{-1} L-52	50	175	9	67
0 P, 24 L ha^{-1} L-52	32	117	5	20
6.5 P, 24 L ha^{-1} L-52	47	105	12	48
13 P, 24 L ha^{-1} L-52	36	165	10	61
20 P, 24 L ha^{-1} L-52	44	129	11	81

Table 5.15 Statistical analysis of canola B accumulation at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

		Soil Type							
		Chernozem				Luvisol			
		20d		35d		20d		35d	
Source	df	MS	F-Value	MS	F-Value	MS	F-Value	MS	F-Value
Rep	3	94724	7.95**	485703	5.70*	1271	1.64	18123	0.96
Prate	3	113106	9.49**	850630	9.98**	9850	12.72**	710606	37.69**
Lrate	2	12627	1.06	116597	1.37	2544	3.28*	4639	0.4
Lform	1	64	0.01	71166	0.84	836	1.08	7531	0.25
Prate*Lform	3	3883	0.33	27408	0.32	400	0.52	7192	0.38
Prate*Lrate	6	5699	0.48	295801	3.47*	308	0.4	43499	2.31*
Lform*Lrate	2	2110	0.18	22375	0.26	1595	2.06	15372	0.82
Prate*Lform*Lrate	6	3794	0.3	53595	0.61	358	0.44	9179	0.47
Error	75	11914		85210		775		18852	

* = significant at 0.05 level
** = significant at 0.0001 level
df = degrees of freedom
MS = Means Squares
P = Phosphorous
L = Leonardite

Table 5.16 Contrast analysis of canola B accumulation at 20 and 35 day after emergence on a Chernozem and Dark Grey Luvisol in 1999.

Contrast	Chernozem		Luvisol	
Phosphorus rate	**20 d**	**35 d**	**20 d**	**35 d**
0 vs 15	*	*	**	**
0 vs 30	ns	*	**	**
0 vs 45	**	**	**	**
15 vs 30	ns	ns	*	ns
15 vs 45	ns	*	**	**
30 vs 45	*	ns	*	*
Leonardite rate				
Control vs Low	ns	ns	ns	ns
Control vs High	ns	ns	*	ns
Low vs High	ns	ns	ns	ns
L formulation				
L45 vs L52	ns	ns	ns	ns

ns = non-significant
* = significant at 0.05 level of probability
** = significant at 0.001 level of probability

Effect of Humic Acid on Canola Seed Yields

Phosphorus fertilizer significantly increased canola yields on the Luvisolic soil (Table 5.17 and 5.18). However, humic acid had no effect on canola seed yield.

On the Chernozemic soil, neither P fertilizer nor the application of humic acid had a significant effect on the yield of canola (Table 5.17 and 5.18). This was interesting since canola tissue P concentrations were significantly increased by both P fertilizer and humic acid 20 and 35 d after emergence.

Table 5.17 Effect of humic acid on canola seed yields (kg ha^{-1}) on a Chernozem and Dark Grey Luvisol in 1999

	Soil Type	
Treatment	Chernozem	Luvisol
0 P, No Humic acid	1771	677
6.5 P, No Humic acid	2006	1502
13 P, No Humic acid	1839	1935
20 P, No Humic acid	1886	2146
0 P, 12 L ha^{-1} L-45	1788	705
6.5 P, 12 L ha^{-1} L-45	1652	1504
13 P, 12 L ha^{-1} L-45	1750	1619
20 P, 12 L ha^{-1} L-45	1823	2055
0 P, 24 L ha^{-1} L-45	1588	932
6.5 P, 24 L ha^{-1} L-45	1598	1609
13 P, 24 L ha^{-1} L-45	1863	1813
20 P, 24 L ha^{-1} L-45	1881	2165
0 P, 12 L ha^{-1} L-52	1677	717
6.5 P, 12 L ha^{-1} L-52	1895	1481
13 P, 12 L ha^{-1} L-52	1724	1769
20 P, 12 L ha^{-1} L-52	1891	2205
0 P, 24 L ha^{-1} L-52	1806	665
6.5 P, 24 L ha^{-1} L-52	1703	1422
13 P, 24 L ha^{-1} L-52	1914	1832
20 P, 24 L ha^{-1} L-52	1858	1806

Table 5.18 Statistical analysis of canola yield on a Chernozem and Dark Grey Luvisol in 1999.

		Soil Type			
		Chernozem		Luvisol	
Source	df	MS	F-Value	MS	F-Value
Rep	3	6463	8.98**	4042	4.70*
Prate	3	878	1.22	98471	114.39**
Lrate	2	1208	0.73	136	0.16
Lform	1	527	1.68	880	1.02
Prate*Lform	3	506	0.23	464	0.54
Prate*Lrate	6	4872	1.13	964	1.12
Lform*Lrate	2	175	0.24	1622	1.88
Prate*Lform*Lrate	6	295	0.39	250	0.27
Error	75	720		861	

* = significant at 0.05

* *= significant at 0.0001

df = degrees of freedom

MS = Means Squares

P = Phosphorous

L = Leonardite

Table 5.19 Contrast analysis of canola yields on a Chernozem and Dark Grey Luvisol in 1999.

Contrast	Chernozem	Luvisol
Phosphorus rate		
0 vs 15	ns	**
0 vs 30	ns	**
0 vs 45	ns	**
15 vs 30	ns	*
15 vs 45	ns	**
30 vs 45	ns	*
Leonardite rate		
Control vs Low	ns	ns
Control vs High	ns	ns
Low vs High	ns	ns
L formulation		
L45 vs L52	ns	ns

ns = non-significant

* = significant at 0.05 level of probability

** = significant at 0.001 level of probability

5.5 Discussion

The addition of humic substances has been shown to have positive effects on biomass and growth of plants (Moris 1995). Duplessis and MacKenzie (1983) discovered that application of leonardite significantly increased the dry matter of corn. Coal combustion by-products enhanced the dry matter of wheat by 30% and 33%, on a fresh and dry weight basis, respectively (Malik and Azam 1985). Humic acid, derived from leonardite, significantly elevated the dry matter of tomato plants by 8% and 9%, on a fresh and dry weight basis, respectively (Adani et al. 1998).

The results of this study show that application of humic acid, derived from leonardite, had inconsistent effects on early growth and biomass of canola. On the Chernozemic soil, the application of humic acid did not increase the biomass of canola 20 d after emergence. However, humic acid had a significant effect on canola biomass 35 d after emergence. It was interesting to note than when no P fertilizer was applied, the biomass 20 d after emergence was increased by humic acid.

The addition of humic acid increased the biomass of canola at one of four combinations of site and sampling time. The improved growth with the application of humic acids may have been due to hormonal effects. O'Donnell (1973) discovered that leonardite may possess compounds that act in a similar fashion to auxin. Auxin is a hormone that can improve plant growth. Preliminary studies by Akinremi (1999) showed increased root length associated with application of humic acids, suggesting that leonardite contains growth enhancing compounds. The humic acids used in this study may have contained hormones that enhanced root length and root biomass. The improved rooting density would allow for improved soil exploration and nutrient absorption creating an increase in growth and biomass.

Characterization of the humic acids showed that the humic acids contained nutrients that are essential for the growth of canola. In particular, the B concentration within the humic acids was relatively high. Therefore, the improved plant growth with the addition of humic acids may be due to nutritional response, particularly with B for canola. However, the greatest response in growth to humic acid application occurred when P fertilizer had not been applied, suggesting that the humic acids may have increased the plants' ability to obtain P from the soil. Once the P fertilizer was applied, this would counter any P response from the humic acid treatments.

Humic acids have increased the P concentrations in the tissue of various crop species. Duplessis and MacKenzie (1983) observed that the concentration of P in the tissue of corn was enhanced with application of leonardite. Humic acids have significantly increased the plant total P and P uptake in wheat (Yang et al. 1985). Li and Wang (1988) observed an increase in the P use efficiency of monoammonium phosphate with the addition of humic substances.

The increase in concentration of P in canola may also be due to competition for anion sorption sites in the soil. Vaughan and MacDonald (1971) found that application of humic acids with P significantly increased tissue concentrations of P. They suggested the increase in concentration of P in the plants was due to competition between P and humic acid for sorption sites. Humic acids contain a relatively large number of functional groups that are dissociated and negatively charged in alkaline soils. The negatively charged functional groups from the humic acid can then compete with the P anion for adsorption sites and reduce P fixation reaction by the soil.

Another factor which may be responsible for the increased availability of P fertilizer with humic acids is chelation of cations by the humic acids reducing reactions between Ca and P. Cations such as Ca, Mg, Al, and Fe form sparingly soluble compounds with the phosphate anion rendering the phosphate relatively unavailable to the plant. Humic acids have been shown to complex cations that reduce their capability of reacting with water-soluble phosphate to form insoluble phosphate precipitates (Moreno et al. 1960).

Humic acids reduce the formation of dicalcium phosphate dihydrate (DCPD) by binding to the surface of DCPD crystals (Grossl and Inskeep 1991). The humic acid "blocked" surfaces that would otherwise have been active for new crystal growth. Reduction in fixation of P may be responsible for the increased concentration of P in canola grown on the Chernozemic soil.

The humic acid, as previously mentioned, contains humic acids that may act as hormones (O'Donnell 1972). The humic acids may stimulate root growth, mass, and density that would improve root exploration and consequently absorption of P by the plants. Other suggestions for improved phosphate availability with the application of humic acids include the formation of an unfavorable electrostatic field around an absorbed humic acid molecule or that humic acid may have a proton buffer-power effect on the phosphate ion. The humic acids could donate protons to the water-soluble orthophosphates that would produce a neutral phosphate molecule. The neutral molecule would participate in fewer soil adsorption and precipitation reactions that may increase the concentration of soil solution P and increase P phytoavailability.

Humic acids have been shown to have effects on the chelation and availability of other metals such as Cu, Zn, and Fe (Moris 1985). In this study, the application of humic acid increased the tissue concentration of B. However, the concentration of B in the humic acid was also high, suggesting that the effect of humic acid on tissue concentration of B was simply due to the application of B as a constituent of the humic acid.

Humic acid has been documented to have an effect on final seed yields of various crop species (Duplessis and MacKenzie 1983, Iswaran et al. 1979). However, the results of this study showed that the application of humic acid did not affect the final seed yield of canola. This was interesting since growth early in season tended to be improved by humic acid. Phosphorus concentration at 20 d and 35 d after emergence was also significantly increased with the application of humic acid on the Chernozemic soil.

Canola is a crop that is very aggressive at acquiring phosphorus. For example, canola has a tendency to proliferate root growth towards fertilizer bands and is capable of acidifing the rhizosphere to solubilize soil P. It would have been interesting to continue this research using a crop that is less efficient in acquiring phosphorus since the results of this study may have been much different. Although leonardite did not have a beneficial effect on canola, a less aggressive crop such as wheat may have seen positive results.

In this study reported herein, canola was used as a test crop. Canola generally utilizes a very high percentage of fertilizer P compared to other crops such as wheat (Strong and Soper 1973). Effects of humic acids on fertilizer P utilization by a crop such as wheat may thus be different than that found for canola.

5.6 Conclusions

The results of this study showed that the application of humic acid applied with ammonium polyphosphate as a side-band application may enhance the early season growth of canola. Humic acids significantly increased the P concentrations in canola on the Chernozemic soil 20 and 35 d after emergence. However, this was not evident on the Luvisolic soil suggesting that soil properties may be important. The humic acids used in this study significantly increased the B concentration of the canola tissue most likely due to the relatively high concentration of B within the humic acids.

Humic acids, although showing a tendency to improve early season growth and P tissue concentrations, had no effect on the final seed yields of canola. Therefore, the application of humic acids with ammonium polyphosphate did not have an overall beneficial effect. Further research is warranted on the effects of humic acid on phosphorus nutrition of plants particularly during early growth stages.

6.EFFECT OF CU-FULVIC ACID ON CU CONTENT AND YIELD OF WHEAT

6.1 Abstract

A field study was performed to compare the effectiveness of foliar application of three Cu-fulvic acids to two commonly used Cu fertilizers. Wheat *Triticum aestivum* cv. AC Barrie grown on a loamy sand soil, was treated with foliar applications of $CuCl_2$, Cu-EDTA, or three Cu complexes with fulvic acid (F.A.-1, F.A.-2, F.A.-3). Four rates of each Cu fertilizer were applied, 0, 0.2, 0.4, or 0.6 kg ha^{-1}. Foliar application of the 0.2 and 0.6 kg ha^{-1} occurred at the 4-leaf stage. The 0.4 kg ha^{-1} treatment was applied as a split application of 0.2 kg ha^{-1} at the 4-leaf stage and a subsequent 0.2 kg ha^{-1} ten days later. The Cu content of the wheat plants was analyzed prior to application and again three weeks following treatment. Biomass and yield at maturity were also determined. All foliar applications increased Cu concentration of the plants with Cu concentrations in the tissues significantly decreasing in the order 0.4>0.2=0.6>0 kg Cu ha^{-1}. Differences in concentration of Cu in plants, for the various Cu fertilizers were not significantly different. Biomass at maturity was significantly increased by Cu application, with yield decreasing in the order 0.4 > 0.6 > 0.2 > 0 kg Cu ha^{-1}. $CuCl_2$ was more effective in increasing biomass than Cu-EDTA and the three Cu-Fulvic Acid complexes. Grain yield also decreased in the order 0.4 > 0.6 > 0.2 > 0 kg Cu ha^{-1}. Grain yield was higher with $CuCl_2$ than with Cu-EDTA or the various fulvic acids. Timing or number of applications of Cu generally had more effect on measurements than the form of Cu applied.

6.2 Introduction

Efficiencies of micronutrient fertilizers by plants are very low due to the formation of stable surface complexes with organic matter, sesquioxides, clay silicates, and porphyrin (Bibak 1994, Goodman and Chesire 1976). Consequently, low efficiency of micronutrient fertilizer may lead to micronutrient deficiency even when fertilizers are applied (Srivastava and Gupta 1996). The applications of humic substances have been shown to increase the phytoavailability of micronutrient fertilizers (Metwally et al. 1976). Humic and fulvic acids have the ability to bind with metal ions to produce a water-soluble complex (e.g. chelate) that is available to the plant. However, the complexing or chelating effect of the humic or fulvic acid is related to the pH of the soil and the metal that is being adsorbed.

Copper is an essential micronutrient that has very low fertilizer use efficiencies when applied to soil. These low efficiencies are due to the strong surface complexation with organic matter, as well as sesquioxides. Humic and fulvic acids also have a strong affinity for Cu (Bunzl et al. 1976, Senesi et al. 1986), however, these complexes remain water-soluble. The binding of Cu to organic acids is in a simple exchangeable form or as complexes with dissociated acidic groups (Davies et al. 1969). The complexation of Cu is primarily through the oxygenated and nitrogenated ligands of the humic or fulvic acid. The addition of humic acid has been shown to increase the Cu concentration, Cu uptake, and dry weight of barley (El Gala et al. 1978) while soil application of humic acid generated an increase in available Cu by 50% (Davies et al. 1969).

This study was conducted in order to determine whether or not fulvic acid (derived from leonardite) can act as a carrier for Cu and thus improve the availability of

Cu relative to traditional Cu fertilizers. The fertilizer was applied as a foliar application since previous work in this area is limited and foliar application is becoming the popular and most effective method of Cu fertilization.

6.3 Materials and Methods

A field study was conducted to determine whether or not foliar application of fulvic acid prepared with Cu salts would improve the absorption and availability of Cu for wheat relative to traditional sources of Cu. The crop used was Canadian Western Red Spring Wheat *Triticum aestivum* cv. AC Barrie and the experiment was designed as a 5x4 (5 Cu treatments and 4 Cu rates) factorial with 5 replicates. Soil analysis was conducted by Norwest labs. Procedures specific for determination of the various soil chemical characteristics are described in Appendix Xa. A summary of the soil characteristics for the Black Chernozem soil is presented below (Table 6.1).

Table 6.1 Characteristics of a loamy sand used for a foliar Cu study in wheat in 1999.

Characteristic	
pH	7.5
texture	loamy sand
O.M. (%)	2.2
E.C. (dS m^{-2})	0.3
soil test NO_3^- (kg ha^{-1})	54
soil test P_20_5 (kg ha^{-1})	16
soil test K_20 (kg ha^{-1})	80
soil test SO_4^{2-} (kg ha^{-1})	19

Cu Treatments

Five sources of Cu were evaluated. $CuCl_2$ and Cu-EDTA (ethylene diamine tetraacetic acid) as well as 3 fulvic acid formulations. The fulvic acid L-16 was formulated with Cu salts to produce the fulvic acid treatments. A description of L-16 is presented in Appendix (I).

All Cu sources were applied in four combinations of rates and methods. The four rates were 0, 0.2, 0.4, and 0.6 kg ha^{-1}. The 0.2 and 0.6 kg ha^{-1} rates were applied as a single application at the 4-leaf stage of the wheat crop. The 0.4 kg ha^{-1} treatment was applied as a split application, 0.2 kg ha^{-1} at the 4-leaf stage and a subsequent 0.2 kg ha^{-1} application ten days following the first application. Therefore, the 0.4 kg ha^{-1} rate can be regarded as a 2 x 0.2 kg ha^{-1} treatment. All treatments were applied as foliar applications diluted with water to apply in 110 L ha^{-1} of solution.

Nitrogen (80 kg ha^{-1}) was applied as a broadcast post plant treatment. Phosphorus was applied with the seed at 9 kg ha^{-1} P at time of seeding. Soil analysis showed that K and S levels in the soil were adequate for a wheat crop.

The plot was initially seeded on May 19; however, excessive moisture resulted in variable emergence, requiring reseeding of the plots. The plots were reseeded to the same variety of wheat *Triticum aestivum* (cv. AC Barrie) on June 11 at a rate of 84 kg ha^{-1}. Plots were 1.6m wide x 5m in length.

Plant Harvest

Tissue samples of plants were taken immediately prior to application of the foliar Cu. Total area sampled for tissue analysis was 0.1 m^2 for selected treatments. Plant samples were also taken three weeks following treatment. Air-dried samples were washed with a mild detergent, followed by a dilute acid, and finally washed with deionized water, as recommended in Walsh and Beaton (1973). The plants samples were dried, weighed, and finely ground using a stainless steel blade. The Cu concentration in the plants was determined using graphite furnace atomic absorption following digestion of samples with a nitric-perchloric acid mixture. The tissue samples were also analyzed for Fe, Zn, and Mn by flame atomic absorption. At maturity, the total above-ground portion of 6 meter of row (4 rows x 1.5 meter in length for the 0.2 and 0.4 kg ha^{-1} treatments, 2 rows x 3 meter in length for the 0.6 kg ha^{-1} treatments) was harvested, dried, threshed, and straw and grain weight obtained.

Statistical analyses of the data were conducted using ANOVA and contrast procedures using the Statistical Analysis System (SAS Institute (Canada) Inc., Version 8.0, BCE Place, Suite 2220, 181 Bay Street, P.O. Box 819, Toronto, ON, M5J 2T3). Treatments were considered significant if the Pr>F value was less than 0.05.

6.4 Results

Concentration of Cu in plant tissue was increased by several treatments (Table 6.2 and 6.5). A significant treatment effect of Cu was obtained in which the concentration in tissue decreased in the order (2 x 0.2) > 0.6 = 0.2 >0 kg ha^{-1} (Table 6.5). This suggests that split applications of Cu were superior to single applications and/or that the Cu fertilizer applied at the later growth stage was much more effective in increasing Cu concentration in tissues than the Cu fertilizer applied at the earlier growth stage.

Differences in Cu concentration due to source of Cu were not observed (Table 6.5). It was interesting to note that EDTA was not superior to other forms of Cu; EDTA is highly recommended for foliar application of micronutrients.

Iron, Zn, and Mn concentrations in wheat tissues were not affected by Cu application (Appendix IV).

Table 6.2 Effect of foliar Cu application on Cu content of wheat at the four leaf stage (mg kg^{-1}).

	Rate and Method of Cu Application (kg ha^{-1})		
Cu Source	**0.2 kg ha^{-1}**	**(2 x 0.2) kg ha^{-1}**	**0.6 kg ha^{-1}**
$CuCl_2$	2.1	9.5	2.6
EDTA	1.9	4.3	2.1
F.A.-1	2.1	4.4	3.1
F.A.-2	2.1	7.2	3.9
F.A.-3	3.0	4.5	2.3
Control		2.0	

Wheat biomass at maturity was significantly affected by the application of Cu (Table 6.3 and 6.5). A significant treatment effect of Cu application was evident, in which yield decreased in the order (2 x 0.2) > 0.6 > 0.2 >0 kg ha^{-1} which was consistent with concentration of Cu in the tissues at early growth stages. Wheat biomass increased with Cu application and the split applications of Cu (2 x 0.2) were significantly better than the single application of Cu (1 x 0.2) at an earlier growth stage.

In comparisons of source of Cu, the $CuCl_2$ was significantly superior to all other treatments for increasing biomass (Table 6.5 and 6.6). This was interesting since the $CuCl_2$ treatment was not superior to other forms in increasing Cu concentration. This suggests that the $CuCl_2$ had an effect on growth of wheat other than the increase in Cu concentration, which is likely the addition of chloride. The EDTA treatment in this study behaved similarly to the fulvic acid treatments.

Table 6.3 Effect of foliar Cu application on total above ground biomass of wheat at maturity (kg ha^{-1}).

	Rate and Method of Cu Application (kg ha^{-1})		
Cu Source	0.2 kg ha^{-1}	(2 x 0.2) kg ha^{-1}	0.6 kg ha^{-1}
CuCl2	2514	4290	3031
EDTA	1717	3209	2585
F.A.-1	1747	2527	2540
F.A.-2	2064	3325	2187
F.A.-3	1865	2949	1824
Control		1415	

Application of Cu significantly increased final grain yields of wheat (Table 6.4 and 6.5). There were also significant rate effects in which the 0.6 > 0.2 > 0 kg ha^{-1}. This was the same trend that was noted for the tissue Cu concentrations at the 4-leaf stage and wheat biomass at maturity. The highest yield was obtained with split applications of the Cu fertilizers which was consistent with Cu levels in plant tissues after Cu fertilization.

$CuCl_2$ increased yield compared to the F.A.-1 and F.A.-2 treatments. Comparison between the fulvic acid treatments showed that the F.A.-3 was superior to F.A.-2 in increasing grain yields. This was interesting since the F.A.-2 treatment had resulted in greater Cu concentration in tissues at the 4 leaf stage than the F.A.-3 or F.A.-1. Yield with EDTA was not significantly different than yield with other Cu treatments.

Table 6.4 Effect of foliar Cu application on wheat grain yields (kg ha^{-1}).

	Rate and Method of Cu Application (kg ha^{-1})		
Cu Source	0.2 kg ha^{-1}	(2 x 0.2) kg ha^{-1}	0.6 kg ha^{-1}
CuCl2	374	1063	316
EDTA	56	813	326
F.A.-1	67	620	253
F.A.-2	159	404	189
F.A.-3	146	808	628
Control		26	

Table 6.5 Statistical analysis of Cu application on Cu concentration at four leaf stage, wheat biomass at maturity, and yield on a loamy sand.

		Cu Concentration		Wheat Biomass		Wheat Yields	
Source	df	MS	F-value	MS	F-value	MS	F-value
Rep	4	4.76	2.21	23357	8.81**	5575	5.08*
Cu Source	4	2.81	1.31	22420	8.45**	2655	2.42
Rate	3	44.86	20.85**	168693	63.61**	27481	25.05**
Rate*Cu Source	12	1.71	0.79	6097	2.30*	1202	1.10
Error	76	2.15		2652		1097	

*** = significant at 0.05 level**

**** = significant at 0.0001 level**

df = degrees of freedom

MS = Means Squares

Table 6.6 Contrast analysis of Cu application on Cu concentration, wheat biomass and yield on a loamy sand.

Cu Rate	Cu Concentration	Biomass	Yields
0 vs 0.2	ns	**	ns
0 vs (2 x 0.2)	**	**	**
0 vs 0.6	*	**	*
0.2 vs (2 x 0.2)	**	**	**
0.2 vs 0.6	ns	*	*
(2 x 0.2) vs 0.6	**	**	**
Cu Source			
CuCl2 vs EDTA	ns	*	ns
CuCl2 vs FA1	ns	**	ns
CuCl2 vs FA2	ns	*	ns
CuCl2 vs FA3	ns	**	ns
EDTA vs FA1	ns	ns	ns
EDTA vs FA2	ns	ns	ns
EDTA vs FA3	ns	ns	ns
FA1 vs FA2	ns	ns	ns
FA1 vs FA3	ns	ns	ns
FA2 vs FA3	ns	ns	ns

ns = non-significant

*** = significant at 0.05 level of probability**

**** = significant at 0.0001 level of probability**

6.5 Discussion

The results of this study showed that foliar application of Cu significantly increased the Cu concentrations of wheat tissue grown on a Cu-deficient sandy soil. These results are consistent with previous work on foliar application of Cu. (Srivastava and Gupta 1996). A significant rate effect of Cu was also evident in which the higher application rates of Cu increased Cu concentration in tissues more than the lower rates. The study also suggested that a split application of Cu may be more effective in increasing tissue Cu concentration than a single application and/or applying Cu at a date later than the 4-leaf stage may be more effective than Cu applied at the 4-leaf stage. These findings are also consistent with previous work (Grundon 1980).

The F.A.-2 and $CuCl_2$ treatments tended to be the most effective in increasing the Cu concentration of wheat whereas EDTA was the least effective. Ferrandon and Chamel (1988) discovered that the foliar application of metals as EDTA complexes result in less absorption than metals applied in an inorganic form. A suggested reason for the lower absorption of Cu supplied as a chelate may be due to the limited number of metal cations available for retention by the negative sites on the plant cuticle (Ferrandon and Chamel 1988). The increased size of the complex may also decrease the rate of binding on the surface and access to inner sites. EDTA has also been shown to be inactivated in the presence of sunlight (Wallace et al. 1957). Since the EDTA was applied as a foliar application, inactivation of the EDTA by sunlight is quite possible. The Cu may then be precipitated as copper hydroxides on the leaf surface and not capable of diffusing through the plant cuticle.

Inorganic forms of Cu have been very effective in correcting Cu deficiencies (Zekri and Koo 1992). The cuticle has been shown to be the most limiting factor in absorption by the leaf (Kannan 1986). Since the cuticle is considered to be non-living, the movement of Cu across the cuticle can be described by Fick's law (Kannan 1986). Studies show that the movement of Cu across the cuticle is very slow when applied in distilled water without Cl but when the Cu was applied as $CuCl_2$ the rate of absorption is much greater. A possible explanation has to do with the salt concentration of the solution and the negative charges in the cuticular membranes at physiological pH values. When the salt replaces pure water in the receiver, conditions for self-diffusion may be produced (Kannan 1986). Thus the $CuCl_2$ form may have greater absorption due to the presence of the Cl^- anion. This charge balance effect has been shown for NH_4^+ and PO_4^{3-} in which the absorption of NH_4^+ increases the absorption of PO_4^{3-} due to the electrostatic charge balance within the cells of the plant. Perhaps this is the same mechanism for the increased absorption of Cu in the presence of Cl.

EDTA did not perform as well as the F.A.-2 treatment in increasing Cu concentration. The molecular weight of the EDTA molecule is 292 g mol^{-1} while the molecular weight for fulvic acid is between 1,000 to 30,000 g mol^{-1} (Paul and Clark 1996). Since the smaller molecule should be more easily absorbed than the larger molecule, the low uptake of Cu from EDTA is not likely due to the size of the molecule.

Solubility of the Cu-complex may be a factor affecting absorption of Cu. Ethylene diamine tetraacetic acid (EDTA) is very soluble and sensitive to washings (Ferrandon and Chamel 1988) and thus rainfall events may have removed Cu-EDTA from tissues to a greater extent than for the other forms resulting in less contact and consequently less uptake.

Significant scorching of the wheat tissue was noted for the $CuCl_2$ treatments. Scorching of the wheat tissue may play a role in improving the absorption of the Cu^{2+}. Scorching of leaves through foliar application of inorganic fertilizers is common. Scorching of leaves is enhanced particularly in warm, sunny conditions (Brennan 1990), which were present at the time of application of treatments in this study. Scorching of the leaves may in fact reduce Cu runoff through rainfall events since the Cu may be embedded within the tissue. This may reduce the amount of Cu that is water soluble and lost in rainfall events, which would increase the Cu concentration from $CuCl_2$ relative to EDTA. Another factor to consider is that the scorching of the leaves may have reduced photosynthetic capability of the wheat resulting in reduced growth. The reduced growth would have increased the concentration of Cu by decreasing the biomass.

Since fulvic acid is water-soluble at any pH, the fulvic acid should be as water soluble as the EDTA. However, the F.A.-2 treatment significantly increased the Cu concentration versus EDTA. No significant scorching of the leaves was evident with the fulvic acid treatments.

The critical Cu concentration of wheat tissue at the 4-leaf stage is 3 ppm while sufficient Cu levels are 4.5 ppm (Srivastava 1996). Therefore, plants with the single applications of Cu were still Cu deficient. Only when a split application of Cu was applied was the tissue level of Cu considered sufficient. This explains the poor growth with the single Cu applications versus the split application.

$CuCl_2$ significantly increased the final wheat biomass relative to all other treatments at all rates of Cu. This was interesting since $CuCl_2$ did not significantly differ from the other treatments in Cu concentration at the 0.2 and 0.6 kg ha^{-1} rates. This leads to the possibility of a confounding factor. The application of $CuCl_2$ may have resulted in a response to Cl^-. This was a loamy sand soil, which suggests that leaching and low levels of Cl^- are possible. Wheat has also been shown to respond to Cl^- application (Grant et al. 1998). Therefore, improvement of yield with the $CuCl_2$ may have been due to nutritional effects of both Cu and Cl.

Final grain yields in this study were poor, even for the greatest yielding treatments. Concentrations of Cu in the plant were considered less than sufficient for most treatments. Copper deficiency in wheat usually results in very low seed yields in relation to total biomass (Srivastava 1996).

Copper is immobile within the plant under deficient conditions (Tisdale et al. 1993). Although treatments at the (2 x 0.2) kg ha^{-1} rate were sufficient at the tillering stage, a Cu deficiency may have occurred at the grain filling stage since Cu is immobile and concentrated in the vegetative tissue. Copper is important for pollen fertility and a Cu deficiency results in sterile pollen (Grundon 1980). This may be a factor in the low grain yields of wheat in this study. Wheat yield could have been increased even more than observed for the (2 x 0.2) kg ha^{-1} rate if more applications and/or higher rates were used.

Comparison of the grain yields shows that the fulvic acid-3 was superior to fulvic acid-2. However, fulvic acid-2 resulted in higher Cu concentrations in tissue. This may reflect differences in mobility of the Cu complexes. Fulvic acid -3 may be more mobile within the plant tissue allowing for mobility of Cu from the lower leaves to the grain. This in turn would allow for increased Cu for grain filling and consequently higher yields. Copper concentration in plants with EDTA was significantly lower than with the fulvic acid-2 but seed yields were statistically not different. The EDTA may be more mobile than the fulvic acid -2 treatment and had similar mobility to the fulvic acid-3 treatment. EDTA has been shown to be much more mobile within the plant versus inorganic sources of Cu (Ferrandon and Chamel 1988)

Finally, stability of the Cu complex is also important. Copper has been shown to undergo fixation reactions within the plant that render the Cu immobile (Loneragan 1981). A complexed form of Cu would be more water soluble and mobile within the plant. However, if the complex is not stable within the plant, the chelate (EDTA or fulvic acid) may not be bound to the Cu. The Cu is then in the free ion form and can then be fixed through reactions with amino acids within the plant. This makes the Cu immobile and if no further sources of Cu are available to the plant, the plant will be Cu deficient at the time of grain filling.

The biomass yield of the wheat in this study was high in comparison to grain; i.e. the harvest index was low. A low harvest index of wheat is a symptom of a Cu defiency. The deficiency would be a result of applied Cu being immobilized in vegetative tissue resulting in a Cu deficiency at the time of grain filling. If the supply of soil Cu is low, there would be insufficient Cu to supply the demand of wheat at grain filling. It would have been interesting to have a high soil Cu treatment that may have overcome the immobilization of Cu in vegetative tissue that is a result of foliar Cu applications.

6.6 Conclusions

Foliar applications of Cu were effective in improving the wheat tissue Cu concentrations, biomass, and yield. Concentration of Cu in plant tissue was similar for the various Cu sources.

Biomass significantly increased with the application of Cu. The $CuCl_2$ treatment was superior to other Cu sources in improving the biomass of wheat. This was due to the increased Cu concentration in plants but may also have been due to effects of Cl^- on plant growth.

Seed yield was also significantly affected by the application of Cu. However, Cu source did not affect wheat grain yields.

Two applications of foliar Cu, one at the 4-leaf stage followed by a second 10 days later, was more effective in increasing Cu concentration in tissue and yield than a single application at the 4 leaf stage at a higher rate.

7. EFFECT OF LEONARDITE ON CU AND ZN PHYTOAVAILABILITY

7.1 Abstract

A growth chamber study was conducted to determine the effect of humic and fulvic acids on Cu and Zn availability to canola. Argentine canola *Brassica napus* (cv. LG 3310) was grown on a loamy sand textured soil. Treatments for the Cu study included $CuCl_2$, Cu-EDTA, Cu-Humic acid, and Cu-Fulvic acid. Treatments for the Zn study included $ZnCl_2$, Zn-EDTA, Zn-Humic acid, and Zn-Fulvic acid. Three methods of application were evaluated, broadcast, band, and foliar. Concentrations of Cu, Zn, Fe, and Mn in plant tissue were determined. Canola biomass was not affected by Cu source but was affected by application method. Foliar application significantly reduced biomass compared to broadcast treatments. Concentration of Cu in canola with Cu-humic acid was significantly lower than with the $CuCl_2$, Cu-EDTA, and Cu-fulvic acid. Cu-fulvic acid was the only treatment that significantly increased the Cu concentration relative to the control plants. Copper accumulation in plants was significantly greater with foliar than with band application. Form of Cu did not affect Cu accumulation. Zinc treatments were ineffective at increasing canola biomass. Foliar application of Zn significantly reduced canola biomass relative to soil applied treatments. Zn-EDTA significantly increased Zn concentration compared to $ZnCl_2$ and Zn-humic acid. Zn-fulvic acid significantly increased Zn concentration compared to Zn-humic acid. Foliar application significantly increased Zn concentration compared to the soil applications. EDTA and fulvic acid significantly increased Zn accumulation compared to the control plants.

EDTA also increased Zn accumulation compared to humic acid. Application method did not affect Zn accumulation.

7.2 Introduction

Humic and fulvic acids added to soil affect the availability of Cu and Zn to crops. Humic acid applied to soil increased the Cu concentration in barley (Elgala et al. 1978, Metwally et al. 1976). The uptake of Zn by wheat was significantly increased by a soil application of fulvic acid (Gupta and Deb 1985). In other studies, the concentration of Zn in rice and wheat significantly increased when humic acid was applied to soil.

In contrast to the results observed above, organic amendments containing humic and fulvic acids have been shown to reduce the availability of Cu and Zn. Copper concentration in bean and corn plants decreased with the addition of coal by-products (Adriano et al. 1978) and humic acid was shown to reduce the concentration and uptake of Zn in barley (Elgala et al. 1978).

Fulvic acids have been shown to be superior to humic acid in improving micronutrient availability (Moris 1985), most likely due to the formation of more stable complexes with humic acids than with fulvic acids (Dkhar et al. 1985). The size of fulvic acids are also smaller than humic acids and thus would be preferred for absorption by plant roots.

This study was conducted to determine the availability of Cu and Zn to canola when using humic and fulvic acids as carriers or chelates of these metals. The plant availability of Cu and Zn from Cu and Zn humic and fulvic acids were compared to the availability of Cu and Zn from traditional sources.

7.3 Materials and Methods

A growth chamber study was conducted to determine the effectiveness of various Cu and Zn fertilizers applied to Argentine canola *Brassica napus* (cv. LG 3310). Three methods of application were evaluated: broadcast and mixed with soil, banded into soil, and foliar. The study was designed as a 4x3 factorial experiment (four sources of Cu or Zn and three application methods) for both the Cu and Zn study. Soil analysis was conducte dby Norwest Labs. Procedures specific to various soil chemical characteristics are described in Appendix Xa. Characteristics of the soil used for the study are presented below (Table 7.1).

Table 7.1 Characteristics of a loamy sand used in Cu and Zn growth chamber studies with humic and fulvic acid in 1999.

Characteristic	
pH	7.5
texture	loamy sand
O.M. (%)	2.2
E.C. (dS m^{-2})	0.3

O.M. = organic matter
E.C. = electrical conductivity

Studies with Cu and Zn were set up as separate experiments. Two kg of soil in 2-liter pots were used, and 3 canola plants per pot were grown.

Four Cu or Zn fertilizers including the inorganic salt ($CuCl_2$ or $ZnCl_2$), the EDTA complexed forms (Cu-EDTA or Zn-EDTA), a humic acid formulation (Cu-humic acid or Zn-humic acid) designated as L-11, and a fulvic acid form designated as L-16 (Cu-fulvic acid or Zn-fulvic acid) were used in this study. Characterization of the humic and fulvic acid is provided in the Appendix (I). The Cu and Zn humic acid and fulvic acids were prepared by reacting inorganic salt with the acids.

The fertilizer was either broadcast (applied and uniformly mixed throughout the soil in the pot) or banded approximately 5 cm below the seed just prior to planting. The fertilizer bands consisted of placing 5 ml of solution dropwise across the center of each pot to provide a band 5 cm long and 0.5 cm wide. Foliar applications were applied at the three leaf stage using a very fine spray in a total volume of 5 ml of solution per pot or treatment. Rates of Cu and Zn used were 5 kg ha $^{-1}$ of Cu or Zn for the soil treatments and 1 kg ha $^{-1}$ for foliar treatments.

A basal application of nutrients consisting of 200 ppm of N as urea, 100 ppm of P as monoammonium phosphate, and 100 ppm of K and S as K_2SO_4 was applied at time of planting. A subsequent application of 200 ppm N was applied at the 4 leaf stage. The growth chamber conditions used were near ideal. Sixteen hours of light was followed by 8 hours of darkness. The daytime temperatures were 25^0C and the night temperatures were 13^0C. The relative humidity remained constant at 60%.

Plants were harvested when the canola plants began to bolt. Plant tissues for foliar treatments were washed as follows: the plants were first washed in a mild detergent solution, followed by a dilute acid solution and finally in deionized water as suggested by Walsh and Beaton (1973). The plants samples were dried, weighed, and finely ground using a grinder equipped with a stainless steel blade.

The Cu, Zn, Fe, and Mn concentrations in plant tissues were determined by digestion of plant material in a nitric-perchloric acid mixture and use of an atomic adsorption spectrometer.

7.4 Results

Cu Study

Copper fertilization did not significantly affect the above-ground biomass production of canola (Table 7.2 and 7.5). However, application method significantly affected biomass of canola. Foliar applications of Cu significantly resulted in lower biomass yields compared to the broadcast application (Table 7.6). There was no significant difference in biomass between the band and foliar applications or between the band and broadcast applications (Table 7.6).

Table 7.2 Effect of Cu fertilizer on biomass (g pot^{-1}).

	Application Method		
Cu Source	Broadcast	Band	Foliar
CuCl2	16.4	16.5	14.1
EDTA	16.0	14.3	13.2
Humic Acid	16.0	14.8	16.0
Fulvic Acid	15.8	15.3	12.6
Control		14.0	

The Cu concentration in tissue with the Cu-humic acid was significantly lower than with $CuCl_2$, Cu-EDTA, and Cu-fulvic acid (Table 7.3 and 7.6). However, Cu-fulvic acid was the only treatments that significantly increased the Cu concentration versus the control (Table 7.6).

Copper treatments were ineffective in increasing the Cu accumulation of canola (Table 7.4 and 7.5). However, significant effects of application method were observed. Foliar applications of Cu significantly increased Cu accumulation of canola versus the band treatments. No significant differences were observed between the foliar applications and the broadcast applications or between band and broadcast application.

Zinc, Mn, and Fe concentrations in canola tissue were also measured and are presented in the Appendix (V, VII).

Table 7.3 Effect of Cu fertilization on concentration of Cu in plant tissues (mg kg^{-1}).

	Application Method		
Cu Source	Broadcast	Band	Foliar
CuCl2	4.4	3.5	5.4
EDTA	4.8	3.9	5.9
Humic Acid	3.4	3.3	4.0
Fulvic Acid	4.2	4.0	6.9
Control	4.1		

Table 7.4 Effect of Cu fertilization on accumulations of Cu in plant tissues (mg pot^{-1}).

	Application Method		
Cu Source	Broadcast	Banded	Foliar
CuCl2	0.072	0.058	0.076
EDTA	0.076	0.056	0.078
Humic Acid	0.054	0.049	0.064
Fulvic Acid	0.066	0.061	0.087
Control	0.057		

Table 7.5 Statistical analysis of Cu application on canola biomass, Cu concentration, and Cu accumulation on a loamy sand.

		Canola Biomass		Cu Concentration		Cu Accumulation	
Source	df	MS	F-value	MS	F-value	MS	F-value
Rep	2	1.83	0.69	1.01	1.23	390.19	1.52
Cu Source	4	4.75	1.79	3.27	3.96*	515.45	2.01
Application	2	10.74	4.04*	9.16	11.11*	1151.77	4.49*
Treatment*Application	8	2.91	1.09	1.40	1.70	139.97	0.55
Error	28	2.66		0.82		256.28	

* = significant at 0.05 level

** = significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

Table 7.6 Contrast analysis of Cu application on Cu concentration, wheat biomass and yield on a loamy sand.

Cu Source	Canola Biomass	Cu Concentration	Cu Accumulation
Control vs $CuCl_2$	ns	ns	ns
Control vs EDTA	ns	ns	ns
Control vs Fulvic Acid	ns	*	ns
Control vs Humic Acid	ns	ns	ns
$CuCl_2$ vs EDTA	ns	ns	ns
$CuCl_2$ vs Fulvic Acid	ns	ns	ns
$CuCl_2$ vs Humic Acid	ns	*	ns
EDTA vs Fulvic Acid	ns	ns	ns
EDTA vs Humic Acid	ns	*	ns
Fulvic vs Humic Acid	ns	*	ns
Application Method			
Band vs Broadcast	ns	ns	ns
Band vs Foliar	ns	**	*
Broadcast vs Foliar	*	*	ns

ns = non-significant

*** = significant at 0.05 level of probability**

**** = significant at 0.0001 level of probability**

Zn Study

Zinc source did not affect the biomass of canola (Table 7.7 and 7.10). However, a significant application method effect was observed. Foliar applications of Zn significantly reduced the biomass of canola versus the soil applied treatments. A significant difference was not observed between the band or broadcast applications (Table 7.11). The reductions in yield of biomass of canola with the foliar applications was probably due to scorching of the leaves and reduced photosynthetic capability of the plants.

Table 7.7 Effect of Zn fertilization on biomass of canola (g pot^{-1}) on a loamy sand in 1999.

	Application Method		
Zn Source	Broadcast	Band	Foliar
ZnCl2	14.2	14.0	11.4
EDTA	12.5	15.7	13.2
Humic Acid	15.1	14.1	13.4
Fulvic Acid	14.0	13.6	11.8
Control		15.3	

Zinc concentration in canola was significantly increased by all Zn fertilizers (Table 7.8 and 7.10). Zn concentration in tissue with EDTA was significantly greater than with $ZnCl_2$ and Zn-humic acid. The fulvic acid treatment significantly increased the Zn concentration in canola compared to the humic acid treatments (Table 7.8 and 7.10). Application method also had a significant effect on Zn concentration. Foliar application of Zn significantly increased the Zn concentration of canola compared to the band and broadcast applications. No difference was observed between the band and broadcast applications. This is the inverse to the biomass results suggesting that the increase in Zn concentration with the foliar applications was due to a reduction in biomass.

Accumulation of Zn in canola was affected by Zn treatment (Table 7.9 and 7.10). In treatment comparisons, the EDTA and the fulvic acid treatments significantly increased Zn concentration versus the control. EDTA also significantly increased Zn concentrations compared to the humic acid treatment. Application method did not affect the Zn accumulation in canola.

Copper, Mn, and Fe concentrations of canola were also measured and are presented in the Appendix (VI, VIII).

Table 7.8 Effect of Zn fertilization on concentration of Zn in canola tissue (mg kg^{-1})

	Application Method		
Zn Source	Broadcast	Band	Foliar
ZnCl2	26.2	24.5	37.3
EDTA	33.7	39.7	34.9
Humic Acid	25.4	26.3	26.3
Fulvic Acid	28.1	21.0	56.6
Control	19.2		

Table 7.9 Effect of Zn fertilization on accumulaton of Zn in canola tissue (mg pot^{-1}) on a loamy sand in 1999.

	Application Method		
Zn Source	Broadcast	Banded	Foliar
ZnCl2	0.373	0.344	0.423
EDTA	0.403	0.623	0.481
Humic Acid	0.383	0.371	0.357
Fulvic Acid	0.401	0.284	0.682
Control	0.293		

Table 7.10 Statistical analysis of Zn application on canola biomass, Zn concentration, and Zn accumulation on a loamy sand in 1999.

		Canola Biomass		Zn Concentration		Zn Accumulation	
Source	df	MS	F-value	MS	F-value	MS	F-value
Rep	2	0.11	0.03	7.15	0.19	3575	0.21
Zn Source	4	7.50	2.34	436.43	11.36**	59217	3.42*
Application	2	12.82	4.00*	366.09	9.53*	25239	1.46
Treatment*Application	8	1.39	0.43	219.43	5.71*	35698	2.06
Error	28	3.21		38.42		17290	

* = significant at 0.05 level
** = significant at 0.0001 level
df = degrees of freedom
MS = Means Squares

Table 7.11 Contrast analysis of Zn application on Zn concentration, canola biomass and Zn accumulation on a loamy sand.

Zn Source	Canola Biomass	Zn Concentration	Zn Accumulation
Control vs $ZnCl_2$	ns	*	ns
Control vs EDTA	ns	**	*
Control vs Fulvic Acid	ns	**	*
Control vs Humic Acid	ns	*	ns
$ZnCl_2$ vs EDTA	ns	*	ns
$ZnCl_2$ vs Fulvic Acid	ns	ns	ns
$ZnCl_2$ vs Humic Acid	ns	ns	ns
EDTA vs Fulvic Acid	ns	ns	ns
EDTA vs Humic Acid	ns	*	*
Fulvic vs Humic Acid	ns	*	ns
Application Method			
Band vs Broadcast	ns	ns	ns
Band vs Foliar	*	*	ns
Broadcast vs Foliar	*	*	ns

ns = non-significant

*** = significant at 0.05 level of probability**

**** = significant at 0.0001 level of probability**

7.5 Discussion

The application of Cu, regardless of form did not affect the biomass of canola. A reduction in biomass from foliar application of fulvic acid was likely due to stress as a result of scorching of the leaves due to the acidity of the fulvic acid solution. Foliar applications were applied using the same volume of solution. The volume of water added however, was not dilute enough to reduce the acidity of the fulvic acid solution.

The lack of response in biomass to Cu application was probably due to two reasons. The level of Cu in the soil was above the critical level for canola even though the soil was coarse and had a low organic matter content. Secondly, the critical level of Cu for canola is relatively low. The sufficiency level of Cu in tissues for canola at the flowering stage is 2.7 ppm (Srivastava and Gupta 1996). The plants in the control treatments, (no Cu had been applied) were above the sufficiency level of 2.7 ppm. Therefore, a response to Cu fertilization should not have been expected.

Concentration of Cu in canola was not increased by soil application of Cu (broadcast or banded). This suggests that the humic acid may have formed very stable complexes with the added Cu as well as soil-Cu decreasing the amount of Cu in solution and phytoavailable Cu. It is also possible that the humic acid-metal complex may be too large for the absorption of the complex by the plant.

Foliar application of Cu increased the Cu concentration of canola. This is probably due to scorching of the leaves which would have reduced the photosynthetic capabilities of the plants. The reduced biomass would have increased the concentration of the Cu even if the uptake was unchanged. This seems quite possible as the biomass of the canola was significantly lower when foliar treatments were applied. Another possibility is that the scorching of the leaves would allow for the Cu to penetrate the plant cuticle, the most limiting step in absorption through leaves. This is consistent with results found in the field study in which Cu treatments that scorched the wheat leaf tissue resulted in the highest tissue Cu concentration and final grain yield.

The application of Zn, regardless of source of Zn or application method did not affect biomass of canola likely due to sufficient levels of phytoavailable Zn in the soil. Control plants (no Zn applied) had a tissue concentration of 19 ppm Zn. The sufficient level of Zn required by canola is about 15 ppm (Srivastava 1996). Thus, the control plants had sufficient levels of Zn without fertilization.

All Zn-treatments significantly increased the Zn concentration of canola. However, in comparison of treatments, Zn-EDTA significantly increased the Zn concentration versus $ZnCl_2$ and Zn-humic acid and Zn-fulvic acid significantly increased the Zn concentration versus Zn-humic acid. Therefore, Zn-EDTA was a highly effective form of Zn fertilizer. The increase in Zn concentration with Zn-EDTA in this study as well as the increase in Zn concentration when Cu-EDTA was applied in the Cu study suggests that EDTA forms a more stable chelate with Zn than with Cu at the soil pH encountered. Zn-fulvic acid performed similarly to Zn-EDTA suggesting that fulvic acid may be effective as a carrier for Zn

Foliar application of Zn treatments significantly increased the Zn concentration of canola. However the increase in Zn concentration was likely due to the reduced biomass of canola. The foliar application of Zn treatments scorched the canola tissue. This may have decreased the photosynthetic capabilities of the canola plants, which would have reduced canola biomass. The reduction in biomass would have increased the concentration of Zn. The other possibility is that the scorching of the leaves may have increased the permeability of the plant cuticle. The plant cuticle is the most limiting step in absorption of nutrients through the leaf. Therefore, scorching of the leaf tissue may have enhanced Zn absorption. Changes in Cu, Mn, and Fe tissue concentrations with Zn application was due to biological dilution.

7.6 Conclusion

$CuCl_2$, Cu-EDTA, and Cu-humic acid were not effective in increasing the Cu concentration of canola tissue when applied to soil. Cu-fulvic significantly increased the Cu concentration of canola versus the control. Cu-humic acid significantly lowered the Cu concentration of canola versus $CuCl_2$, Cu-EDTA and Cu-fulvic acid. Foliar application of Cu treatments significantly increased Cu concentration of canola but significantly lowered canola biomass.

Zinc treatments were ineffective at increasing the biomass of the canola. This is due to the level of available Zn being more than sufficient for the canola plants at the bolting stage. However, the fertilizers used in this study increased the Zn concentration of canola tissue.

8. EFFECT OF LEONARDITE ON THE SOLUBILITY AND CONCENTRATION OF CD IN DURUM WHEAT DUE TO NITROGEN FERTILIZATION

8.1 Abstract

A growth chamber study was conducted to determine the effective of leonardite on Cd solubility and uptake in durum wheat treated with N fertilizer. Durum wheat *Triticum durum* (cv. Sceptre) was grown on a clay loam soil. Three rates of N were applied as urea; 0, 250 and 500 ppm N. Two forms of leonardite were used in this study, a dry powder form (L-31) and a liquid formulation (L-11). Both forms of leonardite were applied at two rates. The dry leonardite was applied at 100 and 400 ppm while the liquid leonardite was applied at 0.05 and 0.2 μL g^{-1} of soil. The leonardite was used to coat the urea granules, which were then applied as a broadcast or band application. Biomass and Cd, Zn, Cu, and Mn concentrations in plant tissues were determined. Nitrogen fertilizer significantly increased the Cd concentration of the durum wheat tissue. The application of leonardite significantly increased the Cd concentration of durum wheat when applied with the N fertilizer but only when the N fertilizer was broadcast. Cadmium content of plants was greater with banded than with broadcast N.

8.2 Introduction

Cadmium is a heavy metal that has received considerable interest in recent years because of its association with various health problems (Mitchell 1997). Research has shown that the addition of fertilizers can increase the phytoavailability of soil Cd due to its effects on pH, ionic strength, complexation, and plant growth. Cadmium is also a contaminant within various fertilizers.

Nitrogen fertilizers containing ammonium are known to have acidifying effects on soil. Cadmium solubility has been shown to be pH dependent. As the pH of the soil becomes more acidic, the availability of Cd is magnified (Mitchell 1997). Erickson (1990) found that as the amount of ammonium added to soil was increased, the solubility, and consequently, plant accumulation of Cd increased. This was due to lowering of the soil pH from acidifying ammonium fertilizers. Acidifying fertilizers such as ammonium sulphate, ammonium nitrate, and urea has been shown to increase the exchangeable and water-soluble Cd in soil (Willaert and Verloo 1992).

Application of humic acid has reduced the phytoavailability of Cd. The addition of humic acid increased the adsorption of Cd in the pH range of 3.69 - 9.16 (Bolton et al. 1996) while humic acid was shown to increase the adsorption of Cd in an acid soil (Hanafi and Salwa 1998). Warwick et al. (1998) discovered that addition of humic acid resulted in greater adsorption of Cd and reduced the mobility of complexed Cd. This reduced the amount of Cd that was absorbed by the plant roots.

The addition of humic acid reduced the activity of Cd in soil solution and consequently reduced the Cd absorption by plants (Tyler and McBride 1982).

This study was conducted to determine the effect of adding leonardite and humic acid on Cd phytoavailability when applied in intimate association with N fertilizer. Leonardite may be able to form a complex with Cd, which may be solubilized when N fertilizers are added, making the Cd less available to the plant and thereby reducing concentration of Cd in durum.

8.3 Materials and Methods

A growth chamber study was conducted in order to determine the effect of an organic amendment, in a solid or liquid phase, on concentration and accumulation of Cd in Canadian Western Amber Durum *Triticum durum* cv.Sceptre treated with N fertilizer. The study was conducted as a factorial experiment with five treatments, three rates of N, two application methods, and three replicates. Soil analysis was conducted by Norwest labs. Procedures specific for the various soil chemical characteristics are described in Appendix Xa. Characteristics of soil used in this study is presented below (Table 8.1).

Table 8.1 Characteristics of a Dark Grey Luvisol used for a Cd study in 1999.

Characteristic	
pH	7.5
texture	clay loam
O.M. (%)	3.6
E.C. (dS m^{-2})	0.2

O.M. = Organic Matter
E.C. = Electrical Conductivity

Forms and Rates of Leonardite

Two forms of leonardite, a powder material designated as L-31 and a liquid form of leonardite (L-11) was used (Appendix I). The powder form of leonardite was applied at two rates: 100 and 400 ppm whereas the liquid leonardite was applied at 0.05 and 0.2 $\mu L\ g^{-1}$ of soil.

Each treatment consisted of a 2 L plastic pot with 2 kg of soil. Each pot contained 4 durum wheat plants. Growth chamber conditions were set to simulate optimal growing conditions. There were 16 hours of daylight followed by 8 hours of darkness with the temperature set at 25°C during daylight hours and 12°C during darkness. The relative humidity remained constant at 60%. Each pot was watered so that water stress did not occur. Field capacity and PWP were calculated for the soil type. Water was added so that the available water remained between 60 and 100% of field capacity.

Urea (46-0-0) was used as the source of N and was applied at rates of 0, 250, and 500 ppm N.

Application of Treatments

The leonardite and urea were either broadcast or banded. For the broadcast application, the leonardite material was coated onto urea fertilizer granules, which were then mixed throughout the entire pot (2 kg). For the banded application, the leonardite-coated urea fertilizer granules were placed in a single band, 5 cm below the seed.

Fertility

A basal application of fertilizer was applied to each pot for adequate growth and consisted of 100 ppm of P and 100 ppm of K. The P was applied as monoammonium phosphate and K as potassium sulphate that also supplied adequate S for the growth of durum wheat.

Harvest

The durum wheat plants were harvested at the boot stage by cutting the plants close to the soil surface. The plant material was dried, weighed, and ground using a grinder equipped with a stainless steel blade.

One-gram samples were then placed in digestion tubes to which 5ml of nitric and 2.5ml of perchloric acid were added. The test tubes were then left for 24 hours at room temperature and were then digested at 230°C for 2 hours. The plant samples were analyzed for Cd, Cu, Zn, and Mn tissue concentration. Cadmium was analyzed by graphite furnace atomic adsorption. Cu, Zn, and Mn were analyzed by flame atomic adsorption. Total biomass and accumulation of Cd, Cu, Zn, and Mn were also determined.

8.4 Results

Effect of Leonardite on Durum Wheat Biomass

Wheat biomass was significantly affected by N fertilizer, with yields significantly decreasing in the order: 250 > 0 > 500 ppm N when N broadcast and 250 > 500 > 0 ppm N when the N was applied in a band (Table 8.2 and 8.4) Leonardite, in the liquid (L-11) or the dry form (L-31) at 400 uL g^{-1}, did not have an effect on the dry matter production of durum wheat. Leonardite at 100 uL g^{-1} of the dry form significantly increased biomass.

Table 8.2 Effect of leonardite and nitrogen application on durum wheat biomass at the boot stage (g pot^{-1})

	Application Method					
	Broadcast			Banded		
	Rate of N (ug g^{-1})			Rate of N (ug g^{-1})		
Leonardite Source and Rate	0	250	500	0	250	500
0 Leonardite	5.2	5.6	2.7	3.9	5.7	5.6
100 uL g^{-1} L-31	5.3	6.2	4.3	4.4	5.9	5.9
400 uL g^{-1} L-31	4.8	5.9	2.9	4.2	5.6	4.4
0.05 uL g^{-1} Humic Acid	3.5	6.6	2.7	4.4	5.5	5.0
0.2 uL g^{-1} Humic Acid	3.6	6.7	3.0	3.7	6.2	3.5

Effect of Leonardite on Cd Concentration in Durum Wheat Tissue

Nitrogen fertilization significantly increased the Cd content of tissue (Table 8.3 and 8.4) with concentration of Cd significantly increasing in the order 0< 500 < 250 ppm N.

Leonardite, when broadcasted in the liquid and dry forms, significantly increased Cd content of durum wheat. In contrast, banding of the leonardite had no effect on the tissue concentration of Cd. Cadmium concentrations in plants were significantly greater with banded than with broadcast treatments. Rate effects were not evident for either form of the leonardite.

Table 8.3 Cd content of durum tissue at boot stage as affected by N fertilization and leonardite (ug g^{-1}).

	Application Method					
	Broadcast			Banded		
	Rate of N (ppm)			Rate of N (ppm)		
Leonardite Source and Rate	0	250	500	0	250	500
0 Leonardite	0.195	0.282	0.201	0.215	0.435	0.483
100 uL g^{-1} L-31	0.289	0.556	0.286	0.211	0.440	0.393
400 uL g^{-1} L-31	0.290	0.606	0.245	0.228	0.427	0.384
0.05 uL g^{-1} Humic Acid	0.217	0.369	0.273	0.297	0.449	0.536
0.2 uL g^{-1} Humic Acid	0.210	0.350	0.248	0.291	0.445	0.462

Table 8.4 Statistical analysis of nitrogen and leonardite application on durum biomass, Cd concentration, and Cd accumulation on a Grey Luvisol in 1999.

Source	df	Durum Biomass		Cd Concentration	
		MS	F-value	MS	F-value
Rep	2	0.19	0.28	0.001	0.07
Nrate	2	33.94	49.31**	0.290	30.25**
Lsource	4	2.09	3.05*	0.004	0.41
Application	1	2.31	3.35	0.077	8.15*
Nrate*Lsource	8	1.43	2.07	0.021	2.24*
Nrate*Application	2	11.95	17.37**	0.060	6.36*
Lsource*Application	4	0.41	0.59	0.032	3.39*
Nrate*Lsource*Application	8	1.10	1.75	0.008	0.82
Error	66	0.69		0.009	

* = significant at 0.05 level

** = significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

Table 8.5 Contrast analysis of nitrogen and leonardite application on durum biomass and Cd concentration on a Grey Luvisol.

Contrast	Durum Biomass	Cd Concentration
N rate		
0 vs 250 ppm	**	**
0 vs 500 ppm	ns	**
250 vs 500 ppm	**	*
Leonardite Source and Rate		
0 vs 0.05	ns	ns
0 vs 0.2	ns	ns
0 vs 100	ns	ns
0 vs 400	ns	ns
0.05 vs 0.2	ns	ns
0.05 vs 100	*	ns
0.05 vs 400	ns	ns
0.2 vs 100	*	ns
0.2 vs 400	ns	ns
100 vs 400	*	ns
Application		
Band vs Broadcast	ns	*

ns = non-significant

* = significant at 0.05 level of probability

** = significant at 0.0001 level of probability

Effect of Leonardite on Micronutrient Concentration in Durum Wheat Tissue

Application of N significantly increased the Zn concentration of durum wheat tissue (Table 8.6 and 8.9). Zinc content with broadcasted N was significantly greater than with banded application. The liquid form (L-11) of leonardite, at 0.2 uL g^{-1}, reduced the concentration of Zn in durum wheat. However, the dry form (L-31) had no significant effects on the Zn concentration in the plants.

Table 8.6 Zn concentration of durum as affected by N fertilization and leonardite (mg kg^{-1}).

	Application Method					
	Broadcast			Banded		
	Rate of N (ppm)			Rate of N (ppm)		
Leonardite Source and Rate	**0**	**250**	**500**	**0**	**250**	**500**
0 L-31	26.3	41.5	38.8	30.2	37.0	49.5
100 ppm L-31	36.1	44.4	45.5	24.0	37.6	39.5
400 ppm L-31	27.3	43.6	47.7	25.5	36.1	34.6
0.05 uL g^{-1} Humic Acid	28.0	36.4	40.2	28.3	36.7	38.0
0.2 uL g^{-1} Humic Acid	22.8	35.5	38.3	25.9	36.3	34.9

Tissue concentrations of Mn were significantly affected by N application (Table 8.7 and 8.9). Rate effects of N were highly significant in which Mn contents decreased in the order 500>250>0 ppm N. Manganese concentration with broadcast application of N were significantly greater than with banded N application. No effects of leonardite on Mn tissue concentration were evident.

Table 8.7 Mn Concentration of durum as affected by N fertilization and leonardite (mg kg^{-1}).

	Application Method					
	Broadcast			Banded		
	Rate of N (ppm)			Rate of N (ppm)		
Leonardite Source and Rate	0	250	500	0	250	500
0 L-31	31	549	1817	30	169	378
100 ppm L-31	34	407	1392	28	155	420
400 ppm L-31	31	389	2025	34	168	548
0.05 uL g^{-1} Humic Acid	37	406	1275	32	180	434
0.2 uL g^{-1} Humic Acid	26	337	1316	24	184	443

Nitrogen application had a significant effect on the Cu concentration in durum wheat tissue (Table 8.8 and 8.9). The N rate effects which were evident were 0 ppm > 500 ppm > 250 ppm N. Method of application did not affect the Cu concentration of durum wheat tissue. An interaction between N rate and application method was also evident. No rate or form effects of leonardite were evident.

Table 8.8 Cu concentration of durum as affected N fertilization and leonardite (mg kg^{-1}).

	Application Method					
	Broadcast			Banded		
	Rate of N (ppm)			Rate of N (ppm)		
Leonardite Source and Rate	0	250	500	0	250	500
0 L-31	3.8	2.7	4.3	3.3	2.8	2.6
100 ppm L-31	4.6	2.1	3.0	3.3	2.0	3.0
400 ppm L-31	6.2	1.3	3.1	5.1	2.8	4.6
0.05 uL g^{-1} Humic Acid	4.4	1.7	3.1	3.9	3.5	2.9
0.2 uL g^{-1} Humic Acid	4.0	2.6	2.2	3.3	2.4	2.3

Table 8.9 Statistical analysis of nitrogen and leonardite application on Zn, Mn, and Cu concentration of durum on a Grey Luvisol.

		Zn Concentration		Mn Concentration		Cu Concentration	
Source	df	MS	F-value	MS	F-value	MS	F-value
Rep	2	6.63	0.26	58622	0.75	2.29	2.46
Nrate	2	1522.01	59.61**	7620266	97.46**	14.94	16.03**
Lsource	4	88.81	3.48*	78960	1.01	1.37	1.47
Application	1	133.86	5.24*	4680270	59.86**	1.49	1.60
Nrate*Lsource	8	10.35	0.41	66708	0.85	0.70	0.75
Nrate*Application	2	9.50	0.37	2591911	33.15**	4.27	4.58*
Lsource*Application	4	109.71	4.30*	66668	0.85	0.54	0.57
Nrate*Lsource*Application	8	38.62	1.63	44845	0.54	1.05	1.14
Error	66	25.53		78187		0.93	

* = significant at 0.05 level

** = significant at 0.0001 level

df = degrees of freedom

MS = Means Squares

Table 8.10 Contrast analysis of nitrogen and leonardite application on Zn, Mn, and Cu concentration of durum wheat on a Grey Luvisol.

	Zn concentration	Mn Concentration	Cu Accumulation
N rate			
0 vs 250 ppm	**	*	**
0 vs 500 ppm	**	**	*
250 vs 500 ppm	ns	**	*
Leonardite Source and Rate			
0 vs 0.05	ns	ns	ns
0 vs 0.2	*	ns	ns
0 vs 100	ns	ns	ns
0 vs 400	ns	ns	ns
0.05 vs 0.2	ns	ns	ns
0.05 vs 100	ns	ns	ns
0.05 vs 400	ns	ns	ns
0.2 vs 100	*	ns	ns
0.2 vs 400	*	ns	ns
100 vs 400	ns	ns	ns
Application			
Band vs Broadcast	*	**	ns

ns = non-significant

* = significant at 0.05 level of probability

** = significant at 0.0001 level of probability

8.5 Discussion

Leonardite had no consistent effect on biomass of durum wheat. It was interesting to note, however, that treatments with leonardite tended to produce the greatest biomass. Nitrogen fertilization had a significant effect on biomass of durum wheat. The yield with 500 ppm N was significantly lower than in the controls. This was most likely due to NH_3 or Mn toxicity. Ammonia is considered toxic to plants at high levels and urea applied on high pH soils has a preference to produce NH_3. The amount of NH_3 that was produced by the 500 ppm N rate may have exceeded crop tolerance levels resulting in a toxicity. Tissue Mn concentrations greater than 500 ppm are considered toxic to most plants (Srivastava and Gupta 1996). Manganese concentrations in plant tissues in many treatments with high rates of N were in excess of 500 ppm. Manganese solubility and availability increase with greater soil acidity. Since, ammonium containing fertilizers, such as urea, are known to acidify the rhizosphere, the acidification of the rhizosphere will increase the availability of Mn and consequently the uptake of Mn (Wilson 1977).

Nitrogen fertilizer, as urea, significantly increased concentration of Cd in durum wheat tissue. This is consistent with previous research (Grant et al. 1996; Mitchell 1997). Reasons for the increase in phytoavailability of Cd due to N fertilization are reduced pH in the rhizosphere (Eriksson 1990), competition from other cations for adsorption sites (Garcia-Miragaya and Page 1976), and formation of complexes (usually with chloride and sulfate fertilizers) (Mitchell 1997).

The addition of leonardite (L-31) and liquid humic substances (extracted from leonardite) (L-11) significantly elevated Cd concentration of durum wheat tissue when applied as a broadcast treatment. These results do not agree with those of Tyler and McBride (1982) who showed that application of humic acid reduced the activity of Cd in solution and Cd absorption by plant roots. Band application of leonardite had no effect on the Cd concentration of durum wheat tissue.

The results from this study suggest that application of organic amendments containing organic acids in intimate association with N fertilizers increased the phytoavailability of Cd. It is likely that the N fertilizer, due to acidification or increased ionic strength of soil solution, caused the Cd to become solubilized. The organic acids (humic or fulvic acids) that are in close association with the urea granules likely formed a complex with the Cd^{2+}. Humic and fulvic acids have been shown to form stable complexes with Cd (Stevenson 1976). This complex was likely water-soluble and thus the re-adsorption and precipitation of Cd was reduced increasing plant available Cd for a greater length of time (i.e. the leonardite had acted as a chelate for the Cd^{2+} ion). It is also possible that some of the acids may not be fully dissociated. The functional groups of the acids would be positively charged and could compete with Cd for sorption sites.

Application of leonardite did not affect Cd tissue concentrations when applied in a band. The high concentration of urea likely solubilized so much Cd that the chelation of Cd by leonardite was not a factor. The concentration of the Cd in the urea band will be much greater than the amount of Cd that is taken up by the plant roots. Therefore, the leonardite may be chelating Cd but the subsequent absorption of Cd by plant roots is not affected since the water-soluble Cd concentration is already high.

The application of N fertilizer also resulted in a significant increase in the availability of Mn, Cu, and Zn. This is consistent with previous work (Thomson et al. 1993). Copper, Zn, and Mn metals occur in soil solution as cations. It is possible that increases in Cu, Zn, or Mn in solution would compete with Cd for adsorption sites that would result in increase phytoavailability of Cd. Another possibility is that the functional groups of humic and fulvic acids may not be completely dissociated, therefore the positively charged functional groups will compete with cations for sorption sites.

The pH of the soil used in this study was 7.5. The formation and stability of Cd complexes becomes greater as the pH becomes more alkaline (Bolton et al. 1996). Bolton et al. (1996) showed that the addition of humic acid increased the adsorption of Cd in the pH range of 3.69 – 9.16, which had reduced Cd availability to the plant. The pH of the soil used in this study is within this range but had resulted in a significant increase in availability of Cd to the durum wheat.

Zinc concentration was significantly reduced by the addition of liquid leonardite. This is in agreement with previous work (Warwick et al. 1998, Gupta and Deb 1985). Zinc concentration has been reduced in plants due to increased adsorption and consequently reduced mobility of the complexed Zn (Warwick et al. 1998). Zinc chelated by fulvic acid reduced uptake of Zn by plant roots (Gupta and Deb 1985). The humic or fulvic acid may also form a complex that is so stable that Zn is unavailable to the plant.

High concentrations of Cd have also been shown to have an antagonistic effect on Zn absorption by plant roots. This may be possible due to the solubilization of Cd by urea fertilizer granules.

8.6 Conclusions

The application of leonardite (containing humic and fulvic acids) in intimate association with urea fertilizer granules did not affect the concentration and uptake of Cd by durum wheat when the N fertilizer was banded in soil. In contrast, the same fertilizer materials significantly increased the concentration and uptake of Cd by durum wheat when broadcast. The increase in Cd uptake with the addition of leonardite was contrary to that expected.

9. GENERAL DISCUSSION

The results of these studies suggest that the use of leonardite as an amendment for some agricultural soils is limited. In field studies, the application of leonardite as a soil amendment at high rates did not significantly or consistently affect the emergence or yield of canola or wheat either as pre-plant or post-plant application.

Leonardite, applied as a pre-plant or post-plant application treatment, did not affect the yield of canola or wheat, beneficially or detrimentally. Analysis on the chemical composition of wheat grain had shown that the application of leonardite did not affect chemical composition of plants. Thus, leonardite, if proven to be beneficial to soil quality could be utilized on agricultural land without any detrimental effects on food safety.

Application of humic acid, derived from leonardite, enhanced the P concentration of canola tissue in the early stages of plant development. However, the increase in P concentration did not significantly affect grain yield at maturity.

Soil application of humic and fulvic acids extracted from leonardite did not improve the availability of Cu and Zn to canola. However, foliar application of fulvic acid containing Cu and Zn elevated the Cu and Zn tissue concentrations of canola. This may be due to improved absorption of the fulvic acid complex or through scorching of canola tissue due to the low pH of the solution.

Foliar application of a fulvic acid-Cu complex increased the wheat tissue concentration of Cu comparable to that of EDTA. The fulvic acid-Cu complex also caused an improvement in wheat biomass and yield similar to EDTA. However, the inorganic source of Cu was the most effective form of Cu application. This may be explained through increased Cu^{2+} adsorption sites with the plant cuticle, scorching of the leaf tissue, and self diffusion.

The intimate association of leonardite and urea granules enhanced the concentration and uptake of Cd by durum wheat when the nitrogen fertilizer was broadcast. Nitrogen has been shown to increase the solubilization and uptake of Cd by durum wheat (Mitchell 1997). However, the application of leonardite with the urea granules resulted in a further increase in the Cd concentration of durum wheat. This is likely due to the formation of a stable water-soluble complex that is available to durum wheat plants.

10. SUMMARY AND CONCLUSIONS

The application of leonardite as a pre-plant or post-plant application has no or limited potential to increase the emergence of small seeded crops on soils such as used in this study.

The yield of canola and wheat was not affected by the application of leonardite, as a pre-plant or post-plant application. Chemical composition of the wheat grain was also unaffected. The studies conducted, eventhough limited, showed that leonardite could be added without compromising food safety.

The application of humic acid, derived from leonardite, applied in the same fertilizer band as ammonium polyphosphate was effective in increasing the P concentration of canola. However, the increase in P concentration did not generate an improvement in yield.

Humic and fulvic acids, derived from leonardite, did not improve the bioavailability of Cu or Zn when soil applied. Foliar application of fulvic acid enhanced the Cu and Zn concentrations of canola.

Foliar application of a fulvic acid-Cu complex was as effective as EDTA in improving the Cu concentration of wheat tissue. However, the inorganic source of Cu was superior to both fulvic acid and EDTA in increasing Cu concentration, wheat biomass, and wheat yield.

The application of leonardite in intimate association with urea fertilizer granules applied broadcast, resulted in greater Cd concentration of durum wheat tissue. This is likely due to the formation of a water-soluble complex formed by the organic acids of leonardite and Cd^{2+}. However, the field application of leonardite as a broadcast amendment did not affect the concentration of Cd in the wheat grain.

11. CONTRIBUTION TO KNOWLEDGE

Leonardite, and products of leonardite, were used in an attempt to improve soil structure, act as a carrier for Cu and Zn, to improve P fertilizer use efficiency, and reduce Cd concentration and accumulation in durum. The studies generally showed that these uses of leonardite in agricultural production in Manitoba have low potential. Leonardite and its products that were tested had little or no effects on emergence and final grain yield of crops but had no detrimental effects on the chemical composition of crops.

Use of fulvic acid derived from leonardite may have potential use as a minor element carrier if improvements are made to the complex. Data from field and growth chamber studies conducted in this thesis had shown that fulvic acid has potential to act as a carrier for Cu and Zn when applied as a foliar fertilizer. This was shown in concentration, biomass, and final grain yield.

These studies overall showed that the use of leonardite per se as a soil amendment had limited value and that further research was needed on leonardite products before use as a minor element fertilizer was viable.

12. REFERENCES

Adani, F., Genevini, P., Zaccheo, P., and Zocchi, G. 1998. The effect of commercial humic acid on tomato plant growth and mineral nutrition. Journal of Plant Nutrition. **21:** 561-575.

Adhikari, M. and Ray, J.N. 1975. Stability constants of humic acids and their phosphorylated derivatives with Cu^{2+} and Al^{3+}. J. Indian Chem. Soc. 238-241.

Adriano, D.C., Woodford, T.A., and Ciravolo, T.G. 1978. Growth and elemental composition of corn and bean seedlings as influenced by soil application of coal ash. J. Environ. Qual. **7:** 416-421.

Akinremi, O. 1999. Personal communication. Lethbridge Research Center.

Appelt, H., Coleman, N.T., and Pratt, P.F. 1975. Interactions between organic compounds, minerals, and ions in volcanic-ash-derived soils: Effects of organic compounds on the adsorption of phosphate. Soil Sci. Soc. Amer. Proc. **39:**628-630.

Ashley, J.T.F. 1996. Adsorption of Cu(II) and Zn(II) by estuarine, riverine and terrestrial humic acids. Chemosphere. **33:** 2175-2187.

Ayuso, M., Hernandez, T., and Garcia, C. 1996. Effect of humic fractions from urban wastes and other more evolved organic materials on seed germination. J. Sci. Food Agric. **72:** 461-468.

Barrow, N.J. 1974. On the displacement of adsorbed anions from soil: Displacement of phosphate by arsenate. Soil Sci. **117:** 28-33.

Bedrock, C.N., Chesire, M.V., and Shand, C.A. 1997. The involvement of iron and aluminum in the bonding of phosphorous to soil humic acid. Commun. Soil. Sci. Plant Anal. **28:** 961-971.

Bedrock, C.N., Chesire, M.V., Chudek, J.A., Fraser, A.R., Goodman, B.A., Shand, C.A. 1995. Effect of pH on precipitation of humic acid from peat and mineral soils on the distribution of phosphorous forms in humic and fulvic acid fractions. Commun. Soil Sci. Plant Anal. **26:** 1411-1425.

Bermudez, D., Juarez, M., Sanchez-Andreu, J., Jorda, J.D. 1993. Role of EDDHA and humic acids on the solubility of soil phosphorus. Commun.Soil Sci. Plant Anal. **24:** 673-683.

Bibak, A. 1994. Cobalt, copper, and manganese adsorption by aluminum and iron oxides and humic acid. Commun. Soil Sci. Plant Anal. **25:** 3229-3239.

Bolton, K.A., Sjoberg, S., and Evans, L.J. 1996. Proton binding and cadmium complexation constants for a soil humic acid using a quasi-particle model. Soil Sci. Soc. Am. J. **60:** 1064-1072.

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

Boyd, S.A., Sommers, L.E., Nelson, D.W., West, D.X. 1983. Copper(II) binding by humic acid extracted from sewage sludge. An electron spin resonance study. Soil Sci. Soc. Am. J. **47:** 43-46.

Boyd, S.A., Sommers, L.E., and Nelson, D.W. 1981. Copper(II) and iron(III) complexation by the carboxylate group of humic acid. Soil Sci. Soc. Am. J. **45:** 1241-1242.

Brady, B. and Pagenkopf, G.K. 1978. Cadmium complexation by soil fulvic acid. Can. J. Chem. **56:** 2331-2336.

Brennan, R.F. 1990. Effectiveness of some copper compounds a foliar sprays in alleviating copper deficiency of wheat grown on copper-deficient soils of western Australia. Aust. J. Exp. Agric. **30:** 687-691.

Bremner, J.M., Mann, P.J.G., Heintze, S.G., and Lees, H. 1946. Metallo-organic complexes in soil. Nature. **158:** 790-791.

Broadbent, F.E. and Ott, J.P. 1957. Soil organic matter-metal complexes: Factors affecting retention of various cations. Soil Sci. **83:** 419-427.

Bunzl, K. 1974. Ion exchange during continuous addition of Pb^{2+}-ions to humic acid and peat. Journal of Soil Science. **25:** 349-356.

Bunzl, K., Wolf, A., and Sansoni, B. 1976. Kinetics of ion exchange in soil organic matter. Differential ion exchange reactions of Cu^{2+}-, Cd^{2+}-, Zn^{2+}- and Ca^{2+}-ions in humic acid. J. Plant Nutr. Soil. Sci. **4:** 475-485.

Butler, J.H.A. and Ladd, J.N. 1969. The effect of methylation of humic acids on their influence on proteolytic enzyme activity. Aust. J. Soil Res. **7:** 263-268.

Cairns, R.R. and Moschopedis, S.E. 1970. Coal, sulfomethylated coal and sulfonated coal as fertilizers for solonetz soil. Can. J. Soil Sci. **51:** 59-63.

Campbell, G.D., Galicia, H.F., and Schindler, P.W. 1987. Binding of cadmium by montmorillonite-humic acid mixtures: Miscible-displacement experiments. Aust. J. Soil. Res. **25:** 391-403.

Cheam, V. and Gamble, D.S. 1974. Metal-fulvic acid chelation equilibrium in aqueous $NaNO_3$ solution. Hg(II), Cd(II), and Cu(II) fulvate complexes. Can. J. Soil Sci. **54:** 413-417.

Cheng, B.T. 1977. Soil organic matter as a plant nutrient. Proc. Int. Symp. Soil Organic Matter Studies. **1:** IAEA, Vienna.

Chesire, M.V. and Cranwell, P.A. 1972. Electron-spin resonance of humic acids from cultivated soils. Journal of Soil Science. **23:** 424-430.

Cotton, F.A. and Wilkinson, G. 1972. Advanced Inorganic Chemistry. Interscience Publishers. John Wiley & Sons. U.S.A.

Csicsor, J. Gerse, J. and Titkos, A. 1994. The biostimulant effect of different humic substance fractions on seed germination. In: Humic Substances in the Global Environment and Implications on Human Health, eds. Senesi, N. and Miano, T.M. Elsevier, New York.

Dalton, J.D., Russell, G.C., and Sieling, D.H. 1952. Effect of organic matter on phosphate availability. Soil Sci. **73:** 173-177.

Davies, R.I., Chesire, M.V., and Graham-Bryce, I.J. 1969. Retention of low levels of copper by humic acid. Soil Sci. **20:** 65-70.

Davis, A.P., and Bhatnagar, V. 1995. Adsorption of cadmium and humic acid onto hematite. Chemosphere. **30:** 243-256.

Deb, D.L. and Datta, N.P. 1967. Effect of associating anions on phosphorus retention in soil: Under variable anion concentration. Plant and Soil. **26:** 432-444.

DeKock, P.C. 1960. The effects of natural and synthetic chelating substances on the mineral status of plants. 7th intern. Congress of Soil Science, Madison, Wisc., U.S.A.

Dkhar, G.D., Prasad, B., and Sinha, M.K. 1985. Nature of zinc complexes of humic and fulvic acids isolated from forest and cultivated soils. J. Indian Soc. Soil Sci. **33:** 168-170.

Duplessis, G.L. and MacKenzie, A.F. 1983. Effects of leonardite applications on phosphorous availability and corn growth. Can. J. Soil Sci. **63:** 749-751.

Duval, J.R., Dainello, F.J., Haby, V.A., and Earhart, D.R. 1998. Hortechnology. **8:** 564-567.

El Gala, A.M., Metwally, A.I., and Khalil, R.A. 1978. The effect of humic acid and Na_2EDDHA on the uptake of Cu, Fe, and Zn by barley in sand culture. Plant and Soil. **49:** 41-48.

Evans, L.J. 1987. Retention of boron by agricultural soils from Ontario. Can. J. Soil Sci. **67:** 33-42.

Fagbenro, J.A. and Agboola, A.A. 1993. Effect of different levels of humic acids on the growth and nutrient uptake of teak seedlings. J. Plant Nutr. **16:** 1465-1483.

Ferrandon, M., and Chamel, A.R. 1988. Cuticular retention, foliar absorption and translocation of Fe, Mn, and Zn supplied in organic and inorganic form. Journal of Plant Nutrition. **11:** 247-263.

Gamble, D.S. 1970. Titration curves of fulvic acid: the analytical chemistry of a weak acid polyelectrolyte. Can. J. Chem. **48:** 3197-3204.

Gamble, D.S., Schnitzer, M., and Skinner, D.S. 1977. Mn^{2+}-fulvic acid complexing equilibrium measurements by electron spin resonance spectrometry. Can. J. Soil Sci. **57:** 47-53.

Garcia-Serna, J., Juarez, M., Jorda, J., and Sanchez-Andreu, J. 1996. Influence of organic compounds on nitrogen-fertilizer solubilization. Commun. Soil Plant Anal. **27:** 2485-2491.

Geering, H.R., Hodgson, J.F., and Sdano, C. 1969. Micronutrient cation complexes in soil solution: The chemical state of manganese in soil solution. Soil Sci. Soc. Amer. Proc. **33:** 81-85.

Goodman, B.A. and Chesire, M.V. 1976. The occurrence of copper-porphyrin complexes in soil humic acids. Journal of Soil Science. **27:** 337-347.

Goodman, B.A., Chesire, M.V., and Chadwick, J. 1991. Characterization of the Fe(III)-fulvic acid reaction by Mossbauer spectroscopy. Journal of Soil Science. **42:** 25-38.

Grant, C.A., Bailey, L.D., and Therrien, M.C. 1996. Effect of N, P and KCl fertilizers on grain yield and Cd concentration of malting barley. Fert. Res. **45:** 153-161.

Grant, C.A., McLaren, D.L., and Mohr, R.M. 1998. Chloride's role in maximizing wheat variety performance. Brandon Research Centre Soil and Crop Management Field Day. July 15, 1998.

Grossl, P.R. and Inskeep, W.P. 1991. Precipitation of dicalcium phosphate dihydrate in the presence of organic acids. Soil Sci. Soc. Am. J. **55:** 670-675.

Grundon, N.J. 1980. Effectiveness of soil dressings and foliar sprays of copper sulphate in correcting copper deficiency of wheat (Triticum aestivum) in Queensland. Aust. J. Exp. Agric. Anim. Husb. **20:** 717-723.

Gu, B. and Lowe, L.E. 1990. Studies on the adsorption of boron on humic acids. Can. J. Soil Sci. **70:** 305-311.

Gupta, G.N. and Deb, D.L. 1985. Influence of fulvic acid on diffusive flux of zinc to roots of rice and wheat plants in an alluvial soil. J. Indian Soc. Soil Sci. **33:** 162-167.

Hanafi, M.M. and Salwa, H. 1998. Cadmium and zinc in acid tropical soils: Influence of humic acid addition on soil properties and their adsorption. Commun. Soil. Sci. Plant Anal. **29:** 1933-1947.

Hammond, D.W. 1997. Effect of Band Chemistry on Fertilizer Phosphorous Availability. Master of Science Thesis. Dept. of Soil Science. University of Manitoba.

Harrell, Jr., J.W. and Saeed, M. 1977. Effect of leonardite on diffusion of phosphorous in coal mine spoils. Soil Sci. **124:** 285-290.

Hatton, D. and Pickering, W.F. 1980. The effect of pH on the retention of Cu, Pb, Zn and Cd by clay-humic acid mixtures. Water, Air, and Soil Pollution. **14:** 13-21.

Helanova, I. And Sladky, Z. 1967. The effect of glycine, humus substances and sucrose on the growth of tomato roots *in vitro*. Biologia Plantarum. **9:** 276-284.

Hoffman, G.L., Nikols, D.J., Stuhec, S., and Wilson, R.A. 1993. Evaluation of leonardite resources in Alberta. Energy, Mines, and Resources Canada, Contract #23403-2-0031/01-XSF. Alberta Research Council Open File 1993-18.

Hue, N.V. 1991. Effects of organic acids/anions on P sorption and phytoavailability in soils with different mineralogies. Soil Sci. **152:** 463-471.

Ishac, Y.Z., El-Haddad, M.E., Daft, M.J., Ramadan, E.M., and El-Demerdash, M.E. 1986. Effect of seed inoculation, mycorrhizal infection and organic amendment on wheat growth. Plant and Soil. **90:** 373-382.

Iswaran, V., Jauhri, K.S., and Sen, A. 1980. Effect of charcoal, coal, and peat on the yield of moong, soybean, and pea. Soil Biol. Biochem. **12:** 191-192.

Kafkafi, U., Bar-Yosef, B., Rosenberg, R., and Sposito, G. 1988. Phosphorus adsorption by kaolinite and montmorillonite: Organic anion competition. Soil Sci. Soc. Am. J. **52:** 1585-1589.

Kodama, H. and Schnitzer, M. 1977. Effect of fulvic acid on the crystallization of Fe(III) oxides. Geoderma. **19:** 279-291.

Ladd, J.N. and Butler, J.H.A. 1969. Inhibition and stimulation of proteolytic enzyme activities by soil humic acids. Aust. J. Soil Res. **7:** 253-261.

Leaver, J.P. and Russell, E.W. 1957. The reaction between phosphate and phosphate-fixing soils. J. Soil Sci. **8:** 113-126.

Lee, Y.S. and Bartlett, R.J. 1976. Stimulation of plant growth by humic substances. Soil Sci. Soc. Am. J. **40:** 876-879.

Levesque, M. and Schnitzer, M. 1967. Organo-metallic interactions in soils: Preparation and properties of fulvic acid-metal phosphates. Soil Sci. **103:** 183-190.

Li, S. and Wang, Z. 1988: *In*: Wang et al. 1995. The effect of humic acids on the availability of phosphorous fertilizers in alkaline soils. Soil Use and Management. **11:** 99-102.

Linehan, D.J. 1976. Some effects of fulvic acid component of soil organic matter on the growth of cultured tomato roots. Soil Biol. Biochem. **8:** 511-517.

Linehan, D.J. 1977. A comparison of the polycarboxylic acids extracted by water from an agricultural topsoil with those extracted by alkali. J. Soil Sci. **28:** 369-378.

Linehan, D.J. 1978. Humic acid and iron uptake by plants. Plant and Soil. **50:** 663-670.

Linehan, D.J. and Shepherd, H. 1979. A comparative study of the effects of natural and synthetic ligands on iron uptake by plants. Plant and Soil. **52:** 281-289.

Loneragan, J.F. 1981. Distribution and movement of copper in plants. Copper in Soils and Plants. Academic Press. Sydney, Australia.

Lopez-Hernandez, D., Flores, D., Siegert, G., Rodriguez, J.V. 1979. The effect of some organic anions on phosphate removal from acid and calcareous soils. Soil Sci. **128:** 321-326.

Lowe, L.E. and Bustin, R.M. 1989. Forms and hydrolytic behaviour of sulphur in humic acid and residue fractions of four peats from the Fraser lowland. Can. J. Soil Sci. **69:** 287-293.

Malik, K.A. and Azam, F. 1985. Effect of humic acid on wheat (*Triticum aestivum*) seedling growth. Environmental and Experimental Botany. **25:** 245-252.

Martin, A.E. 1960. Chemical studies of podzolic illuvial horizons. Flocculation of humus by ferric and ferrous iron and by nickel. J. Soil Sci. **11:** 382.

Martin, A.E. and Reeve, R. 1960. Chemical studies of podzolic illuvial horizons. Flocculation of humus by aluminum. J. Soil Sci. **11:** 369.

Martinez, M.T., Romero, C., and Gavilan, J.M. 1984. Solubilization of phosphorous by humic acids from lignite. Soil Sci. **138:** 257-261.

Matsuda, K., and Ito, S. 1970. Adsorption strength of zinc for soil humus: Relationship between stability constants of zinc-humic and zinc-fulvic acid complexes and degree of humification. Soil Sci. Plant Nutr. (Tokyo) **16:** 1-10.

McBride, M.B. 1978. Transition metal bonding in humic acid. An ERS study. Soil Sci. **126:** 200-208.

Metwally, A.I., El Gala, A.M., and Khalil, R.A. 1976. Effect of humic acid and $CaCO_3$ on the content of Cu, Fe, and Zn in barley. Egypt. J. Soil Sci. **16:** 163-169.

Mitchell, L.G. 1997. Solubility and Phytoavailability of Cadmium in Soils Treated With Nitrogen Fertilizers. Master of Science Thesis. Dept. of Soil Science. University of Manitoba.

Mittal, S.B., Mehta, S.C., and Rastogi, S.K. 1984. Influence of humic acid and calcium carbonate on copper adsorption in soils. J. Indian Soc. Sci. **32:** 365-367.

Moreno, E.C., Lindsay, W.L., and Osborn, G. 1960. Reactions of dicalcium phosphate dihydrate in soils. Soil Sci. **90:** 58-68.

Michalyna, W. and Smith, R.E. 1972. Soils of the Portage la Prairie Area. Canada Dept. of Agriculture, Manitoba Dept. of Agriculture, Manitoba Dept. of Mines and natural Resources, Dept. of Soil Science, The University of Manitoba.

Moris, N. 1985. The effects of humic substances and micronutrients on plant growth. In Soils and Nutrition of Perennial Crops. Malaysian Society of Soil Science. 209-219.

Nagarajah, S., Posner, A.M., and Quirk, J.P. 1968. Desorption of phosphate from kaolinite by citrate and bicarbonate. Soil Sci. Soc. Amer. Proc. **32:** 507-510.

Nuttall, W.F. 1970. Effect of organic amendments on some physical properties of luvisolic soils in relation to emergence of rapeseed in a growth chamber. Can. J. Soil Sci. **50:** 397-402.

O'Donnell, R.W. 1973. The Auxin-like effects of humic preparations from leonardite. Soil Science. **116:** 106-111.

Olk, D.C. and Cassman, K.G. 1995. Reduction of potassium fixed by two humic acid fractions in vermiculite soils. Soil Sci. Soc. Am. J. **59:** 1250-1258.

Paul, E.A. and Clark, F.E. 1996. Soil Microbiology and Biochemistry. Academic Press. Madison, Wisconsin, USA.

Petrovic, P., Vitorovic, D., and Jablanovic, M. 1982. Investigations of biological effects of humic acids. Acta Biol. Med. Exp. **7:** 21-25.

Piccolo, A., Nardi, S., and Conchieri, G. 1992. Structural characteristics of humic substances as related to nitrate uptake and growth regulation in plant systems. Soil Biol. Biochem. **24:** 373-380.

Pinton, R., Cesco, S., Santi, S., Varanini, Z. 1995. Effect of soil humic substances on surface redox activity of oat roots. Journal of Plant Nutrition. **18:** 2111-2120.

Pratt, L.E. Ehrlich, W.A., Leclaire, F.P., and Barr, J.A. 1961. Manitoba Soil Survey Report No. 12. Report of Detailed-Reconnaissance Soil Survey of Fisher and Teulon Map Sheet Areas.

Rowberry, R.G. and Collin, G.H. 1977. The effects of humic acid derivatives on the yield and quality of Kennebec and Sebago potatoes. American Potato Journal. **54:** 607-609.

Rubinchik, G.F., Tadzhiev, A.T., Adylkhodzhaeva, T.A. 1985. Active reagents from brown coal and hydrolysis lignin and their interaction with calcium phosphates *In*: Wang et al. 1995. The effect of humic acids on the availability of phosphorous fertilizers in alkaline soils. Soil Use and Management. **11:** 99-102.

Saeed, M. 1978. Use of sorption isotherms for evaluating the effect of leonardite on phosphorous availability in a coal mine spoil. Soil Sci. **126:** 157-165.

Saha, S.K., Dutta, S.L., and Chakravarti, S.K. 1979. Polarographic study of metal-humic acid interaction. Determination of stability constants of Cd- and Zn-humic acid complexes at different pH. J. Indian Chem. Soc. **56:** 1129-1134.

Salisbury, F.B. and Ross, C.W. 1992. Plant Physiology 4th Edition. Wadsworth Inc. California, U.S.A.

Samir, P. and Sengupta, M.B. 1985. Nature and properties of humic acid prepared from different sources and its effect on nutrient availability. Plant and Soil. **88:** 71-91.

Sanchez-Andreu, J., Jorda, J., Juarez, M. 1994. Humic substances. Incidence on crop fertility. Acta Horticulturae. **357:** 303-315.

Schisler, D.A. and Linderman, R.G. 1989. Influence of humic-rich organic amendments to coniferous nursery soils on Douglas-Fir growth, damping-off and associated soil microorganisms. Soil Biol. Biochem. **21:** 403-408.

Schnitzer, M. and Poapst, P.A. 1967. Effects of a soil humic compound on root initiation. Nature (Lond.) **213:** 598-599.

Schnitzer, M. 1969. Reactions between fulvic acid, a soil humic compound and inorganic soil constituents. Soil Sci. Soc. Amer. Proc. **33:** 75-81.

Senesi, N., Bocian, D.F., and Sposito, G. 1985. Electron spin investigation of copper(II) complexation by fulvic acid extracted from sewage sludge. Soil Sci. Soc. Am. J. **49:**119-126.

Senesi, N., Bocian, D.F., and Sposito, G. 1985. Electron spin resonance investigation of copper(II) complexation by soil fulvic acid. Soil Sci. Soc. Am. J. **49:** 114-119.

Senesi, N., Spositio, G., and Martin, J.P. 1986. Copper(II) and iron(III) complexation by soil humic acids: and IR and ESR study. The Science of the Total Environment. **55:** 351-362.

Senesi, N., Miano, T.M., Provenzano, M.R., and Brunetti, G. 1991. Characterization, differentiation, and classification of humic substances by fluorescence spectroscopy. Soil Sci. **152:** 259-271.

Sibanda, H.M. and Young, S.D. 1985. Competitive adsorption of humus acids and phosphate on geothite, gibbsite and two tropical soils. Soil Sci. **37:** 197-204.

Singh, P.J., Nagra, P., Mehrotra, R.S. 1981. Effect of organic amendments on root rot of gram (*Cicer arietinum*) and their influence on plant growth. Plant and Soil. **63:** 199-207.

Sinha, M.K. 1972. Organo-metallic phosphates. The solvent action of fulvic acids on insoluble phosphates. Plant and Soil. **37:** 457-467.

Sladky, Z. 1959. The application of extracted humus substances to overground parts of plants. Biologia Plantarum. **1:** 199-204.

Smidova, M. 1962. Effect of sodium humate on swelling and germination of winter wheat. Biologia Plantarum. **4:** 112-118.

Smirl, C. 1999. Personnel communication. Dept. of Soil Science. University of Manitoba.

Srivastava, P.C. 1996. Trace Elements in Crop Production. Science Publishers Inc. New Hampshire, U.S.A.

Stevenson, F.J., Krastanov, S.A., and Ardakani, M.S. 1973. Formation of constants of Cu^{2+} complexes with humic and fulvic acids. Geoderma. **9:** 129-141.

Stevenson, F.J. 1976. Stability constants of Cu^{2+}, Pb^{2+}, and Cd^{2+} complexes with humic acids. Soil Sci. Soc. Am. J. **40:** 665-672.

Stevenson, F.J. 1982. Humus chemistry, genesis, composition, reactions. J. Wiley and sons, New York, N.Y.

Strong, W.M. and Soper, R.J. 1973. Utilization of pelleted phosphorus by flax, wheat, rape, and buckwheat from a calcareous soil. Agron. J. **65:** 18-21.

Struthers, P.H. and Sieling, D.H. 1950. Effect of organic anions on phosphate precipitation by iron and aluminum as influenced by pH. Soil Sci. **69:** 205-213.

Swenson, R.N. Cole, C.V., and Sieling, D.H. 1949. Fixation of phosphate by iron and aluminum and replacement by organic and inorganic ions. Soil Sci. **67:** 3-22.

Swift, R.S. and Posner, A.M. 1972. Nitrogen, phosphorous, and sulphur contents of humic acids fractionated with respect to molecular weight. Journal of Soil science. **23:** 50-57.

Szalay, A. and Szilagyi, M. 1968. Laboratory experiments on the retention of micronutrients by peat humic acids. Plant and Soil. **24:** 219-224.

Tan, K.H. 1978. Effects of humic and fulvic acids on release of fixed potassium. Geoderma. **21:** 67-74.

Tan, K.H. and Nopamornbodi, V. 1979. Effect of different levels of humic acids on nutrient content and growth of corn (*Zea Mays*). Plant and Soil. **51:** 283-287.

Tan, K.H. 1980. The release of silicon, aluminum, and potassium during decomposition of soil minerals by humic acid. Soil Sci. **129:** 5-11.

Tan, K.H. and Tantiwiramanond, D. 1983. Effect of humic acids on nodulation and dry matter production of soybean, peanut, and clover. Soil Sci. Soc. Amer. J. **47:** 1121-1124.

Tan, K.H. and Binger, A. 1986. Effect of humic acid on aluminum toxicity in corn plants. Soil Science. **141:** 20-25.

Taylor, M.D. and Theng, B.K.G. 1995. Sorption of cadmium by complexes of kaolinite with humic acid. Commun. Soil Sci. Plant Anal. **26:** 765-776.

Tisdale, S.L., Nelson, W.L., Beaton, J.D., and Havlin, J.L. 1993. Soil Fertility and Fertilizers 5th Edition. Prentice Hall. U.S.A.

Tyler, L.D. and M^cBride, M.B. 1982. Influence of Ca, pH and humic acid on Cd uptake. Plant and Soil. **64:** 259-262.

Vaughan, D. and MacDonald, I.R. 1971. Effects of humic acid on protein synthesis and ion uptake in beet discs. Journal of Experimental Botany. **22:** 400-410.

Vaughan, D. and MacDonald, I.R. 1976. Some effects of humic acid on cation uptake by parenchyma tissue. Soil Biol. Biochem. **8:** 415-421.

Vaughan, D. and Malcolm, R.E. 1985. Soil organic matter and biological activity. Ed. Martinus Nijhoff. Boston.

Wallace, A. and Romney, E.M. 1980. Effect of powdered coal on plant growth and composition. Journal of Plant Nutrition. **2:** 159-161.

Wallace, A., Shannon, L.M., Lunt, O.R., and Impey, R.L. 1957. Some aspects of the use of metal chelates as micronutrient fertilizer sources. Soil Science. **84:** 27-41.

Wang, X.J., Wang, Z.Q., and Li, S.G. 1995. The effect of humic acids on the availability of phosphorous fertilizers in alkaline soils. Soil Use and Management. **11:** 99-102.

Warwick, P., Hall, A., Pashley, V., Van der Lee, J., Maes, A. 1998. Zinc and cadmium mobility in sand: Effects of pH, speciation, cation exchange capacity (CEC), humic acid and metal ions. Chemosphere. **36:** 2283-2290.

Walsh, L.M. and Beaton, J.D. 1973. Soil Testing and Plant Analysis. Soil Science Society of America, Inc. Madison, Wisconsin, USA.

Wild, A. 1950. The retention of phosphate by soil: A review. J. Soil Sci. **1:** 221-238.

Willaert, G. and Verloo, M. 1992. Effects of various nitrogen fertilizers on the chemical and biological activity of major and trace elements in a cadmium contaminated soil. Pedologie **43:** 83-91.

Xudan, X. 1986. The effect of foliar application of fulvic acid on water use, nutrient uptake and wheat yield. Aust. J. Agric. Res. **37:** 343-350.

Yang, X., Zhang, M., Li, J., Yang, Z. 1985. Study on the effect of ammonium nitro-humus from several materials on fertilizer superphosphate. Application of Atomic Energy in Agriculture. **1:** 45-50.

Zaifnejad, M., Clark, R.B., Ritchey, K.D., Baligar, V.C., and Parrish, D.J. 1996. Growth, photosynthesis, and water relations of wheat grown on acid soil amended with coal combustion by-products. Crop Sci. **36:** 968-974.

Zekri, M and Koo, C.J. 1992. Application of micronutrients to citrus trees through microirrigation systems. Journal of Plant Nutrition. **15:** 2517-2529.

13. APPENDICES

Appendix Ia. Description of leonardite products used in various studies.

Material	Form
L-11	Liquid (Humic Substances)
L-14	Dry
L-16	Liquid (Fulvic Acid)
L-31	Dry
L-45	Liquid (Humic Acid)
L-52	Liquid (Humic Acid)

Appendix Ib. Functional group analysis of leonardite products L-11 and L-31.

Material	Total Acidity	Carboxylic Funtional (meq/g)	Phenolic Functional (meq/g)
L-11	7.7	3.6	4.1
L-31	7.1	1.8	5.4

Appendix Ic. Chemical properties of leonardite products used in various studies.

	Moisture (%)		Carbon (%)		Hydrogen (%)		Nitrogen (%)		Ash (%)		Sulphur (%)		Oxygen (%)		pH
Material	AR	AD	AR	AD	AR	AD	AR	AD	AR	AD	AR	AD	AR	AD	
L-11	91.28	0.59	4.46	51.14	0.30	3.47	0.10	1.18	2.25	25.84	0.04	0.44	1.56	17.92	8.57
L-14	34.30	12.35	28.27	43.02	1.73	2.63	0.71	1.08	23.53	35.81	0.33	0.50	11.13	16.94	3.58
L-16	98.63	2.92	0.51	37.22	0.04	2.61	0.01	0.54	0.37	26.89	0.01	0.49	0.44	32.26	n/a
L-31	35.10	8.68	34.38	52.98	2.59	3.99	0.79	1.22	6.17	9.51	0.31	0.47	20.66	31.84	3.70
L-45	91.90	7.36	3.80	46.91	0.20	2.41	0.10	1.19	2.20	27.20	0.23	2.78	1.58	19.50	5.40
L-52	91.36	3.11	2.82	32.69	0.16	1.84	0.07	0.77	4.27	49.41	0.47	5.49	0.85	9.80	n/a

AR = As received, AD = Air Dried

Appendix Id. Elemental composition of leonardite products.

	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	Hg	K	La	Mg	Mn	P	Pb	Sr	Zn
Material	(%)	(ppm)	(ppm)	(ppm)	(%)	(ppm)	(ppm)	(ppm)	(ppm)	(%)	(ppm)	(%)	(ppm)	(%)	(ppm)	(%)	(ppm)	(ppm)	(ppm)
L-11	0.4	1	31	344	1.70	<1	4	12	9	0.3	0.03	0.8	7	0.21	22	0.011	8	200	28
L-16	0.6	14	27	<1	1.25	<1	11	19	18	0.6	n/a	6.9	13	0.24	62	17.64	<1	92	40
L-45	0.7	2	636	118	0.96	<1	4	6	3	0.4	n/a	6.0	12	0.17	117	0.015	6	151	6
L-52	1.4	<1	70	161	0.84	<1	<1	34	31	0.6	n/a	12.7	16	0.07	98	0.002	21	106	54

Appendix IIa. Chemical composition of wheat grain in section 3 of thesis on a Luvisol soil in 1998.

	N	S	P	K	Ca	Mg	Na	Zn	B	Mn	Cu	Fe	Al	Cd
Treatment	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
Control	3.20	0.20	0.45	0.34	0.04	0.15	<.01	46.2	1.3	34.5	<1.0	45.8	15.7	0.032
50kg P	3.20	0.20	0.46	0.35	0.03	0.15	<.01	50.4	2.4	37.1	<1.0	49.0	14.5	0.031
150kg P	3.30	0.20	0.46	0.40	0.04	0.16	<.01	46.3	0.5	44.0	<1.0	45.3	12.9	0.036
500kg P	3.10	0.19	0.51	0.41	0.04	0.17	0.0	58.2	1.5	47.9	<1.0	58.5	19.8	0.032
1000kg P	3.20	0.20	0.46	0.35	0.03	0.16	<.01	45.8	0.9	40.7	<1.0	48.3	11.0	0.034
5000kg P	3.60	0.21	0.45	0.35	0.03	0.15	<.01	48.9	0.8	41.3	<1.0	47.1	14.2	0.034
50kg M	3.30	0.20	0.48	0.38	0.04	0.16	<.01	50.9	0.9	44.5	1.2	94.4	37.8	0.027
150kg M	3.20	0.19	0.46	0.36	0.03	0.16	<.01	53.6	0.9	40.9	2.2	65.9	28.1	0.034
500kg M	3.40	0.20	0.47	0.35	0.03	0.15	<.01	54.9	0.4	43.3	2.0	56.6	14.6	0.042
1000kg M	3.20	0.19	0.44	0.35	0.03	0.15	<.01	45.9	0.4	35.6	1.3	48.8	13.3	0.030
5000kg M	3.20	0.19	0.45	0.35	0.03	0.15	0.0	49.3	0.7	41.0	1.3	69.4	28.8	0.032
50L	3.30	0.20	0.47	0.37	0.03	0.16	0.0	52.5	0.8	43.7	<1.0	80.0	30.1	0.034
100L	3.20	0.19	0.45	0.35	0.03	0.15	<.01	47.6	1.5	36.5	2.8	51.9	12.6	0.036
300L	3.30	0.20	0.50	0.39	0.04	0.17	<.01	60.3	0.7	48.0	2.1	86.4	33.6	0.034

Appendix IIb. Chemical composition of wheat grain in section 3 of thesis on a Luvisol soil in 1999.

	N	S	P	K	Ca	Mg	Na	Zn	B	Mn	Cu	Fe	Al	Cd
Treatment	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
Control	2.7	0.18	0.36	0.37	0.05	0.14	0.01	47.2	1.2	33.6	4.0	44	18.0	0.027
50kg P	2.6	0.18	0.37	0.37	0.05	0.14	0.01	47.6	1.2	37.0	5.2	53	25.9	0.025
150kg P	2.7	0.19	0.38	0.40	0.04	0.15	<.01	52.9	1.8	38.0	3.9	53	28.2	0.028
500kg P	2.6	0.18	0.37	0.37	0.05	0.14	0.01	47.4	1.1	32.4	4.0	37	19.5	0.029
1000kg P	2.6	0.18	0.37	0.38	0.04	0.15	0.01	49.4	0.9	34.7	5.5	35	15.9	0.024
5000kg P	2.7	0.19	0.36	0.38	0.05	0.14	0.01	49.6	1.6	38.9	3.9	35	22.2	0.028
50kg M	2.6	0.18	0.38	0.39	0.05	0.15	<.01	51.4	2.3	30.9	3.3	48	28.0	0.028
150kg M	2.6	0.18	0.38	0.40	0.05	0.15	0.01	51.6	3.7	37.3	5.4	40	17.4	0.022
500kg M	2.6	0.18	0.37	0.39	0.05	0.14	0.01	47.6	2.6	33.8	4.7	53	28.8	0.029
1000kg M	2.6	0.18	0.37	0.38	0.04	0.14	0.01	47.1	1.7	31.3	4.9	40	16.9	0.027
5000kg M	2.6	0.17	0.36	0.39	0.05	0.14	0.01	48.3	1.6	39.8	5.5	80	41.2	0.028
50L	2.6	0.18	0.37	0.38	0.05	0.15	0.01	51.3	1.6	31.9	5.2	37	22.4	0.030
100L	2.5	0.18	0.37	0.37	0.04	0.14	0.01	50.8	1.8	35.3	3.2	44	20.7	0.027
300L	2.6	0.18	0.38	0.38	0.04	0.15	0.01	52.1	1.7	33.7	4.5	45	24.3	0.028

Appendix IIc. Chemical composition of wheat grain in section 3 of thesis on a Chernozem soil in 1998.

	N	S	P	K	Ca	Mg	Na	Zn	B	Mn	Cu	Fe	Al	Cd
Treatment	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
Control	2.8	0.17	0.44	0.45	0.03	0.15	<.01	42.1	3.4	22.0	3.6	72.9	48.9	0.047
50kg P	2.8	0.17	0.46	0.48	0.03	0.15	0.01	44.5	2.7	23.2	4.1	98.2	76.6	0.052
150kg P	2.9	0.18	0.46	0.46	0.03	0.16	0.01	47.2	2.6	22.0	4.2	50.1	23.7	0.041
500kg P	2.9	0.18	0.42	0.46	0.03	0.15	0.01	40.1	2.3	24.5	4.5	113.4	86.2	0.043
1000kg P	2.9	0.18	0.44	0.44	0.03	0.15	0.01	42.6	2.2	19.7	3.3	42.1	5.9	0.047
5000kg P	2.8	0.18	0.43	0.44	0.03	0.15	0.01	43.5	2.1	27.7	2.6	208.7	205.9	0.045
50kg M	3.0	0.18	0.44	0.48	0.03	0.15	<.01	44.0	2.1	28.1	<1.0	125.8	111.5	0.044
150kg M	2.8	0.18	0.48	0.50	0.03	0.16	<.01	46.4	2.2	25.5	4.3	132.2	109.5	0.048
500kg M	2.9	0.18	0.45	0.47	0.03	0.15	<.01	43.8	2.8	21.9	3.5	98.1	68.0	0.044
1000kg M	3.0	0.17	0.46	0.48	0.03	0.16	0.01	47.7	1.6	23.9	2.7	108.7	59.5	0.047
5000kg M	3.0	0.18	0.43	0.44	0.03	0.14	<.01	41.6	2.2	21.1	2.1	70.4	50.3	0.041
50L	2.9	0.18	0.43	0.46	0.03	0.14	<.01	40.9	2.5	23.1	2.0	69.2	45.1	0.047
100L	2.9	0.17	0.45	0.46	0.03	0.15	<.01	41.3	1.2	24.3	3.2	61.5	32.8	0.042
300L	2.8	0.17	0.45	0.45	0.03	0.15	<.01	44.2	0.7	21.1	3.5	63.8	43.4	0.039

Appendix IId. Chemical composition of wheat grain in section 3 of thesis on a Chernozem soil in 1999.

Treatment	N (%)	S (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Zn (ppm)	B (ppm)	Mn (ppm)	Cu (ppm)	Fe (ppm)	Al (ppm)	Cd (ppm)
Control	2.6	0.18	0.43	0.63	0.05	0.17	0.01	42.2	6.0	30.9	5.8	126	122	0.107
50kg P	2.5	0.17	0.44	0.63	0.04	0.18	0.01	39.4	5.1	29.5	6.4	33	25	0.102
150kg P	2.5	0.18	0.45	0.70	0.05	0.18	0.01	42.5	3.1	32.3	5.7	74	55	0.101
500kg P	2.6	0.20	0.44	0.66	0.04	0.18	0.01	38.5	3.5	29.8	6.4	64	58	0.088
1000kg P	2.4	0.17	0.43	0.67	0.04	0.17	0.01	39.5	3.0	30.2	5.9	40	24	0.115
5000kg P	2.4	0.21	0.44	0.66	0.04	0.18	0.01	40.2	2.0	30.9	6.1	33	13	0.100
50kg M	2.5	0.17	0.45	0.70	0.04	0.18	0.01	41.1	2.3	31.4	6.2	35	14	0.100
150kg M	2.5	0.17	0.45	0.72	0.04	0.18	0.01	43.2	2.8	33.8	5.8	48	37	0.099
500kg M	2.5	0.18	0.44	0.65	0.04	0.18	0.01	41.3	1.7	33.4	6.1	31	13	0.094
1000kg M	2.5	0.17	0.44	0.70	0.05	0.18	0.01	41.4	2.3	30.5	6.2	37	19	0.093
5000kg M	2.5	0.18	0.44	0.65	0.05	0.18	0.01	41.9	4.5	30.1	6.1	52	35	0.098
50L	2.4	0.17	0.46	0.70	0.05	0.18	0.01	45.6	3.8	36.2	6.3	54	36	0.104
100L	2.4	0.17	0.45	0.74	0.04	0.19	0.01	42.4	2.9	34.4	6.3	40	26	0.094
300L	2.4	0.17	0.44	0.68	0.04	0.18	0.01	50.0	1.7	30.4	5.8	35	7	0.109

Appendix IIIa. Concentration of nutrients in canola 20 d after emergence on a Luvisol soil in 1999.

Treatment	N (%)	K (%)	S (%)	Ca (%)	Mg (%)	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
0P, No humic acid	4.95	4.60	1.30	2.68	0.49	4.73	34.40	283	34.3
6.5P, No humic acid	4.33	4.00	1.00	2.33	0.42	4.30	28.10	240	45.9
13P, No humic acid	4.96	3.80	1.00	2.54	0.49	4.35	30.50	284	54.2
20P, No humic acid	4.82	3.93	0.97	2.59	0.45	4.15	27.40	236	52.0
0 P, 12 L ha^{-1} L-45	4.70	4.71	1.25	2.63	0.48	4.75	36.00	351	38.5
6.5 P, 12 L ha^{-1} L-45	4.31	4.09	1.08	2.57	0.43	4.28	28.90	225	45.2
13 P, 12 L ha^{-1} L-45	4.74	4.11	0.99	2.55	0.46	4.15	28.80	261	52.5
20 P, 12 L ha^{-1} L-45	4.53	3.69	0.94	2.38	0.47	4.68	31.20	220	55.0
0 P, 24 L ha^{-1} L-45	4.83	4.67	1.29	2.63	0.45	4.38	36.20	280	36.1
6.5 P, 24 L ha^{-1} L-45	4.56	3.98	0.99	2.53	0.45	4.53	29.20	280	44.3
13 P, 24 L ha^{-1} L-45	4.21	4.03	1.06	2.52	0.44	4.55	29.60	221	45.9
20 P, 24 L ha^{-1} L-45	4.34	3.83	1.01	2.47	0.47	4.20	31.20	278	54.5
0 P, 12 L ha^{-1} L-52	4.36	4.48	1.28	2.59	0.46	4.48	33.50	282	36.4
6.5 P, 12 L ha^{-1} L-52	4.49	3.84	1.07	2.45	0.46	4.27	29.80	221	44.3
13 P, 12 L ha^{-1} L-52	4.35	3.79	0.98	2.45	0.46	4.35	27.00	276	49.9
20 P, 12 L ha^{-1} L-52	4.46	3.73	0.95	2.38	0.46	4.20	29.80	310	53.3
0 P, 24 L ha^{-1} L-52	4.80	4.64	1.36	2.64	0.48	4.75	36.20	270	34.6
6.5 P, 24 L ha^{-1} L-52	4.16	3.89	1.06	2.47	0.44	4.38	30.90	300	50.5
13 P, 24 L ha^{-1} L-52	4.20	3.81	1.00	2.46	0.46	4.00	28.50	256	50.4
20 P, 24 L ha^{-1} L-52	4.28	4.04	0.99	2.45	0.42	4.28	30.00	240	50.9

Appendix IIIb. Concentration of nutrients in canola 35 d after emergence on a Luvisol soil in 1999.

Treatment	N (%)	K (%)	S (%)	Ca (%)	Mg (%)	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
0P, No humic acid	4.91	2.79	0.85	2.83	0.38	3.43	24.0	117	34.3
6.5P, No humic acid	4.55	2.95	0.69	2.61	0.34	3.68	21.0	84	39.7
13P, No humic acid	4.51	2.94	0.73	2.62	0.39	3.78	22.7	93	43.9
20P, No humic acid	4.65	2.85	0.74	2.71	0.35	4.00	22.1	100	48.3
0 P, 12 L ha^{-1} L-45	4.84	2.82	0.78	2.62	0.36	3.18	23.7	97	35.8
6.5 P, 12 L ha^{-1} L-45	4.64	2.95	0.70	2.47	0.35	3.43	20.0	89	38.7
13 P, 12 L ha^{-1} L-45	4.49	2.57	0.72	2.68	0.37	3.95	20.0	94	43.5
20 P, 12 L ha^{-1} L-45	4.55	2.94	0.69	2.56	0.38	3.98	22.2	91	47.3
0 P, 24 L ha^{-1} L-45	5.20	2.98	0.86	2.81	0.39	3.78	29.6	100	33.6
6.5 P, 24 L ha^{-1} L-45	4.20	3.04	0.67	2.44	0.37	3.70	18.4	94	37.3
13 P, 24 L ha^{-1} L-45	4.60	2.80	0.71	2.60	0.34	3.63	21.3	96	40.3
20 P, 24 L ha^{-1} L-45	4.32	2.83	0.73	2.54	0.39	4.03	24.9	91	45.8
0 P, 12 L ha^{-1} L-52	4.85	2.76	0.85	2.72	0.37	3.48	23.1	114	35.1
6.5 P, 12 L ha^{-1} L-52	4.83	2.86	0.69	2.41	0.36	3.65	21.1	85	38.7
13 P, 12 L ha^{-1} L-52	4.41	2.80	0.67	2.44	0.33	4.13	22.9	95	45.8
20 P, 12 L ha^{-1} L-52	4.07	2.79	0.68	2.51	0.38	4.00	22.5	91	43.9
0 P, 24 L ha^{-1} L-52	4.98	2.77	0.82	2.70	0.38	3.28	22.3	108	32.6
6.5 P, 24 L ha^{-1} L-52	4.60	2.72	0.65	2.44	0.34	4.15	20.4	86	39.8
13 P, 24 L ha^{-1} L-52	4.72	2.88	0.72	2.47	0.37	3.90	19.2	90	43.9
20 P, 24 L ha^{-1} L-52	4.01	2.89	0.70	2.62	0.34	3.65	19.8	79	43.2

Appendix IIIc. Accumulation of nutrients in canola 20 d after emergence on a Luvisol soil in 1999.

Treatment	N (g)	K (g)	S (g)	Ca (g)	Mg (g)	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
0P, No humic acid	0.10	0.09	0.02	0.05	0.01	9	190	562	67
6.5P, No humic acid	0.25	0.23	0.06	0.14	0.02	25	487	1285	263
13P, No humic acid	0.23	0.18	0.05	0.12	0.02	20	560	1362	261
20P, No humic acid	0.27	0.21	0.06	0.16	0.03	24	521	1401	303
0 P, 12 L ha^{-1} L-45	0.15	0.14	0.04	0.08	0.01	14	252	1188	121
6.5 P, 12 L ha^{-1} L-45	0.18	0.18	0.05	0.11	0.02	18	521	983	193
13 P, 12 L ha^{-1} L-45	0.30	0.25	0.06	0.16	0.03	26	455	1780	349
20 P, 12 L ha^{-1} L-45	0.26	0.21	0.05	0.14	0.03	27	582	1264	317
0 P, 24 L ha^{-1} L-45	0.13	0.13	0.04	0.07	0.01	12	338	761	99
6.5 P, 24 L ha^{-1} L-45	0.21	0.18	0.05	0.11	0.02	20	269	1214	196
13 P, 24 L ha^{-1} L-45	0.19	0.19	0.05	0.12	0.02	20	468	1050	218
20 P, 24 L ha^{-1} L-45	0.28	0.25	0.07	0.16	0.03	27	727	1794	350
0 P, 12 L ha^{-1} L-52	0.12	0.13	0.04	0.75	0.01	12	173	808	106
6.5 P, 12 L ha^{-1} L-52	0.19	0.16	0.04	0.75	0.02	17	431	915	176
13 P, 12 L ha^{-1} L-52	0.19	0.17	0.04	0.11	0.02	19	420	1224	223
20 P, 12 L ha^{-1} L-52	0.26	0.21	0.05	0.14	0.03	23	594	1827	312
0 P, 24 L ha^{-1} L-52	0.15	0.15	0.04	0.08	0.01	15	229	857	110
6.5 P, 24 L ha^{-1} L-52	0.30	0.28	0.08	0.18	0.03	31	464	2130	363
13 P, 24 L ha^{-1} L-52	0.28	0.25	0.07	0.16	0.03	25	492	1734	333
20 P, 24 L ha^{-1} L-52	0.26	0.24	0.06	0.15	0.03	26	569	1487	317

Appendix IIId. Accumulation of nutrients in canola 35 d after emergence on a Luvisol soil in 1999.

Treatment	N (g)	K (g)	S (g)	Ca (g)	Mg (g)	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
0P, No humic acid	0.38	0.22	0.06	0.22	0.03	27	64	925	273
6.5P, No humic acid	1.05	0.68	0.15	0.59	0.07	82	166	1928	923
13P, No humic acid	1.14	0.73	0.18	0.65	0.09	94	141	2340	1094
20P, No humic acid	1.09	0.66	0.17	0.62	0.09	97	156	2369	1175
0 P, 12 L ha^{-1} L-45	0.52	0.31	0.08	0.28	0.04	34	115	1014	380
6.5 P, 12 L ha^{-1} L-45	1.17	0.76	0.18	0.64	0.10	86	124	2344	993
13 P, 12 L ha^{-1} L-45	1.03	0.58	0.16	0.60	0.09	90	183	2195	1009
20 P, 12 L ha^{-1} L-45	1.30	0.76	0.17	0.66	0.10	103	182	2385	1263
0 P, 24 L ha^{-1} L-45	0.56	0.32	9.00	0.31	0.04	41	99	1090	368
6.5 P, 24 L ha^{-1} L-45	0.59	0.43	0.09	0.35	0.07	56	134	1399	544
13 P, 24 L ha^{-1} L-45	1.00	0.60	0.14	0.55	0.09	78	131	2004	899
20 P, 24 L ha^{-1} L-45	1.30	0.84	0.22	0.76	0.11	120	204	2757	1409
0 P, 12 L ha^{-1} L-52	0.36	0.20	0.06	0.21	0.03	26	98	826	281
6.5 P, 12 L ha^{-1} L-52	0.98	0.53	0.12	0.50	0.07	71	120	1702	775
13 P, 12 L ha^{-1} L-52	0.82	0.51	0.12	0.45	0.06	76	120	1775	853
20 P, 12 L ha^{-1} L-52	1.07	0.74	0.17	0.66	0.09	104	171	2389	1168
0 P, 24 L ha^{-1} L-52	0.46	0.27	0.08	0.27	0.04	32	114	1012	317
6.5 P, 24 L ha^{-1} L-52	1.04	0.61	0.14	0.55	0.08	92	223	2004	903
13 P, 24 L ha^{-1} L-52	1.23	0.76	0.18	0.63	0.10	102	186	2347	1161
20 P, 24 L ha^{-1} L-52	1.15	0.83	0.20	0.75	0.11	106	184	2289	1262

Appendix IVa. Concentrations of Zn (ppm) in wheat tissue on a loamy sand soil after foliar Cu application.

	Rate and Method of Cu Application (kg ha^{-1})		
Cu Source	0.2	0.4	0.6
CuCl2	23.9	24.7	23.8
EDTA	25.2	25.0	21.7
F.A.-1	26.4	26.5	22.1
F.A.-2	31.1	23.7	21.7
F.A.-3	24.7	22.7	26.4
Control	25.1		

Appendix IVb. Concentrations of Fe (ppm) in wheat tissue on a loamy sand soil after foliar Cu application.

	Rate and Method of Cu Application (kg ha^{-1})		
Cu Source	0.2	0.4	0.6
CuCl2	133	119	129
EDTA	143	118	117
F.A.-1	135	103	111
F.A.-2	133	116	123
F.A.-3	120	116	124
Control	129		

Appendix IVc. Concentrations of Mn (ppm) in wheat tissue on a loamy sand soil after foliar Cu application.

	Rate and Method of Cu Application(kg ha^{-1})		
Cu Source	0.2	0.4	0.6
CuCl2	41.6	53.2	48.2
EDTA	54.7	46.9	46.8
F.A.-1	46.6	40.9	41.6
F.A.-2	46.9	45.1	41.8
F.A.-3	49.5	45.1	49.1
Control	45.7		

Appendix Va. Concentrations of Zn (ppm) in canola on a loamy sand following Cu application.

	Application Method		
Zn Source	Broadcast	Banded	Foliar
ZnCl2	26.0	22.1	26.9
Zn-EDTA	29.5	29.9	25.8
Zn-H.A.	22.4	22.7	18.1
Zn-F.A.	24.2	29.8	32.2
Control		24.6	

Appendix Vb. Concentrations of Fe (ppm) in canola on a loamy sand following Cu application.

	Application Method		
Zn Source	Broadcast	Banded	Foliar
ZnCl2	55.0	42.8	45.2
Zn-EDTA	49.3	46.8	56.5
Zn-H.A.	52.0	56.1	47.7
Zn-F.A.	50.1	59.9	59.7
Control		76.6	

Appendix Vc. Concentrations of Mn (ppm) in canola on a loamy sand following Cu application.

	Application Method		
Zn Source	Broadcast	Banded	Foliar
ZnCl2	160	169	162
Zn-EDTA	178	164	159
Zn-H.A.	147	143	122
Zn-F.A.	151	185	176
Control		177	

Appendix VIa. Concentrations of Cu (ppm) in canola on a loamy sand following Zn application.			
	Application Method		
Zn Source	Broadcast	Banded	Foliar
ZnCl2	3.00	2.94	3.18
Zn-EDTA	2.88	3.01	3.64
Zn-H.A.	2.92	2.66	2.77
Zn-F.A.	3.08	3.11	2.94
Control	2.93		

Appendix VIb. Concentrations of Fe (ppm) in canola on a loamy sand following Zn application.			
	Application Method		
Zn Source	Broadcast	Banded	Foliar
ZnCl2	58.2	55.0	73.4
Zn-EDTA	55.8	51.8	69.1
Zn-H.A.	78.1	54.1	56.1
Zn-F.A.	64.3	126.4	203.7
Control	52.1		

Appendix VIc. Concentrations of Mn (ppm) in canola on a loamy sand following Zn application.			
	Application Method		
Zn Source	Broadcast	Banded	Foliar
ZnCl2	135	133	143
Zn-EDTA	121	142	139
Zn-H.A.	127	126	132
Zn-F.A.	146	139	133
Control	139		

Appendix VIIa. Accumulations of Zn (ppm) in canola tissue on a loamy sand following Cu application.

	Application Method		
Cu Source	Broadcast	Banded	Foliar
CuCl2	424	364	379
Cu-EDTA	467	427	338
Cu-H.A.	356	333	291
Cu-F.A.	380	456	400
Control	343		

Appendix VIIb. Accumulations of Fe (ppm) in canola tissue on a loamy sand following Cu application.

	Application Method		
Cu Source	Broadcast	Banded	Foliar
CuCl2	893	701	633
Cu-EDTA	776	668	738
Cu-H.A.	830	835	754
Cu-F.A.	792	917	739
Control	1071		

Appendix VIIc. Accumulations of Mn (ppm) in canola tissue on a loamy sand following Cu application.

	Application Method		
Cu Source	Broadcast	Banded	Foliar
CuCl2	2641	2787	2287
Cu-EDTA	2847	2332	2075
Cu-H.A.	2361	2135	2011
Cu-F.A.	2388	2839	2208
Control	2479		

Appendix VIIIa. Accumulations of Cu (ppm) in canola tissue on a loamy sand following Zn application.

	Application Method		
Zn Source	Broadcast	Banded	Foliar
ZnCl2	42.7	41.4	36.5
Zn-EDTA	36.5	47.7	47.9
Zn-H.A.	43.8	37.6	37.4
Zn-F.A.	43.6	42.4	34.0
Control	44.7		

Appendix VIIIb. Accumulations of Fe (ppm) in canola tissue on a loamy sand following Zn application.

	Application Method		
Zn Source	Broadcast	Banded	Foliar
ZnCl2	823	771	831
Zn-EDTA	694	812	957
Zn-H.A.	1177	760	758
Zn-F.A.	928	1742	2101
Control	794		

Appendix VIIIc. Accumulations of Mn (ppm) in canola tissue on a loamy sand following Zn application.

	Application Method		
Zn source	Broadcast	Banded	Foliar
ZnCl2	1929	1880	1661
Zn-EDTA	1492	2222	1833
Zn-H.A.	1919	1780	1793
Zn-F.A.	2068	1903	1531
Control	2128		

Appendix IXa. Accumulations of Zn (mg pot^{-1}) in durum wheat tissue on a Luvisol soil.

	Application Method					
	Broadcast			Banded		
	Rate of N (ppm)			Rate of N (ppm)		
L Source and Rate	0	250	500	0	250	500
0 L-31	135	232	106	117	209	279
100 ppm L-31	192	275	194	105	220	233
400 ppm L-31	131	256	138	107	200	153
0 Humic Acid	135	232	106	117	209	279
0.05 uL g^{-1} Humic Acid	99	238	107	125	201	190
0.2 uL g^{-1} Humic Acid	82	239	113	96	224	121

Appendix IXb. Accumulations of Mn (mg pot^{-1}) in durum wheat tissue on a Luvisol soil.

	Application Method					
	Broadcast			Banded		
	Rate of N (ppm)			Rate of N (ppm)		
L Source and Rate	0	250	500	0	250	500
0 L-31	160	3074	4942	116	955	2128
100 ppm L-31	181	2523	5944	123	907	2474
400 ppm L-31	149	2283	5852	142	932	2422
0 Humic Acid	160	3074	4942	120	955	2128
0.05 uL g^{-1} Humic Acid	131	2659	3404	141	988	2166
0.2 uL g^{-1} Humic Acid	93	2265	3882	89	1133	1533

Appendix IXc. Accumulations of Cu (mg pot^{-1}) in durum wheat tissue on a Luvisol soil.

	Application Method					
	Broadcast			Banded		
	Rate of N (ppm)			Rate of N (ppm)		
L Source and Rate	0	250	500	0	250	500
0 L-31	19.3	15.0	11.8	12.9	15.5	14.5
100 ppm L-31	24.4	12.9	12.8	14.3	11.7	17.7
400 ppm L-31	29.6	7.8	8.9	21.2	15.3	20.2
0 Humic Acid	19.3	15.0	11.8	12.9	15.5	14.5
0.05 uL g^{-1} Humic Acid	15.6	10.9	8.4	17.3	19.2	14.6
0.2 uL g^{-1} Humic Acid	14.3	17.3	6.4	12.0	14.9	7.8