STUDIES ON THE EXTRACTION AND AVAILABILITY OF MICRONUTRIENTS IN SOIL

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بسم الله الرحمن الرحيم

In the Name of ALLAH

"Most Gracious, Most Merciful"

"He Who taught (the use of) the Pen,"

"Taught man that which he Knew not."

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Contents Page no

Summary

Chapter one includes the survey of dynamic equilbria and the forms of micronutrients which exists in fractions. The sources of micronutrients as influenced by natural conditions and anthropogenic metal addition are also discussed. The presence of micronutrient cation in different pools and the removal of metals from these pools in soil are reviewed. Extractable levels of micronutrients in soil are affected by various factors. The forms of trace metals as influenced by these factors are also discussed. The objectives of the thesis are to study the removal of micronutrient in soil from different pools and also the changes in the distribution of these metals with plant uptake.

Chapter 2 is comprised of the physical properties of the soil study and the slective extraction procedures for the measurement of soil Co, Cu, Fe, Mn, Ni and Zn.

Chapter 3 consisted of the removal of exchangeable metal by using simple metal salts. The removal of micronutrients associated with exchangeable fraction in the soil is affected by the nature of the extracting solution, extractant concentration and by the replacing cation. Extraction of exchangeable metals with different salts of acetate, nitrates and chlorides showed non detectable level of Co, Cu and Ni from all the soils. Which indicated that large proportion of these elements are associated with organic and oxide bounds fractions. Nitrates and chlorides appeared to extract large quantity

of Mn and Zn and very small amount of Fe. While, ammonium acetate extracted more Fe than Mn and Zn from these soils. It indicates that kind of the cation also plays an important role on the exchange of micronutrients. Amount of Fe, Mn and Zn extracted by various salts of acetate, nitrates and chlorides for most of the soils were increased with the increase of extractant concentrations. Which suggested that higher concentrations of different salts extract a fraction of trace metals from these soils, which was retained by the soil when extracted with lower concentrations of the extracts.

Chapter 4 lays emphasis on the removal of metals associated with organic matter and oxide bound fractions. Comparison between the pairs of extractants and individual extractant are studied for the removal of Cu, Fe, Mn and Zn. It is found that there is no big difference beteen the two pairs of extractants. Ammonium EDTA (0.05M) pH 7.0 is suggested a common extractant for the removal of organic matter fraction of Cu, Fe, Mn and Zn in preference to pyrophasphate and sodium hydroxide. As pyrophosphate also affected the oxide pool and NaOH extracted very small amount of Mn and Zn from all the soils. Acid oxalate is suggested for the dissolution of oxide bound fraction, as it extracted more Cu, Fe, Mn and Zn than the hydroxylamine hydrochloride from all the soils. Whereas $NH_2OH.HCl$ extractable copper was below the detection limit. In soil extracts metals are present in suspended materials. In this study, soil extracts were cleared by precipitation of suspended materials and organic matter with filtration through 0.45 µm filters. It is observed that extractable amount of Cu, Fe, Mn and Zn are decreased after filtration through 0.45 µm filters and ultrafiltration of the soil extracts are suggested for further study of these elements.

Chapter 5 investigates the influence of continuous cropping of ryegrass on the distribution of Cu, Fe, Mn and Zn in soil using a pot experiment and assessess this by means of specific extractants. Calcium chloride, acetic acid, ammonium EDTA and acid oxalate were used to extract Cu, Fe, Mn and Zn associated with exchangeable, specific adsorbed, organic matter and oxide bound fractions, respectively. It was found that $CaCl_2$ and acetic acid extractable Cu and Fe were very small or below the detection limit, and large quantity of Mn and Zn were present in these pools. Which indicates that Mn and Zn are more easily available to plants than that of Cu and Fe. As the large quantity of Cu and Fe were present in organic matter and oxide fractions, which indicates that organic and oxide fractions are the big reservoir of Cu and Fe. It is also observed that extractable amount of Cu and Zn decreased in the first 10 weeks and than remain unchanged. whereas the amount of Fe and Mn showed a small increased from initial to ist 10 week of samplings and thereafter it remain unchanged. The amount of Cu and Zn taken up by grass are higher in soils with the higher levels of Cu and Zn. The uptake of Mn was higher in soil with high Mn in readily available pools and the uptake of Fe was higher in the soil of lower Fe content, rather than the higher total amounts, suggesting that total Fe and Mn is not a good indicator for plant uptake.

Chapter 1

Micronutrients in Soils

1.1 Introduction

Those elements which are required in small amounts for plant growth are called 'Micronutrients'. Usually their concentration is less than 100 $\mu g\ g^{-1}$ in the plants. There are seven elements which are required in 'Trace' amounts: B, Mn, Fe, Cu, Zn, Mo and Cl (Shkolnik, 1984). Other elements are 'Functional' in certain circumstances they may increase the yield but they are not necessary. However, there are some elements which are not supplied in adequate amounts by forage and grain but required by the animals:i.e. Co (It is also essential for legume bacteria involved in the fixation of nitrogen from air) I, Se, and Na which are also useful for some plant species (Viets, 1962 and Landon, 1984).

Micronutrient deficiencies often occur on areas where the surface soil has been removed in levelling management practices or by soil erosion. Information on the content and the distribution of micronutrient cations in the soil can be useful in evaluating nutrient deficiencies most likely to limit plant growth.

As the demand for higher yields increases, the plants requirements are more likely to become limiting. To increase in the per acre yield and good quality of crops to fulfil the human need of food, fibre and energy, the use of NPK fertilizers, higher yielding and disease resistant plant varieties with increased nutrient demands and the decreasing availability of farm yard manure (rich in trace metals) in those areas where mixed farming has given way to intensive arable agriculture, have combined to increase the demands made on the soil in terms of its ability to supply trace metals to plants.

The micronutrient cations required for the growth and reproduction of plants and animals include Zn, Fe, Mn, B, Mo, Cl and Cu and the deficiencies of these elements often develop on certain crops and also on certain types of soil. Micronutrient deficiencies are highly important in a few crops but the need for additions of micronutrients will always be less widespread than for the major elements. The uncertainty about their status in any particular soil/climate combination makes their study more difficult and more interesting than that of the major elements. Their behaviour is extremely complicated, and in a number of respects it is still not understood, either in the soil or in the plant.

The most important micronutrients for plant growth are Zn, Cu, Mn, B, Mo, and Fe. Cu, Mn, Fe and Zn occur in soil solution as cations, while B and Mo occur as anions. With the exception of Fe which is a major element, geochemically they are all trace elements (Jenkins and Jones, 1980). Their chemistry differs from one element to the other, although with similarities within small groups. Boron is the only nonmetal among the micronutrients and has a constant valance of 3^+ and a very small ionic radius (Krauskopf, 1972). Zinc and copper exhibit one valance in soils while Mn, Fe and Mo show more than one valance. The most common valencies are 2^+ and 4^+ for Mn and 2^+ and 3^+ for Fe and 6^+ for molybdenum (Krauskopf, 1972).

Table 1.1 Total contents of trace metal ions in the lithosphere and in soil.

Element	Average in lithosphere (mg kg^{-1})	Soil content (mg kg^{-1})
Iron	50,000	10,000-300,000
Manganese	1000	200-3,000
Nickel	100	10-1000
Chromium	100	5-3,000
Zinc	80	10-300
Copper	70	2-100
Cobalt	40	1-50
Lead	10	2-200
Molybdenum	2	0.2-5
Cadmium	0.2	0.01-7

From: Bohn, McNeal and O'Connor, 1985 p311

Distribution of micronutrients has been well described by Hodgson (1963) and Bohn et al. (1985). Table 1.1 indicates the total contents of trace metal ions in the lithosphere and in soils.

The total content of micronutrients in soil has been reviewed by Aubert and Pinta (1977) and Lindsay (1984). The contents of micronutrients in different soils vary according to the rocks they are formed from and the pedogenic processes involved, which in turn are determined by soil forming processes. e.g. vegetation, topography and climate (Jenkins and Jones, 1980 and Krauskopf, 1972).

The availability of micronutrients not only depends upon the total contents but on their chemistry as well. Considering the soil chemistry of micronutrients, Hodgson (1963) divided them into three groups (1) Cu and Zn, which are heavy metal cations held in soil principally on organic or inorganic surfaces or substituted as accessory constituents in common soil minerals. (2) Fe and Mn for which the most important property is that of being oxidized to higher valance states which can form very insoluble oxides and phosphates and (3) B and Mo, which exist in the soil as anions and are the most mobile among the micronutrients. However, under acid conditions Mo combines very strongly with "oxide" minerals and clay minerals.

Different micronutrients do not just vary in total soil contents, they also differ considerably in their requirements by plants. Viets (1962) gave the example of Mo, for which 0.5 µg g^{-1} in most plants is adequate, while

Fe must be present in concentrations of about 100 $\mu g\ g^{-1}$ on a dry weight basis.

Soil solution plays a central role in many soil processes. It is a mobile phase that is responsible for redistribution of solids with in the soil. It is the medium from which all plants take their nutrients, and small amounts of plant constituents may also be released into the soil solution. Although the soil solution is affected by all of the reactions shown in figure 1.1, its composition is ultimately controlled by the mineral phases of the soil.

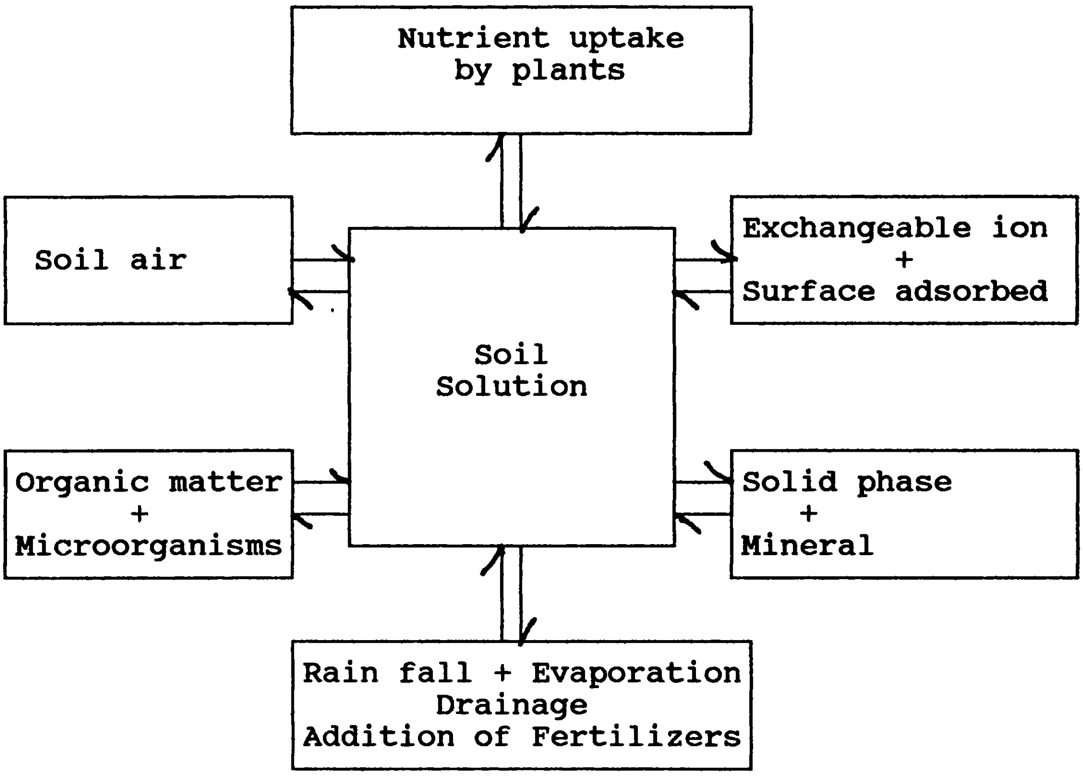


Figure 1.1 The dynamic equilibria that occur in soils from Lindsay (1979).

Lindsay (1979) stated that soil is the "Living phase" includes living organisms which continually breaking down the organic residues and synthesizing many of the products into their body tissue, while others are released to the surroundings. The manner in which the various constituents of the soil interact is represented in figure 1.1.

Different chemical extraction methods are used to distinguish and estimate the different forms of micronutrients. Soil test methods are very important in the study of micronutrients in the soil. They help to assess the amount of nutrient that would be available to the crop over the growing season. With the use of soil tests, a critical level is identified below which plants will be deficient and above which no deficiency occurs. Soil tests are also important for the detection of micronutrient toxicity, and provide some guide to the amount of fertilizer needed to correct a given deficiency (Lindsay and Cox, 1985).

Plant availability of Cu, Fe, Mn and Zn is often reported to be associated with the distribution of these micronutrients among certain soil fractions. Fractionation schemes used to study this relationship generally evaluate the role of solution, exchangeable, organically complexed and oxide (Fe, Mn, Al) bound forms (Iyengar et al., 1981; Sedberry and Reddy, 1976; Shuman, 1979 and Sposito et al., 1982). Variations in critical soil chemical parameters can induce shifts in these pools, which may result in a micronutrient deficiency or toxicity.

Studies involving micronutrient fractionation and plant availability have traditionally attempted to relate the various micronutrient pools to plant concentration or uptake. Iyengar et al. (1981) for example, partitioned soil zinc into six fractions and found, based on zinc uptake, that adsorbed and organically bound Zn were more available than that occluded in Fe or Mn oxides. Studies on Cu uptake by McLaren and Crawford (1973) and Jarvis (1981) point to the importance of the organic and exchangeable fractions, but also to the often inconsistent relationships found between soil Cu and plant uptake. Shuman (1979) in studies with 10 Georgia soils, identified the exchangeable and organic matter fractions as the forms most likely to contain plant available Mn.

Plant analysis is equally important in the agronomic studies. Therefore, both plant and soil analysis together with the availability of micronutrients will be reviewed in this chapter.

1.2 Sources of micronutrients

Mattigod and Page (1983) divided the sources and types of micronutrients into two main groups. (A) Metal concentration under natural conditions and (B) Anthropogenic metal additions. The metal content of a soil is the composition of parent material while the other sources of metal pollution are industrial activities. Such as mining, smelting and refining can result in a widespread dispersal of metals throughout the environment. The manner in which the various sources of anthropogenic and natural inputs of metals in soils is represented diagrammatically in figure 1.2.

1.2.1 Natural sources of trace metals

The main sources of micronutrients in soils are the parent materials from which they are derived. Usually this is weathered bedrock that has been transported by wind , water or glacial activity. Mitchell (1964) stated that ninety five percent of the earth's crust is made up of igneous rocks and five percent of sedimentary rocks. Table 1.2 indicates the mean contents of micronutrients and heavy metals in common igneous and sedimentary rocks. Mitchell (1964) concluded that sandstone contains minerals that weathered slowly and usually contain small amounts of micronutrients. Shales may be of organic or inorganic origin, and usually contain large amounts of micronutrients. Soils derived from these parents materials tended to reflect their chemical composition. Thus those developed from the weathering of coarse-grained sediments such as sands and sand stones, and from acid igneous rocks tend to contain smaller amounts of nutritionally essential elements such as Cu and Co, than those derived from fine-grained sedimentary rocks such as clays and shales and from basic igneous rocks.

Trace element content of the soil may be directly related to bedrock geochemistry, as residual soils are formed from the underlying bedrocks. Where parent materials have been mixed or redistributed by alluvial transport, wind or glacial activity, the influence of the underlying rock may be either modified or completely masked; at times the effect of bedrock composition may be smeared in the direction of water flow or ice movement. The influence of parent materials on the total content and form of trace elements in soils is modified to varying degrees by pedogenetic processes that may lead to the mobilization and redistribution of trace elements both with in the soil profile and between neighbouring soils (Swaine and Mitchell, 1960; Mitchell, 1964).

The processes of gleying, leaching, surface organic matter accumulation and podzolization, together with soil properties such as reaction (pH) and redox potential (E_h) may affect the distribution, the form and the mobility of trace elements in the soil. Soils in mineralized areas are often enriched in the ore metals and contain high concentrations of one or more of the elements Cu, Pb, Zn, Cd, and As. Mortvedt and Giordani (1977) found that differences in micronutrient content of mined phosphate rocks are caused by impurities co-precipitated with phosphates at the time of deposition. Hirai (1989) measured the metal concentrations in snow packs in the Hokuriku mountains along the Japan sea and indicated that chemical enrichment occurred in frozen layers with high density.

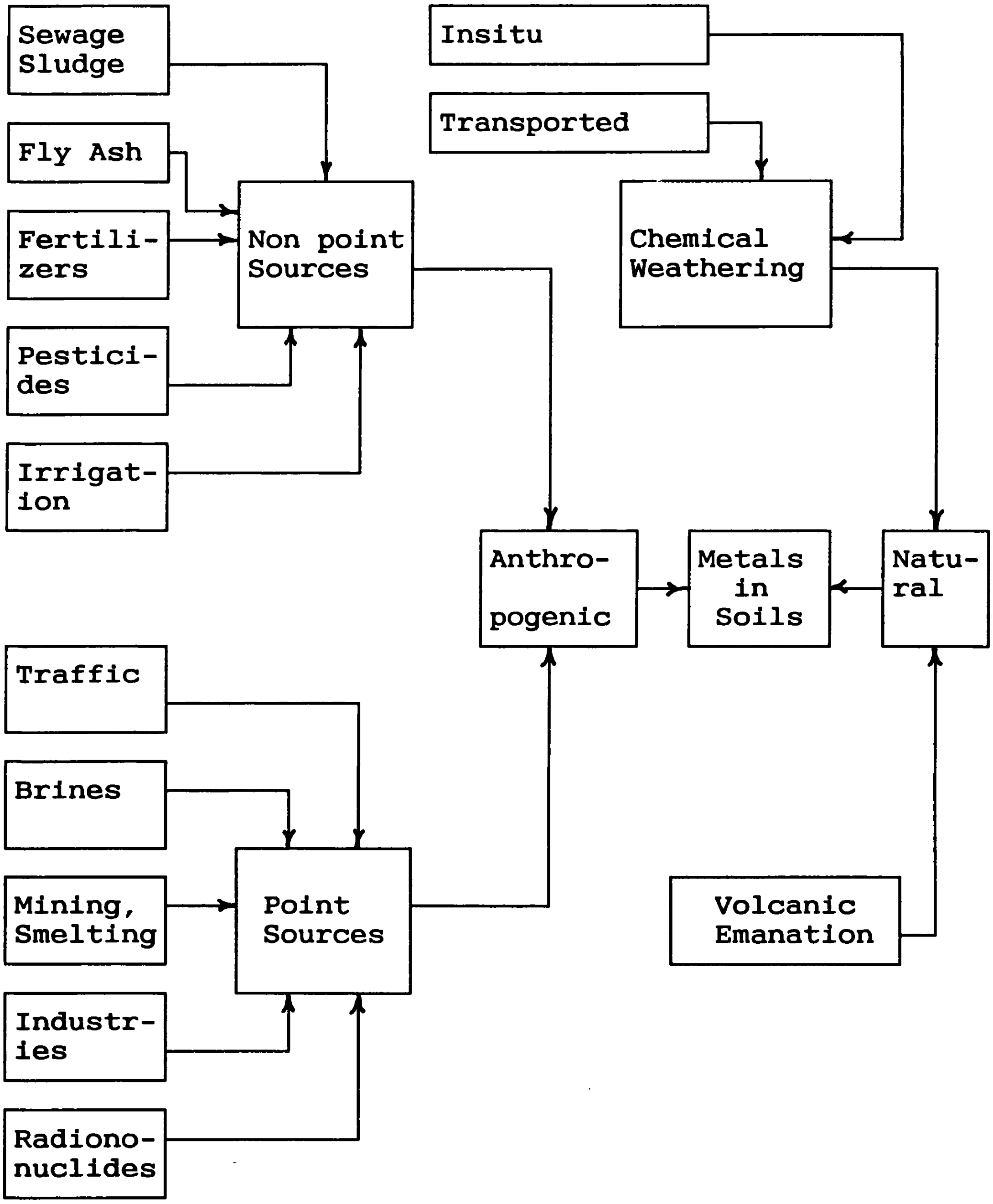


Figure 1.2 Sources of anthropogenic and natural inputs of metals in soils

Table 1.2. Mean concentrations of some metals and metalloids in igneous and sedimentary rocks (PPM).

Metals	1	2	3	4	5	6
As	2.00	2.00	10.00	0.00	1.70	2.00
Cd	0.20	0.15	1.40	1.00	0.05	0.05
Cr	220.00	20.00	120.00	100.00	10.00	35.00
Co	50.00	5.00	20.00	10.00	0.10	0.30
Cu	90.00	15.00	50.00	70.00	4.00	2.00
Hg	0.05	0.06	0.09	0.50	0.04	0.05
Pb	6.00	18.00	20.00	30.00	9.00	12.00
Mo	1.50	1.40	2.50	10.00	0.40	0.20
Ni	140.00	8.00	68.00	50.00	20.00	2.00
Se	0.05	0.05	0.60	00.00	0.08	0.05
Zn	110.00	40.00	90.00	100.00	20.00	16.00

From: Thornton (1983).

1 = Basalic igneous 2 = Granitic igneous 3 = Shales clays
4 = Black shales 5 = Lime stone 6 = Sand stone

1.2.2 Anthropogenic sources of trace elements

Anthropogenic metal inputs can be broadly classified as point or non-point sources. The metal inputs into soils from both point and non-point sources can be wide spread and can occur at considerable distances from the sources. Also, the metal inputs from non-point sources are due to deliberate application of wastes, fertilizers, pesticides, etc. Whereas, metal inputs from point sources are usually unintentional.

Disposal of sewage sludge on agricultural lands has been in practice on a limited scale for several decades. Metal concentrations in sewage sludge are largely dependant on the nature and the intensity of the industrial activity as well as the type of the sewage treatment process. Page (1974) found that maximum amounts of metal extracted from sewage sludges are considerably more than the amount extracted from soils. One of the potential problems of sewage sludges disposal on land is the accumulation of toxic concentrations of heavy metals in soil.

Large quantities of flyash have been disposed of on the soil. It is observed that maximum concentrations of heavy metals encountered in flyash significantly exceed the maximum concentration of some metals in uncontaminated soils. Norton et al. (1988) described from their study that in the stack flyash the most prominent increases in micronutrient concentrations resulting from combustion were for Br, Cd, Pb, Sb, Se, Sn and Zn. Elseevi and Page (1984) found that approximately 70 million Mt of flyash containing Cd, Co, Cu, Fe, Al, Mn, Mo, Ni, and Zn are produced annually.

Chemical fertilizers are potential sources of metal pollution in soils. Soils are deficient in plant available forms of one or more than one of the six micronutrient (Zn, Mn, Fe, Cu, Mo and B) and their application is recommended. The most widely used source of Cu in agriculture is $CuSO_4.5H_2O$ (25.5% Cu) and other sources such as copper oxides, basic copper sulphate and copper carbonate have also been used. The most commonly Mn fertilizers are $MnSO_4.4H_2O$ (24% Mn) and manganese oxide (MnO, 78% Mn). In contrast two types of Zn fertilizers have been reported. 1) Inorganic sources, which includes zinc sulphate, zinc oxide and zinc ammonia. and 2) organic sources, include ZnEDTA, zinc lignin sulphate and zinc polyflavonoids.

Limestone is widely used on agricultural soils. Micronutrient concentration in the soil increased with the repeatedly addition of trace metal-rich limestone. In addition to these micronutrient carriers, other commercial fertilizers contains small amounts of micronutrients.

Inorganic pesticides are also a source of metal contamination of soils, as they are mainly derivatives of Cu, Hg, Pb, Mn, and Zn. Pesticides (herbicides, insecticides, fungicides, rodenticides etc) which are widely used in high production agriculture for the control of insect and disease in fruit, vegetables and other crops, may contain several kilograms of certain elements. Hirst et al. (1961) reported concentrations of 1500 ppm Cu in apple orchards soils. Metal pollution through irrigation is one of the aspects of secondary salinization that is considered a major problem in many parts of the word.

A large number of micronutrients are present in the waste of animals. The variability of elements relate to the class of animal, age of animal, type of ration, type of housing and also waste management practices (Adriano, 1986). Terman (1978) and Page et al. (1979) reviewed coal residues and its characterization, potential for utilization, and potential hazards to plants and animals. They proposed that concentrations of micronutrients in coal residues are extremely variable and depend on the composition of the parent coal, conditions during coal cumbustion, efficiency of emission control devices, storage and handling of the product and climate.

Municipal refuse such as paper, glass, ceramics, metals and food wastes can also enhance trace metal contamination in soils. Purves and Mackenzie (1973) reported that boron may be a problem micronutrient in refuse composts.

Metal contamination of soils near industrial facilities is not as widely documented as water contamination. However, various industries are the sources of metal enhancement in surrounding surface soils (Mattigod and Page, 1983). Soderlund et al. (1988) found that mining and industrial activities along the river Dalalven are main sources of Zn and several other metals. Quevenviller et al. (1989) concluded that the distribution of heavy metal in sediments in the drainage basin and estuary of the Sado river (Portugal) was thought to be in inorganic forms.

Soil, plants and residents along roads with heavy traffic loads are subjected to increasing levels of contamination with heavy metals (Harrison et al., 1981). Yassoglou et al. (1987) reported that the main source of contamination was automobile exhausts. Roadside soils were also enriched with air borne Cd and Zn but to a lesser degree than with Pb. Concentration of Pb in soil increased near the highways. Emission of Pb compounds by vehicles that use fuels with Pb additives is the source of such contamination. Nriagu (1978) showed that Pb concentrations of soils near highways are high and decrease gradually with the increase of distance from the highways. He also concluded that other modes of transportation such as trains and aircraft may also contribute towards Pb contamination of soils.

High concentration of dissolved metal in geothermal brines are potential sources of metal contamination of soils. White (1968) reported that brines are mainly chloride brines of Na and Ca. Some of these metal-rich brines are ore-forming fluids that contain very high concentrations of Fe, Mn, Pb, Zn, Ba, and B. Contamination with brines can be expected to increase heavy metal concentration in surrounding soils.

Major sources of radionuclides are nuclear weapons testing, nuclear reactors and allied industries. Several of these artificial radionuclides of metals, estimated cumulative releases from major sources and detected activity in soils.

1.3. Pools of micronutrients in soil.

Metal cations in the soil may be present in several different physiochemical forms such as (1) Simple or complex ions in soil solution (2) Easily exchangeable ions (3) Organically bound (4) Occluded or co-precipitated with metal oxides, carbonates or phosphates and other secondary minerals or (5) As ions in a crystal lattice of primary minerals (Viets, 1962; McLaren and Crawford, 1973; Pickering, 1981; Soon and Bates, 1982; Adriano, 1986; Beckett, 1989 and Shuman, 1991).

1.3.1 Micronutrients as simple or complex ions in soil solution.

The soil solution is the medium for many of the chemical and biological interactions which allow it to support the growth of plants under a variety of physical, chemical and environmental conditions but this control is substantially modified by complex interactions involving exchange surface adsorption, organic matter, microbial activity, nutrient uptake by plants and release of organic substances from both living and dead plant roots.

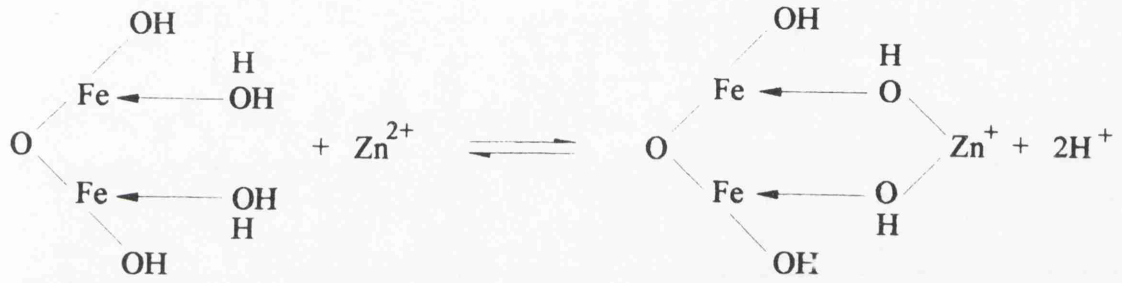
Soil moisture, pH, temperature, oxidation/reduction status, fertilizer additions and plant uptake influence the concentration of micronutrients in soil solution. Soil water content is very important because under wet conditions the elements can move by diffusion and under gravitational influence. As the soil dries, the ions in solution become more concentrated and can precipitate or be adsorbed. Lindsay (1979) defined the soil solution as the central focus of soil chemistry and from this medium the plant absorbs nutrients. It is the mobile phase that is responsible for redistribution of solids within the soil. Soil solution provides the immediate source of nutrient for plant and microorganisms and acts as a temporary sink for some of their waste products (Adam and Odom, 1985). The mobility of a particular element is an extremely complicated question in soil, because the ion may exist in different valency or hydroxy-ion forms, each of which is also in equilibrium with its complexes in solution and sorbed forms on the various soil organic and inorganic constituents (Tinker, 1985). Sandar (1982) stated that the total concentration of copper in the soil solution often varies little with pH, provided the organic matter in the solution remains constant, even though the concentration of the free Cu^{++} ions varies widely. In general, about 25% of the Zn, 90% of the Mn and more than 90% of the Cu were complexed in solution (Hodgson et al., 1965 and 1966). Concentrations of Fe in soil solution have been reported from 0.4 to 5 µM (Oliver and Barber, 1966). Linehan et al. (1989) stated that the concentrations of Co, Cu, Zn, and Mn in soil solution were $< 0.02 < 0.015 < 0.05$ and 0.18 µM, respectively. They also showed the low concentration of Cu, Mn, Zn and Co in soil solution in late winter with increase in spring and early summer. The micronutrients in soil solution are generally too low for the total quantity present at any one time to sustain plant growth. The micronutrients are rapidly replenished from the solid phase to provide sufficient for plant growth.

1.3.2 Easily exchangeable and surface adsorbed micronutrients.

Heavy metal cations in the soil solution are readily adsorbed on the surface exchange sites of clay minerals. Mitchell (1964) gave the approximate order of difficulty of displacement from clays as Cu > Pb > Ni > Co > Zn > Ba > Rb > Sr > Ca > Mg > Na. However, the relative position in this series may be altered by the concentration of the ions in solution, the nature of the exchanger and the effect of complexing agents in solution. In addition to being adsorbed as exchangeable ions, heavy metals are also sorbed in a non-exchangeable manner. This type of sorption has been termed as specific sorption. Tiller and Hodgson (1962) demonstrated two types of specifically sorbed Co in studies on montmorillonite. One form was exchangeable with other Co ions or ions of other heavy metals while the other form, present in small amounts, was not exchangeable. Jenne (1968) described that in many situations the concentration of ions in natural water including the soil solution is controlled by adsorption on the surface of oxides, particularly those of Fe and Mn. McLaren and Crawford (1973) reported that the amount of copper held on these sites decreased with increased pH. Quantities of manganese and iron held on these sites were greatly affected by the pH and redox potential (Sims and Patrick, 1978). The amount of zinc held on exchangeable sites was inversely related to pH, but positively correlated with cation exchange capacity of the soil (John, 1974). Soon and Bates (1982) showed the increase in exchangeable Cd, Ni, and Zn with a decrease of pH. Goldberg and Smith (1984) found less than 3% of the total manganese in the water soluble plus exchangeable form in selected U.K. soils. Bowman et. al. (1981) showed how the presence of soluble complex-forming ligand in solution can alter the whole adsorption isotherm of Ni, which behaves in a similar way to Mn and Zn. Sorption of the micronutrients can involve both simple cation exchange, with purely electrostatic bonds for ions such as Zn^{++} or Mn^{++} and ligand exchange, where a coordinated water molecule is exchanged for a H_2O or OH group on the solid surface, with liberation of proton.

$\text{Surface}^{-OH} + M(H_2O)_6^{2+} \rightarrow \text{Surface-OM}(H_2O)_5^{+} + H^{+} + H_2O.$

Adsorption of this type accordingly increases with pH. The removal of the hydrolysis reaction products by the surface causes displacement of the solution equilibrium and appreciable amounts of the metal ion in the hydrolysed form are adsorbed. The adsorption of Zn (II) at an oxide surface may be represented as a bridging ligand between two neutral sites.



In above scheme an olation bridge and ring structure are postulated to form. Such adsorption can be viewed as a growth or an extension of the surface and its properties would accord with the lack of complete reversibility (Quirk and Posner, 1975). Exchangeable cations are isotopically exchangeable and may also be displaced by the other cations already present in the soil solution (Beckett, 1989). Covalant bonds have an important control on the amount of metal sorbed by Fe and Mn oxides (McBride, 1989 and Stahl and James, 1991a,b). McBride (1989) thought that sorption of heavy metal on these oxides occurs via formation of covalent bonds, resulting in decreased bioavailability. Stahl and James (1991) reported from their study that Zn was retained in an exchangeable form at low pH but became nonexchangeable as pH increased in soil materials. The increase in pH primarily resulted in a redistribution of the Zn from an exchangeable to a nonexchangeable form.

Shuman (1991) reported that micronutrients are present in solution as charged ions and these ions are attracted towards the charged surfaces of the collids. He concluded that exchangeable and adsorbed ions are grouped with each other and are extracted together. However, some of them adsorbed weakly (non specific sites) and some are strongly held ions (specific sites).

1.3.3 Micronutrients associated with organic matter.

Micronutrient cations are strongly bound by the soil organic matter. Research into the character of bonding that takes place between soil organic matter and various micronutrient elements has been well described by Hodgson (1963); Lindsay (1974) and Stevenson and Ardakani (1972).

Ionisation of the carboxylic acid groups on humic surfaces will lead to the electrostatic bonding of metals as exchangeable ions. However, organic matter can also adsorb metal ions in a less readily exchangeable form. Fixation is generally attributed to complexing or chelation of metals by humic compounds. Most of the studies reviewed are interpreted as indicating that heavy metal ions and hydroxymetal ions react with carboxylic acid and phenolic hydroxyl group on the humus surface and are bound as chelates at those positions where the ligands are suitably oriented. The nature of the ligands and their relative configurations determine the strength of bonding. Ennis (1962) suggested that thiol groups may be important for the binding of Cu.

The ability of soil organic matter to form complexes with metals has been questioned by a number of workers who proposed that binding is purely electrostatic and fixation of ions is a result of changes in the configuration of the humus molecules following neutralization of internal coulombic forces by the sorbed metal. Matsuda and Ikuta (1969) found that the percentage of organically complexed Zn was higher in humic soil. They observed that complexation increased with humification of organic matter.

Most of the humified organic material in soils is associated with clay minerals. Heavy metals have been suggested as being involved with these linkages, acting as bridging ions to form clay-metal organic complexes. The bonding of metal ions to the colloidal organic fraction of soil has the effect of removing these ions from solution. However, smaller organic compounds could also have the opposite effect and may be important in maintaining the soluble metal concentration in soil solution. McLaren and Crawford (1973) found that there was a considerable variation in the proportion of total Cu present in various fractions, however, 30% of the total Cu was associated with organic matter.

Some soil organic matter will be water soluble and could be flocculated or precipitated by complexing cations like calcium or trace metals such as copper. Organic matter is closely associated with iron or aluminium oxides (Smith and Mitchell, 1984). Singh et al. (1988) reported that concentrations of Cu, Fe, Mn and Zn associated with organic matter fraction was very low in the calcarious soils of India. Liang et al. (1991) found that 7.9% of the total copper is associated with organic fractions in Saskatchewan soils.

1.3.4 Micronutrients associated with oxide materials and carbonates.

Manganese, iron and aluminium oxides are abundant hydrous oxides and are present in most soils. Taylor and Mackenzie (1966) showed that most of the Co in soils is associated with Mn oxides. Cobalt is adsorbed much more strongly on the Mn than the Fe oxides. The Fe oxide may hold the Co only in the absence of Mn oxides. In soil solution many competing ions will be present and perhaps some of the other ions prevent the adsorption of Co by Fe oxides.

Stanton and Burger (1967) found from their study that OH^- and HPO_4^{2-} are the most important mechanisms for adsorption of Zn in ferric and aluminium oxides. Jenne (1968) concluded that the principal processes of metal adsorption are the occlusion and coprecipitation of micronutrients with oxides of Fe and Mn. Jenne (1977) and McLaren et al. (1981) concluded that the oxides of Fe, Mn and organic matter are the most important constituents. They proposed the adsorption maxima among different soil as follows Mn oxide > organic matter > Fe oxide > clay minerals. These oxides have the great ability to absorb other micronutrients. Sposito (1983) reported that micronutrients associated with oxides by adsorption, surface complex formation, ion exchange, penetration of the crystal lattice and coprecipitation. Most of the Zn is associated with the oxides of Fe and Mn in heavily polluted samples (Hickey and Kittrick, 1984). Goldberg and Smith (1984) reported that 75% of the total indigineous soil Mn in the selected U.K. soils occurred in oxide bound fractions.

Stahl and James (1991a,b) stated that mineralogy of Fe and Mn oxides affects the distribution of exchangeable and non exchangeable forms of Zn. They concluded that oxyhydroxides retained Zn in a predominantly non exchangeable form, while oxides retained Zn in both exchangeable and non exchangeable forms at all pHs (3-7). The same authors Stahl and James (1991ab), reported that oxides and oxyhydroxides of Fe, Mn and Al also have the sorption sites and from these sites they allow the non exchangeable sorption of metals.

1.3.5 Micronutrients associated with primary and secondary minerals.

Those minerals which are formed directly from the parent rock material through the process of physical weathering are called primary minerals. They have not been subjected to either geochemical or pedochemical weathering and they tend to be found in the larger particle size fraction of soils, which are less reactive chemically because of their smaller specific surface area. With the proceeding of chemical weathering, the primary minerals dissolve and their elemental components subsequently can precipitate as secondary minerals, form complexes with inorganic surfaces or remain temporarily in the soil solution. Shuman (1985) defined from his study that majority of the trace metals are present in the crystal structures of highly resistant primary and secondary minerals, which comprise the residual fraction after all other extractants have been employed. Sposito (1983) quoted the principal modes by which several environmentally important trace metals have been found to occur in primary and secondary soil minerals which are given below

A) Modes of occurence of transition and heavy metals in primary soil minerals.

Vanadium

Isomorphous substitution for Fe in pyroxenes and amphiboles and for Al in micas; substitution for Fe in oxides.

Chromium

($FeCr_2O_4$) isomorphous substitution for Fe or Al in other minerals of the spinel group.

Cobalt

Isomorphous substitution for Mn in oxides and for Fe in pyroxenes, amphiboles and micas.

Nickel

Sulphide inclusions in silicate; isomorphous substitution for Fe in olivines, pyroxenes, amphiboles, micas and spinels.

Copper

Sulphide inclusions in silicates; isomorphous substitution for Fe and Mg in olivines, pyroxenes, amphiboles, and micas and for Ca, K or Na in Feldspars.

Zinc

Sulphide inclusions in silicates; isomorphous for Mg and Fe in olivines, pyroxenes, and amphiboles, and for Fe and Mn in oxides.

Cadmium

Sulphide inclusions and isomorphous substitution for Cu, Zn, Hg and Pb in sulphides.

Molybdenum

Molybdenite (MoS_2) isomorphous substitution for Fe in oxides.

Lead

Sulphide and phosphate inclusions; isomorphous substitution for K in feldspars and micas, for Ca in feldspars, pyroxenes and phosphates and for Fe and Mn in oxides.

B) Coprecipitated trace metals in secondary soil minerals.

1) Fe oxides	V, Mn, Ni, Cu, Zn, Mo
2) Mn oxides	Fe, Co, Ni, Zn, Pb
3) Ca carbonates	V, Mn, Fe, Co, Cd
4) Illites	V, Ni, Co, Cr, Zn, Cu, Pb

5) Smectites	Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Pb
6) Vermiculites	Ti, Mn, Fe

Beckett (1989) concluded from his study that new precipitates of carbonates, sulphides, phosphates and hydroxides contain more than one trace metal and often form mixed crystals with the corresponding salts of major elements, usually calcium or iron. Shuman (1991) divided these minerals into two types (1) sulphides of Zn, Cu, and Fe and (2) carbonates of Zn, Cu, Mn and Fe. He concluded that these are less resistant to extraction and easily separated or dissolve with one of the other fractions. Most of the micronutrients in this class are associated with silicate minerals in isomorphous substitution sites with in primary or secondary minerals.

1.4 Selective extraction

Developing extraction methods to estimate the availability of plant nutrients has not been easy. Extraction of soil should measure the portion of the solid phase that is controlling the availability of micronutrients and it should also characterize how readily the solid phase would dissolve (Cox and Kamprath, 1972). It should extract nutrients from the same labile pool that the plant does. It cannot extract exactly the same quantity of nutrients as the plant does, but the extracted amount must relate to that taken up by the plants (Lindsay and Cox, 1985). Campbell et al. (1988) proposed that these methods are not specific but are only selective, and are employed to study the distribution among principal components. Different extracting methods have been reviewed by (Cox and Kamprath, 1972; Viets and Lindsay, 1973; Beckett, 1989 and Shuman, 1991).

The succeeding section will describe the most frequently used procedures for selective fractionation. Some recent work on the extracting methods of Co, Cu, Ni, Zn, Mn and Fe is discussed with particular emphasis on their usefulness in the study of micronutrients in the soil.

1.4.1 Water soluble fraction.

Water soluble fraction is the first fraction to be dissolved. Several workers have measured a water soluble fraction of the micronutrients by shaking soil with water, usually distilled or deionized water (Viets, 1962; Sims and Patrick, 1978; Iyengar et al., 1981; Miller et al., 1986a,b; Beckett, 1989 and Shuman, 1991). Sims and Patrick (1978) divided the water soluble fraction into free ions and ions complexed by organic matter by extracting the former with cation and anion exchange resins. Haq et al. (1980) reported that water soluble Zn in a contaminated soil ranged from 0.00 to 3.38 mg kg^{-1}. Sposito et al. (1982) used very pure distilled and deionized water as an extractant for non specifically "sorbed" or "adsorbed" ions, after removing exchangeable ions with 0.5M KNO_3. Lund et al. (1985) demonstrated that H_2O reagent successfully removed cadmium, nickel, and zinc recently adsorbed on to freshly prepared hydrous iron oxides. Miller et al.(1986) conducted a study on fresh freeze dried and dried samples and found that water soluble Cu increased from 2% to 30% with increasing severity of drying. They concluded from their study that H_2O is more effective than calcium and lead salts for the extraction of Cu. Levesque and Mathur (1986) showed that on an average 0.85% of the soil copper was water soluble. They observed that water soluble copper had the best and most frequent correlation with other forms. Singh (1986) found that water soluble plus exchangeable fraction affected the availability of plant zinc in three Saskatchewan soils. Bakhsh (1988) reported smaller amounts of zinc extracted with water, ranging from 0.00 to 0.33 mg kg^{-1} and Mathur and Levesque (1988) reported that on an average, 0.2%, 0.5%, and 1.9% of the total Fe, Mn and Zn were water soluble. Abdel-Sabour et al. (1988) used deionized water for the removal of water soluble cadmium and Zn. According to Levesque and Mathur (1988) Cu, Fe, Mn and Zn in cultivated organic soils were considered to be mainly water soluble. Shuman (1991) concluded from his study that concentration of metal in the water soluble pool is very low and cannot be detected by analytical methods. He also concluded that significant amounts of metal may be extracted only at the time of recently applied micronutrient fertilizer or sewage sludge.

1.4.2 Extraction of exchangeable fraction.

Majority of the exchangeable metals are held on specific sites with high affinity for the metal and are replaced by an excess of cations, such as Ca^{2+}, Mg^{2+} and NH_4^+. Divalent ions have greater replacing power than the monovalent ions but monovalent ions sometime replace ions held between the clay mineral layers. Anions mostly used for exchangeable salts are Cl^-, NO_3^- and CH_3COO^- (OAC^-). Many workers estimated exchangeable or neutral salt exchangeable ions of micronutrients from soil by extraction with relatively strong solutions of the chlorides or nitrates of potassium, magnesium and calcium. Separation of various forms of metal in soil has been useful in studying the retention of metals by the soil and their release to the plant. Different workers have used different types of extractants depending on the ability to extract different types of metal from a wide range of soils. Amounts of micronutrients extracted from the exchangeable fraction are most closely related to plant uptake. Stewart and Berger (1965) found that 1M $MgCl_2$ solution was a suitable predictor of zinc uptake. Zinc concentration in corn and oats grown on different samples were best correlated with amounts extracted by 1M $MgCl_2$, acidic CH_3COONH_4 and 1M KCl (John, 1974). Khokhlova (1967) used a KCl extractant for available Zn and Cu, and persulfate method for available Mn to establish distribution patterns of these micronutrients. Exchangeable Cu and Zn have been extracted with 0.05M $CaCl_2$ (McLaren and Crawford, 1973a and Elsokkary, 1979).

Shuman (1979), (1985) used 0.5M $Ca(NO_3)_2$ and $Mg(NO_3)_2$ for the removal of exchangeable ions. Selvarajah et al. (1982) used 1M CH_3COONH_4 (pH 4.6), 0.01M $CaCl_2$ and 0.2M $MgSO_4$ for the extraction of exchangeable Zn and Cu. They concluded that more Zn was extracted by the ammonium acetate (pH 4.6) than the other two salts. This may be due to its acidic nature which made it able to extract some precipitated forms of zinc in paddy soils. Iyenger et al. (1981) used 0.05M $CaCl_2$, while McLaren et al. (1986) used 0.5M $CaCl_2$ for the extraction of exchangeable micronutrients. Gibbs (1973, 1977) used 0.5M $MgCl_2$ for the extraction of exchangeable metals. Soon and Bates (1982); and Mandal and Mandal (1986) used 1M CH_3COONH_3 for the extraction of exchangeable Cd, Zn and Ni. Neilsen et al. (1986) reported that 1M $MgCl_2$ extracted more Zn than M CH_3COONH_4 (pH 7.00).

Jopony (1985) used 0.5M KNO_3 to remove soluble plus exchangeable forms of Cu, Zn, Ni and Co in the soil and sediment samples of Lohan area in Ranau Sabah (India) and found that exchangeable plus soluble fraction was very low. Miller et al. (1986) used 0.025M $Ca(NO_3)_2$, 0.5M $Ca(NO_3)_2$, 0.05M $Pb(NO_3)_2$ and 0.44M CH_3COOH for the extraction of exchangeable and specifically bound trace metals and found that CH_3COOH extracted more Cu, Mn and Fe than the $Pb(NO_3)_2$. Leavesque and Mathur (1986) reported that on an average 0.67% of the soil Cu was extracted with $CaCl_2$.

The selection of reagent for extracting a given form of the metal from the soil and the sequence of extraction affect not only the partitioning of metals in different fractions, but also the total amount of metals in each fraction. For example, $MgCl_2$ or $Mg(NO_3)_2$ has been preferred over NH_4OAC for extracting exchangeable metals, as the latter dissolve oxides coatings and may attack carbonates (Shuman, 1985). Singh et al. (1988) used 1M $Mg(NO_3)_2$ for the removal of exchangeable Cu, Fe, Mn and Zn and found an average of 2% of total Cu and Zn while exchangeable Fe and Mn were non significant, averaging < 0.1% of the total Fe and Mn. They observed that exchangeable metal did not show significant correlation with any soil properties except exchangeable Zn and free Fe. Abdel-Sabour et al. (1988) used 0.5M KNO_3 to extract exchangeable Cd and Zn. Mathur and Levesques (1988) applied $CaCl_2$ for the removal of exchangeable Cu, Fe, Mn and Zn and found an average of 0.1%, 6.5% and 4.5% of Fe, Mn and Zn in Ca exchangeable fraction, respectively.

Nielsen et al (1990) used 0.02M $CaCl_2$ for extraction of exchangeable Mn and found minimum amount of Mn in this fraction. Exchangeable Zn in prairie soils were reported from 0.2 to 2.4% of the total Zn. Recently, Liang et al. (1991a) reported that 0.5M $Ca(NO_3)_2$ extracted an average of 2.2% of the exchangeable Cu in 27 prairie soils and more recently, Mandal et al. (1992) used 1M $Mg(NO_3)_2$ pH 7.0 to dissolve water soluble plus exchangeable fraction of Zn.

1.4.3 Extraction of sorbed fraction.

Metals on specifically sorbed sites are not exchangeable by salt extracts. This fraction has a high correlation with plant uptake. Several schemes have been devised to remove the metal from this fraction. Himes and Barber (1957) concluded that copper acetate extracted more zinc than 2M KCl. McLaren and Crawford (1973) suggested that smaller amounts of exchangeable Cu in soil solution are in equilibrium with specifically absorbed forms constituting the bulk of the "available copper reserves". John (1974) extracted more "plant available" zinc with Morgans solution (7% sodium acetate and 3% acetic acid, pH 4.8) than acidic ammonium acetate. Acetic acid has been successfully used as an extractant to estimate the specifically adsorbed forms of Co, Zn, Ni and Pb (Berrow and Mitchell, 1980). In a cool temperature climate such as that in Britain arable top soils are generally acid and often require liming to about pH 6-6.5 for good crop growth. Thus for many soils in Britain acetic acid has been found to be a suitable extractant for available cobalt, nickel and zinc. Stover et al. (1976) reported that 2.5% acetic acid dissolved zinc and copper carbonate and a little lead carbonate or zinc and copper sulphides, but it could not dissolve cadmium carbonate. Iu et al. (1981) reported that under waterlogging conditions acetic acid extractable Cu and Zn increased with time, possibly because specific inorganic adsorption sites preferentially took up Zn and Cu at the expense of the organic sites. Selverajah et al. (1982) used 0.05M HCl for the extraction of specific absorbed Zn and Cu. Reaves and Berrow (1984a) gave the mean acetic acid extractable lead content as 0.24 $\mu g\ g^{-1}$ in Scottish soils, with a range of 0.016-3.4 $\mu g\ g^{-1}$. Jarvis (1986) and McLaren et al. (1986) reported that 0.5M acetic acid extractable soil cobalt was correlated with the total iron contents of the same soils but not with their total manganese. The availability of plant nutrients in soil is measured by acetic acid alone or buffered by acetate salt (Iyengar et al., 1981; Miller et al., 1986b and Rappaport et al., 1986) used 0.5M acetic acid to extract "specifically bound" or "specifically adsorbed" metals. Miller et al. (1986) used somewhat more complex fractionation to determine the distribution of trace metals in contaminated soils, based on the ability of certain solvents to remove specifically bound forms of the metal and they concluded that $Pb(NO_3)_2$ extracted lesser amount of metals than CH_3COOH and was probably more specific in replacing metals covalently bound to adsorption sites. 0.3M $LaCl_3$ extracted more Al from soils than 1M KCl (Jarvis, 1986). Jin et al. (1987) measured specifically sorbed B with mannitol. Bakhsh (1988) reported from his study that acetic acid extracted significantly higher amounts of Zn than the modified Morgan's solution from all the soils studied. While, Shuman (1991) reported that mobility of metals in this fraction was very low.

1.4.4 Extraction of organic fraction.

In this fraction metals are released by the oxidation of organic matter. Selecting the oxidation procedure in this fraction is very difficult because it often dissolves other fractions as well. Shuman (1979) used sodium hypochloride (NaOCl) pH 8.5 to oxidize the organic fraction Mn, Cu, Fe and Zn instead of H_2O_2. Singh et al. (1988) used 0.7M NaOCl (pH 8.5) for the extraction of organically bound Cu, Fe, Mn and Zn in the calcarious soils of India and found that the amount of metal associated with organic fraction was very low, generally < 1.0% of the total soil Cu, Fe, Mn and Zn. More recently Mandal et al. (1992) used 0.7M NaOCl pH 8.5 to dissolve organically complexed Zn.

Different workers used different extractants for the removal of organically bound micronutrients. Smith and Mitchell (1984) observed that 0.47M hot sodium carbonate dissolved gibbsite, allophane and poorly ordered aluminosilicates and they also reported that cold 0.47M Na_2CO_3 removed only the aluminosilicates. However, Beckett (1989) concluded that sodium carbonate extracted a smaller amount of metals than sodium hydroxide. Lund et al. (1985) used 0.5M NaOH to mobilize organically bound micronutrients and observed that it also dissolved Pb, Zn carbonates and small proportion of Cd, Cu and Ni carbonates. Miller et al. (1986) extracted more copper from pig manure with NaOH than with 0.1M pyrophosphate.

Lindsay and Norvell (1978) are the first investigators to suggest the use of DTPA (diethylenetriamine penta acetic acid), for the simultaneous determination of Zn, Fe, Mn, and Cu. The extracting solution was 0.005M DTPA, 0.01M $CaCl_2$ and 0.1M triethanolamine, adjusted to pH 7.30. This method gave a good separation of Zn and Fe deficient soils as determined in the greenhouse with corn and sorghum. Hickey and Kittrick (1984) showed that DTPA-Zn is correlated with pyrophosphate Zn and assumed that DTPA extracts organically bound zinc. Mathur and Levesque (1988) applied DTPA to dissolve strongly complexed Cu, Fe, Mn, and Zn.

The ethylenediamine tetra acetic acid (EDTA) method, developed by Viro (1955) has been used widely. Gajbhiye et al. (1984) used 0.02M EDTA for the evalution of available Fe, Mn, Zn and Cu in soil. Emmerich et al. (1982) and Sposito et al. (1982) used 0.05M Na_2-EDTA and 0.5M NaOH for the removal of organically bound trace metals. McLaren et al. (1986a) used 0.4M di-sodium EDTA for the extraction of organically bound Co. Burridge and Hewitt (1987) and Archer and Hodgson (1987) used 0.05M EDTA to determine the extractable Cd, Co, Cu, Ni, Pb, Se, Zn and Mn. Bakhsh (1988) used 0.05M NH_4EDTA for the removal of organically bound Zn. Cadmium, copper, nickel and zinc were extracted with 0.05M Na_2-EDTA (Aller and Deban, 1989).

Bascomb (1968) was the first investigator to suggest the use of pyrophosphate ($K_4P_2O_7$) to extract the organically bound micronutrients. McLaren and Crawford (1973) indicated that the bulk of "available" Cu reserves in the organic fraction, were extracted by 0.1M $K_4P_2O_7$. Pyrophosphate dissolves "free" oxide associated with organic collids, particularly of iron and aluminium but less "free" iron from inorganic materials (Rendell et al., 1980). Jeanroy et al. (1986b) reported that pyrophosphate dissolves less iron that acid ammonium oxalate. They assume that it dissolved "hydroxyferric-organic complexes" but it did not dissolve inorganic iron compounds. Miller et al. (1986) stated that pyrophosphate mobilized more organic matter than H_2O_2 or NaOCl and its efficiency increased with pH. Niskanen (1989a,b) used 0.1M $Na_4P_2O_7$ and 0.05M $K_4P_2O_7$ to mobilize organically bound Al, Fe and Mn. He concluded that pyrophosphate extractable aluminium closely correlated with EDTA and acetate extractable aluminium. Liang et al. (1991) used 0.1M $K_4P_2O_7$ for the estimation of organically bound copper in Prairie soils and found that 7.9% of the total soil Cu was present in this fraction.

Hamblin and Posner (1979) used acetyl acetone for the extraction of organically bound micronutrients in the soil. Mandal and Mandal (1987a,b) used copper acetate to remove metals strongly held on organic sites.

1.4.5 Extraction of oxide, carbonate and sulphide fractions

Trace metals associated with oxides or held by oxide materials are solubilized by a variety of extractants. Different workers used different types of extractant to dissolve the trace elements from this fraction. Chao (1972) used 0.1M $NH_2OH.HCl$ (pH 1) (hydroxylamine hydrochloride in 0.01M HNO_3) to dissolve most of the Mn, but only few percent of the Fe, in various soils. While 0.1M $NH_2OH.HCl$ (pH 2) (hydroxylamine hydrochloride in 0.01M HNO_3) dissolved somewhat less manganese and very little iron. Chao and Zhou (1983) evaluated 0.25M $NH_2OH.HCl$ in 0.25M HCl at 70°C to dissolve substantial amounts of amorphous iron oxides and significant amounts of crystalline oxides. Miller et al. (1986b) reported that 0.01M $NH_2OH.HCl$ in 0.1M HNO_3 dissolves reducible soil manganese. Mn oxide, amorphous Fe oxides, and crystalline Fe oxides fraction of Cu, Fe, Mn and Zn were dissolved with 0.1M $NH_2OH.HCl$ (pH2, HNO_3), 0.25M $NH_2OH.HCl$+0.25M HCl and 0.2M $(NH_4)_2C_2O_4$+0.2M $H_2C_2O_4$ (pH3) + 0.1M ascorbic acid (Singh et al., 1988). Xian (1989) used 0.04M $NH_2OH.HCl$ in 25% acetic acid (v/v) to dissolve Mn and Fe oxide fraction of Cd, Zn, and Pb in polluted soils. Liang et al. (1991b) used $NH_2OH.HCl$ + 0.1M HNO_3 to dissolve the Mn oxide occluded Zn in Saskatchewan soils and found that most of the Zn was recovered in the Mn oxide fractions after a 7-days incubation. And more recently Mandal et al. (1992) used 0.1M $NH_2OH.HCl$ (pH2.0), 0.2M $(NH_4)_2C_2O_4$ + 0.2M $H_2C_2O_4$ (pH3.0) and 0.2M $(NH_4)_2C_2O_4$ + 0.2M $H_2C_2O_4$ + 0.1M ascorbic acid to dissolved the Mn oxides bound amorphous Fe oxide bound and crystalline Fe oxides bound Zn, respectively.

Gajbhiye (1984) used 0.1N HCl for the estimation of available Cu, Fe, Mn and Zn in soil. Tiwari and Mohankumar (1982) concluded that 0.1N HCl extracted copper showed significant relationship with plant uptake. Mathur and Levesque (1988) used 0.1M HCl and aqueous KCN to dissolve carbonates and sulphides fraction of the Cu, Fe, Mn and Zn and found that an average of 4% and 3% of the total iron was associated with sulphide and carbonates fraction and 0.45 and 0.9% of the total Mn was associated in sulphide and carbonates fraction. Aller and Deban (1989) reported an average of 5.5 to 7.3 $\mu g\ g^{-1}$ and 0.010 to 0.027 $\mu g\ g^{-1}$ of mercury extracted by using 0.1N HCl and 0.5N HCl from the agricultural soils of the Valderas area, Spain. Soon and Abboud (1990) reported that oxide bound Cd, Cr, Cu, Mn, Ni, Pb and Zn were dissolved with 0.1M HCl.

McLaren and Crawford (1973a) used acid oxalate (oxalic acid 0.1M, and ammonium oxalate 0.175M, pH 3.25) for the removal of copper occluded by free oxides. Hydrous iron oxide bound micronutrients were removed with the solution of 0.2M ammonium oxalate and 0.2M oxalic acid (pH 3) (Shuman, 1979). Jarvis (1984) used acid ammonium oxalate to extract manganese in some acidic soils. McNeal et al. (1985) used hydroquinone, oxalate (0.2M ammonium oxalate) and 0.1M HCl to dissolve trace elements occluded with Mn oxides, Fe oxides and carbonate minerals. Nielson et al. (1986) used 0.2M ammonium oxalate and 0.2M oxalic acid pH 3.0 for the removal of iron and aluminium oxide associated Zn and found 1.3 to 15% of the total Zn in this fraction in orchard soils. Miller et al. (1986) used 0.175M $(NH_4)_2C_2O_4$ + 0.1M $H_2C_2O_4$ (oxalate reagent) in the dark and oxalate reagent at 85°C under ultraviolet irradiation to dissolve amorphous Fe oxide and crystalline Fe oxide fraction of the micronutrients. Archer and Hodgson (1987) extracted Ni and Mo with ammonium oxalate. Wang et al. (1987) used hydroxylamine and acid ammonium oxalate solution for the extraction of Al, Fe and Si and found that acid oxalate extracted more Al and Fe and slightly less Si than the hydroxylamine. Bakhsh (1988) used acid oxalate (0.1M oxalic acid, 0.175M ammonium oxalate pH 3.25) (Tamm's solution) to dissolve the zinc associated with oxide fraction and found that 11.32% to 38.69% of the total Zn was associated in this fraction. Liang et al. (1991a) used 0.113M ammonium oxalate + 0.087M oxalic acid in darkness for the measurement of Fe and Al oxide bound copper and found that 11.1% of the total Cu were present in Fe and Al oxide fraction in Prairie soils.

1.4.6 Extraction of total or residual fraction.

Total amount of micronutrients is of little value in predicting the sufficiency or deficiency except where the total content is very low (Viets, 1962). The availability of micronutrients does not only depend upon their total content but on their chemistry as well. The total concentrations can give a good guide to determine the percentage of the total amount in each individual pool of trace metals in soils. The 'residual' trace metal remaining from the previous extraction or the total quantity of micronutrients have been estimated by the digestion in strong acids.

McLaren and Crawford (1973) used hydrofluoric acid and perchloric acid for the digestion of Cu from soils. Mills and Zevarich (1975) used nitric acid and perchloric acid to digest the soil for the measurement of total quantity of Cd, Cu, Ni and Zn. Aitang and Hani (1983) and

Jarvis (1984) treated the soil with HF and digested the residue with a mixture of HNO_3, $HClO_4$ and H_2SO_4 acids for the determination of total Mn in acidic soils. Nitric acid and perchloric acid were also used for the estimation of total Zn (Mandal and Mandal, 1986).

Archer and Hodgson (1987) used HNO_3 and $HClO_4$ for the measurement of total quantity of As, B, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se and Zn in the soils of England and Wales. Bakhsh (1988) used the mixture of H_2SO_4 and HNO_3 to dissolve the total quantity of Zn in soils. Singh et al.(1988) used HF, $HClO_4$ and HCl for the determination of total quantity of Cu, Fe, Mn and Zn. Total concentration of Fe, Mn and Zn were measured by digestion of the residue with HNO_3-$HClO_4$-HF (Mathur and Levesque, 1988). Aller and Deban (1989) digested the soil with perchloric acid, hydrofluoric acid and hydrochloric acid for the measurement of total concentration of Cd, Cr, Cu, Hg, Li, Ni, Pb, Sr and Zn.

Liang et al. (1991b) used HF, HNO_3 and $HClO_4$ to determine the residual fraction or total Zn in Saskatchewan soils. The same method of acid digestion was used by the same authors for the measurement of the residual fraction of the Cu in Prairie soils.

1.5 Factors affecting the availability of micronutrients in the soil to plants.

Plant availability of micronutrients is often reported to be associated with the distribution of micronutrients among certain soil fractions. Fractionation schemes used to study this relationship generally evaluate the role of solution, exchangeable, organically complexed and oxide bound forms. Variations in critical soil chemical parameters can induce shifts in these pools, which may result in a micronutrient deficiency or toxicity. Extractable levels of micronutrients in soil are known to be affected by different factors such as organic matter, pH, interaction with other elements, soil moisture and aeration, light, temperature and time.

1.5.1 Organic matter.

The amount of organic matter in soil has a great impact on many soil properties. The organic fraction generally contributes a major portion of the exchange capacity of a soil as well as acting to increase the concentration of ions in the soil solution through the action of low molecular weight, organic complexing agents. The colloidal organic fractions however, also fixes metals ions in forms unavailable to plants. Crops grown on organic rich soils are often found to be deficient in copper. These deficiencies are commonly attributed to the fixation of Cu in unavailable form by soil organic matter (Ennis and Brogen, 1961 and Ennis, 1962a).

The concentrations of soil copper associated with the occurrence of deficiencies in plants are also considerably higher for organic soils, though if account is taken of their lower bulk densities it is apparent that on a volume basis the satisfactory Cu levels for these and for mineral soils are more alike (Lucas and Knezek, 1972). Sanchez and Kamprath (1959) and Fujimoto and Sherman (1948) reported that adding organic matter to soil will increase the exchangeable Mn and manganese uptake by plants. In soils with high amounts of organic matter, Mn may form sufficiently stable complexes to compete with precipitation as the mechanism controlling Mn availability (Ellis and Knezek, 1972).

Waterlogging released Fe and Mn from the organic and oxide fractions and moved them to the soluble, exchangeable and inorganic forms (Iu et al., 1981a). In this experiment adding organic matter accelerated the distribution of the metals, but did not apparently alter the movement among forms. The affects of organic matter additions on Zn and Cu are usually less than those for Fe and Mn because they are not as sensitive to redox changes. However, both are subject to complexation by organic matter with waterlogging, zinc and copper increased in the amorphous Fe oxide fraction (Iu et al., 1981b).

Albasel and Cottonie (1985) indicated that increasing lime rate reduced the uptake of heavy metals, and adding chelating agents enhanced the uptake of heavy metals. Martini and Mutters (1985) found that extractable quantity of Fe, Cu and Zn decreased with the addition of lime. Soluble organic matter causes an increase in microbial substrate for microbial activity, and hence may be rapidly degraded, leaving the microelements to be precipitated or to be complexed by less soluble organic materials. Most microbiological effects on the availability of micronutrients involve the oxidation and reduction of Fe and Mn. The microbial decomposition of organic complexing agents that serve to stabilize reduced forms of Fe and Mn provides indirect means of promoting oxidation of these elements. Such decomposition can also serve to convert other elements to less available form.

Mandal and Mandal (1987a,b) reported that addition of organic matter increased organic and exchangeable Zn and decreased the oxide fraction Zn. Shuman (1988) reported that adding of organic matter mobilized the Zn to Mn and Fe oxide fractions from the other fractions. The same author, Shuman (1988b) reported that increasing organic matter caused Mn and Fe to move from the less soluble forms to more plant available forms. Adding of organic matter to soil will increase the applied Zn in all fraction except the crystalline Fe oxides and the residual fractions. McGrath et al. (1988) reported that Cu associated with organic fraction was higher in organic soils than the mineral soils.

1.5.2 Soil pH.

Soil pH is a major factor governing micronutrients availability in soils. Mitchell (1964) described the availability of soil zinc as being decreased as the pH is raised above 6, whereas copper is most available between pH 5 and 6. Lucas and Knezek (1972) stated that Cu availability is not appreciably increased until the soil pH is reduced below this lower value of pH 5. Christenson et al. (1950) reported 20 to 50 fold decrease in exchangeable Mn on raising a soil pH from 4.6 to 6.5. Hodgson (1963) observed that the amount of Cu, Zn and Co extracted with chemical extractants varied more with soil pH than the amounts removed by plants. This is due to an increased efficiency in the process of plant uptake at higher pH values. Plant uptake of zinc is considerably more sensitive to changes in the soil pH than is that of Cu. It is suggested that the behaviour of Cu is less affected by pH variations because of the strong complexing of Cu^{2+} ions by organic molecules in the soil solution (Hodgson et al., 1965). The solubility of both Cu^{2+} and Zn^{2+} ions is highly pH dependant and decreases with increasing pH. The solution concentration of Zn^{2+} for example decreases 100 fold for each unit increase in pH (Lindsay, 1972b).

McLaren et al. (1973) found increased Cu adsorption as pH increased, but in later work McLaren et al. (1981) using lower Cu levels found minimal changes in adsorption over pH range of 5 to 7. Zinc uptake by rice plants under both aerobic and anaerobic conditions progressively decreased as soil pH was raised from 5.0 to 8.0 (Jugsujinda and Patrick, 1977). They also found a sharp decrease in uptake under aerobic conditions at pH 7 to 8. Plant available Mn was high at low soil pH levels (<5.2)

(Sims, 1986). Jahirudin et al. (1986) reported that extractable Fe decreased with the increase of soil pH. Neilsen et al. (1986) found that Zn in exchangeable and organic fractions was negatively correlated with pH and recently Shuman (1986 and 1988) found that increasing pH decreased exchangeable and organic fraction Zn.

The adsorption sites on oxides and organic matter are pH dependant. Stahl and James (1991a,b) coated sands with the oxides of Fe and Mn for examining the importance of pH dependant charge on the oxides for the retention of Zn. Mineralogy of Fe and Mn oxides affected the distribution between exchangeable and non exchangeable forms of Zn. Oxyhydroxides retained Zn in a predominantly non-exchangeable form, while oxides retained Zn in both exchangeable and non-exchangeable forms at all pHs in the range of 3 to 7.

1.5.3 Interaction with other elements.

Plant uptake of ions of one element may influence other ions, either in the soil or in the plant itself. Interactions that occur in the soil can be generally regarded as affecting the solubility of the ions concerned. Whereas interactions in the plant may involve effects on uptake or translocation within the plant. All apparent interactions are not the result of one element directly influencing the behaviour of another and interpretation of observed effects must take into consideration possible indirect relationships. For example, increased plant growth resulting from the addition of a nutrient to the soil may be accompanied by decrease in the plant content of another element if the soil supply of this element is insufficient to satisfy the increased plant requirement. There would therefore, appear to be a relationship between these two elements in this situation if plant contents alone were considered although, in fact, no direct interaction would exist. Phosphorus appears to interact with micronutrients. There are frequent reports of deficiencies of Zn and Cu in citrus on soils containing high levels of available P, either naturally or from fertilizer applications (Lindsay, 1972a and Olsen, 1972). Added phosphorus shifted Cu from the residual to the exchangeable fractions, for four fine textured soils, presumably making it more plant available. It shifted Mn from the residual and crystalline Fe oxide to the Mn oxide and amorphous Fe oxide fraction, but had little effect on the more bioavailable exchangeable and organic-fraction Mn. Adding phosphorus to eight soils caused decreases in crystalline Fe oxide and residual Fe, and increases in amorphous Fe-oxide Fe (Shuman, 1988a). Basak et al. (1982) observed a significant decrease in Cu content in soils with Mo application. They also indicated an antagonism between Mn and Mo in soils.

1.5.4 Soil moisture and aeration.

Mitchell et al. (1957) and Swaine and Mitchell (1960) concluded from the study of a range of Scottish soils that the extractable trace element contents and the amounts taken up by plants were influenced to a considerable extent by the drainage status of the soil. Poor drainage conditions increased the extractable levels of several micronutrients. For several of these elements, particularly Ni and Co, plant uptake showed a corresponding increase for poorly drained soils. Nambiar and Cotenie (1971) investigated the uptake of Cu and Zn by maize and beans from soils at different moisture contents, though soils were not wetted sufficiently to become water logged. Increasing soil moisture level had no consistent effect on Cu or Zn contents of either plant though uptake was increased by the high soil moisture treatments. When Cu and Zn were added to the soil, uptake was again increased in both beans and maize. The copper content however, was only increased by the high moisture in maize and was highest in beans at the low moisture level. Estepp and Keefer (1969) reported that continuous moisture moved Zn into the inorganic fraction.

The development of anaerobic conditions cause the solution of Fe and Mn through reduction. Occluded micronutrients will be released on dissolution of Fe and ferromanganese minerals, although ions will be readsorbed and fixed on subsequent reoxidation. Although, poor aeration is usually the result of high moisture content, the composition of the soil atmosphere is also influenced by factors, such as microbial activity, temperature and the bulk density of the soil (Lucas and Knezek, 1972).

1.5.5 Light, temperature and time.

Temperature, light intensity and day length influence the uptake of ions by plants and several workers have stressed the growth and nutrient uptake studies (Hallsworth et al., 1957 and Bates, 1971). Zinc deficiencies are most prevalent early in the growing season and it is suggested that cold and wet spring conditions limit the availability of soil Zn by either delaying root development or limiting microbial activity and release of bound Zn (Lindsay, 1972a). The effect of temperature on the availability of Zn in soil was investigated by Bauer and Lindsay (1965) by incubating the soils at different temperatures for varying periods of time before seeding with maize. Yield and Zn uptake were increased in soils that had previously been incubated at 43°C for 1 to 3 weeks. Longer incubation periods reduced this effect. The addition of fertilizer Zn showed that in the lower temperature treatments, growth was limited by the supply of available Zn. Macmillan and Hamilton (1971) grew carrots in soils maintained at different temperatures and though the higher soil temperature was found to generally increase the Cu content of leaves, no similar effect was observed for Zn unless the element had been applied to the soil. Rufty et al. (1979) found that Zn from sewage sludge increase in the carbonate fraction with time.

Micronutrients have also been shown to respond differently to different lighting conditions. Millikan (1953) demonstrated that whereas Cu deficiency in both clover and lucerne was unaffected by light intensity, the severity of Zn deficiency was significantly less in plants under reduced light than in those receiving full light. Ozzanne (1955b) similarly demonstrated that clover showed an increasing response to added Zn with increasing light intensity up to a maximum, beyond which the response decreased. The duration of lighting was also important, with a longer period of illumination also increasing the response to Zn application.

Miller et al. (1987) reported that copper-enriched manure added to soils initially existed in specifically adsorbed forms, but after 12 days, some Cu was found in the Mn and amorphous Fe oxides forms and are not readily available to plants.

Using no tillage as opposed to conventional tillage caused Mn to increase in the exchangeable and organic fractions. The effects of no tillage were greater for Mn and Fe than for Cu and Zn and in general moved manganese and iron from less to more plant available forms. Controlled oxidation-reduction experiments have shown that more Mn, Cu, Fe and Zn move into the exchangeable and organic fractions at low pH and Eh (reducing condition) than at high pH and Eh (oxidizing conditions) (Sims and Patrick, 1978). Kuo et al. (1983) observed that Cu in all fractions decreased with the depth of the soil. Sajwan and Lindsay (1986) observed that controlled redox conditions decreased the availability of Zn concentration in rice plants. Exchangeable and organic Zn decreased and amorphous and crystalline Fe oxide Zn increased with the reducing conditions (Hazra et al., 1987). Zurera-Cosano et. al. (1989) demonstrated that the uptake of heavy metal from soil to plant is a function of the physical and chemical nature of the soil and is altered by innumerable environmental and human factors.

1.6 Aims of thesis

The review part of thesis presents an outline of the present state of knowledge on metal reactions and availability in soil, together with the method of trace metal extraction. This discussion in turn provides the basis for future investigative work.

Deficiency of several metals such as Cu, Fe, Mn and Zn at present is a major limiting factor for the production of crops in Pakistan. The main objectives of the present study, is to understand the chemistry and behaviour of trace elements regarding their distribution and solubility in the soil and availability to plants. Copper, cobalt, iron, manganese, nickel and zinc were used as study examples for micronutrients through the use of selective extraction.

A greenhouse experiment was conducted to see the uptake of plant and seasonal changes on the distribution of Cu, Fe, Mn and Zn in various soil pools by using different extractants.

Chapter 2

Material and Methods

2.1. Introduction

A total of nine soils, which varied greatly in physical and chemical properties, were collected from various locations in Scotland and used in the present research project. Grass samples and the five soils used in a greenhouse study were collected by A. Bakhsh in connection with his Ph.D research programme, and description of these soils and greenhouse experimental procedures are available in his thesis (Bakhsh, 1988). The four Scottish soils, Dreghorn, Caprington, Darleith, and Dunlop were collected by the author. Thus, these four soils are briefly described below.

This chapter also includes the description of the analytical techniques and methods common to different experiments or specific to particular experiments of this work. The physical and chemical characteristics of the soils used are presented in table 2.1.

2.2. Collection and preparation of soil samples.

Soil samples were taken in the fresh condition from the upper 0-15 cm depth of soil profile and were brought to the laboratory in labelled plastic bags as soon as possible. The samples were spread on clean plastic sheets in the laboratory and air dried just sufficiently to pass through a sieve. All the samples were collected from different locations in Scotland. The soil survey of Scotland classifies them into Soil Associations. The soil associations are subdivided into Soil Series on the basis of point in a Catena and drainage status. The Soil Association is the Catenary sequence of soils on a particular parent material. Soil Series with in the association are distinguished on the basis of drainage status.

2.3. Description of the soils

A brief description of each of the soil sampling sites is given below.

2.3.1. Dreghorn

The site is situated at West of Scotland College of Agriculture, Auchincruive, Ayr, Scotland. Grid reference No is NS 373232. The soil is under permanent grass adjacent to greenhouses. It belongs to the Dreghorn association which is developed from raised beach deposits. The series is Dreghorn which has been classed as freely drained brown forest soil.

2.3.2. Caprington.

The site is situated at West of Scotland College of Agriculture, Auchincruive, Ayr, Scotland. Grid reference

No is NS 376232. It is devoted to permanent grass and managed for grazing by dairy cows. The soil belongs to the Rowanhill Association which is developed from glacial till derived from sandstone and shale of the productive Coal measures. The soil comes under the Caprington Series which is classed as an imperfectly drained brown forest soil.

2.3.3. Darleith

The site is located at South Drumboy Farm, Fenwick Ayrshire, Scotland. Grid reference No is NS 498485. The soil is under poor pasture and used for sheep grazing and beef cattle. The soil belongs to Darleith Association which is developed on till derived from carboniferous age igneous rocks (Basalt). The soil series is Darleith which has been classed as freely drained brown forest soil.

2.3.4. Dunlop

The site is located at South Drumboy Farm, Fenwick, Ayrshire, Scotland. The grid reference No is NS 500484. The soil is under permanent grass and used for sheep grazing and beef cattle. The site receives occasional dressings of lime. The soil belongs to Darleith Association which is developed on till derived from carboniferous age igneous rocks (Basalt). The soil series is Dunlop which is classed as an imperfectly drained brown forest soil.

2.4. Soil analysis

2.4.1. Particle size distribution

This was performed by the pipette sampling method as described by (Khan, 1987). The textural class was determined by plotting the results of % sands % silt and % clay on a triangular coordinate diagram (Soil Survey of England and Wales, 1976).

2.4.1.1. Reagents

(1) 30 percent hydrogen peroxide.(2) Dispersing reagent (Calgon) 50.0 g sodium hexametaphosphate plus 7.0 g sodium carbonate (anhydrous) were dissolved in water and diluted to 1 litre. (3) Silicone antifoaming agent 1 cm^3 of 30 % aqueous emulsion (BDH Chemical Ltd) was diluted to 100 cm^3 in water. (4) 2M hydrochloric acid.

2.4.1.2. Dispersing procedure

The soil was air dried and ground to pass a 2 mm sieve. 10.0 g was weighed accurately into a 600 cm^3 beaker for each soil. Approximately 10 cm^3 of 30 % hydrogen peroxide and two drops of antifoaming agent were than added to each beaker. The initial reaction was allowed to subside for 20 minutes. The beakers were gently heated on a steam bath with occasional stirring with a glass rod. The heating was continued until the reaction ceased. The beakers were cooled, a further 10 cm^3 of 30 percent hydrogen peroxide was added washing down the sides of the beaker and heating continued until the reaction ceased completely. 20 cm^3 of hydrogen peroxide was sufficient for most of the soils except those high in organic matter where additions were continued until no reaction was observed. For soils containing calcium carbonate, approximately 2M hydrochloric acid was added dropwise and the contents of the beaker were stirred continuously until the effervescence ceased. For all soils a further 10 cm^3 of acid was added giving a dispersion of soil in approximately M/5 hydrochloric acid. The beakers were stirred at intervals during an hour and then the soil was allowed to settle. The soil suspension was filtered through a whatman filter paper No 50 under suction. The paper was wasned with 3 successive portions of 50 cm^3 of hot water. The soil was scraped from the filter paper with a spatula and then the filter paper and spatula washed with a jet of hot water letting the washings into the beaker. The sides of the beakers were washed down with distilled water and sufficient water was added to give approximately 2 cm^3 depth of suspension in the beaker then 10 cm^3 of calgon solution was pipetted into each beaker. The solution was dispersed for 5 minutes using the ultrasonic probe (MSE instrument Ltd). After the dispersion, any soil adhering to the probe was washed into the beaker using distilled water.

25 cm^3 of the calgon solution was pipetted into a weighed dish, evaporated to dryness on a steam bath and then dried in 110 C^0 oven overnight and reweighed. This was carried out to find out what weight of calgon was added to the suspension as its weight must be subtracted from the weights for silt plus clay and clay.

2.4.1.3. Fractionation of sand

A one litre graduated cylinder was set-up with a large filter funnel in the neck. A 180 micrometer and a 53 micrometer sieve were banked together and placed in the funnel, with 180 micrometer sieve on the top. These sizes allow the separation of the coarse plus medium and the fine sand fractions. The soil suspension was poured into the 180 micrometer sieve. The sides of the beakers were washed using a wash bottle and rubber coated glass rod to ensure that all the soil was removed from the beaker. The soil was washed through 180 micrometer sieve until the coarse and medium sand appeared clean. The contents of the 53 micrometer sieve were washed in the same way. The sieves and funnel were removed and the volume of the cylinder was made upto 1000 cm^3 with water. The contents of the sieves were washed into weighed and labelled porcelain basins and evaporated to dryness on a steam bath. The basins were then transferred into a 110°C oven and left overnight, cooled in a dissicator for 30 minutes and reweighed. The percent coarse sand plus medium sand and fine sand was then calculated on an oven dry basis in the soil mineral material.

2.4.1.4. Fractionation of silt plus clay

The cylinders were kept in a water bath at a constant temperature of 20°C. The temperature of the suspension was noted and appropriate time for the silt plus clay at a depth of 20 cm and a 10 cm sampling depth for clay particles, were selected from the table prepared by the Soil Survey of England and Wales (1976). Each cylinder was shaken thoroughly for one minute to ensure that all the soil was in suspension. The cylinder was then placed on the pipetting stand. Immediately 25 cm^3 pipette was lowered down into the cylinder with the top closed, until it failed to touch the surface of the liquid and the height on the scale was noted. About 20 seconds before the required time, the pipette was lowered down gently to exactly 20 cm depth. The top was opened at the appropriate time and sample of slightly more than 25 cm^3 of the suspension was taken. The top was closed and the pipette was then removed from the cylinder and the volume was adjusted to 25 cm^3. The solution was poured into a weighed basin. The shaking was repeated to obtained a duplicate sample in the same way. The samples were evaporated to dryness on the steam bath and then dried at 110°C in an oven overnight. The samples were removed, cooled in a desiccator for 30 minutes and weighed.

The cylinders were left undisturbed in a water-bath for the appropriate time to allow the silt particles to settle down. The temperature of the suspension was noted and a sample of clay was taken at a depth of 10 cm by the same procedure as described for silt plus clay except that the cylinders were not shaken between pipetting the samples. Again the solution was run into a weighed and labelled porcelain dish, evaporated to dryness on a steam bath and then left in a 110°C oven overnight. Textural class of soil was determined with the help of a triangular chart prepared by the Soil Survey of England and Wales (Hodgson, 1976).

2.4.2. Moisture contents

Porcelain basins were washed, cleaned and then left in the oven at 110°C for an hour to dry. They were cooled in a desiccator and weighed. 5 gram soil was weighed into each basin and were placed for 24 hours in a oven at 110°C, cooled in a desiccator and reweighed. The percent moisture contents were determined on an oven dry basis.

2.4.3. Soil pH

Soil pH was determined in a 5:1 water/soil mixture by a combined glass/reference electrode. It was first standardized with buffer solutions of pH 7.0 and 4.0. The buffer solutions were prepared by dissolving a tablet in a 100 cm^3 of deionized water. After this 2 g of air dried soil ground to pass a 2 mm sieve was taken in a 4 ounce glass bottle. 10 cm^3 of deionized water was added to the bottle which was then shaken for 15 minutes. The electrode was then immersed in the bottle and the soil suspension stirred by swirling the electrode slightly. The pH was read immediately.

2.4.4. Organic matter measurement

2.4.4.1. Percent loss on ignition

Vitreosin basins were placed in a 110°C oven for one hour, cooled in a desiccator for 30 minutes and weighed. Approximately 5 g of soil was weighed accurately into the basins. The basins were then placed in the oven overnight, after which they were removed, cooled in a desiccator and reweighed. Then the basins were placed in the electric furnace. The furnace ignited and the temperature was increased to 450°C. The furnace was switched off after 6 hours of ignition. The basins were cooled in a desiccator and reweighed. The organic matter content was obtained as a percent loss on ignition. It is very important to make a preplan of basins put in the furnace before igniting the switch, because after 6 hour heating the differentiation of basins is very difficult.

2.4.4.2. Percent organic carbon

Percent organic carbon was measured by the Walkley Black method.

2.4.4.2.1. Reagents

(1) Barium diphenylamine sulphonate (0.5 g barium diphenylamine sulphonate was dissolved in approximately 250 cm^3 of warm water, 50.0 g of barium chloride was added, warming to dissolve, cooled and diluted to 500 cm^3).

(2) Ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) 0.5N. 140 g of reagent grade ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) was dissolved in water, then 15 cm^3 of concentrated sulphuric acid (H_2SO_4) was added into 800 cm^3 of water and diluted to 2 litres.

(3) Potassium dichromate ($K_2Cr_2O_7$), approximately 60.0 g of potassium dichromate powder was dried in a 110°C

oven overnight and then cooled in a desiccator. 49.04 g of the dried salt was dissolved in approximately 800 cm^3 of water and diluted to 1 litre.

(4) Concentrated sulphuric acid (H_2SO_4, 98%).

(5) Orthophosphoric acid (H_3PO_4, 85%).

2.4.4.2.2. Standardization of ferrous sulphate solution

10 cm^3 of potassium dichromate solution was added into a 500 cm^3 thoroughly clean, round bottom flask then 10 cm^3 of 85% orthophosphoric acid (H_3PO_4), 250 cm^3 of 1M H_2SO_4 and 2 cm^3 of the barium diphenylamine sulphonate indicator solution was added dropwise from a 50 cm^3 graduated burette until the colour of the contents of flask changed from dark green through blue grey with a sharp change at the end point to light green. The volume of ferrous sulphate used was noted and the standardization factor was calculated.

Standardization factor of ferrous sulphate = 10/volume of ferrous sulphate used. The standardization was carried out each day immediately before the examination of soils.

2.4.4.2.3. Determination in soils

Air dried soil samples previously ground to pass a 2 mm sieve were ground to a powder form using mortar and pestle. 0.1 to 0.5 g of the sample was accurately weighed into a 500 cm^3 round bottom flask. Then 10 cm^3 of potassium dichromate solution was added and mixed by swirling the flask. 20 cm^3 of concentrated sulphuric acid was added carefully from the dispenser and mixed by rotating the flask for 1 minute. Then the flasks were allowed to stand for 30 minutes. After this all the samples were diluted with 200 cm^3 of water. Then 10 cm^3 of 85% phosphoric acid (H_3PO_4) and 2 cm^3 of barium diphenylamine sulphonate reagent were added. The samples were titrated with ferrous sulphate solution by adding it dropwise until the colour changed from dark green to bluegrey. The volume of the ferrous sulphate used was noted. A blank titration was carried out exactly in the same way except that no soil sample was taken.

2.5. Determination of total trace elements

2.5.1. In soils

An acid mixture was used to dissolve the samples for the determination of total Co, Cu, Fe, Mn and Zn. The air dried soil samples were ground very finely with an agate, mortar and pestle. 100 mg of each sample, in duplicate, were placed in the acid-washed teflon dissolution bombs with screw tops. 5 cm^3 analar H_2SO_4 and 4 cm^3 analar HNO_3 were added to these bombs. The bombs were sealed with screw lids and kept at 200°C for 2.5 hours over the sand bath, then removed and cooled thoroughly.

Approximately 20 cm^3 of deionized water was added to each bomb and the digest was filtered through Whatman filter paper No 50, size 11.0 cm, into 50 cm^3 volumetric flask, and then the volume was made upto the mark with deionized water. Two blank solutions having no soil containing only the acids were made with the same procedure and analysed together with the samples. The standards were made with the same volume of acids to reduce the effect of background electrolyte for the determination of total Co, Cu, Fe, Mn and Zn.

2.5.2. In plants

Plant material was extracted by the wet pressure digestion method adopted by Adrian (1973) and used for calcium determination by Adrian and Stevens (1977). About 0.5 g ground plant tissue was weighed into 60 cm^3 polypropylene bottles with screw caps. 4 cm^3 of concentrated nitric acid and 2 cm^3 of concentrated perchloric acid were delivered to these bottles via 100 cm^3 burettes (0.10 cm^3 divisions), which enhanced the speed and accuracy with which acids could be added. The bottles were swirled to soak all the sample with acid, and kept for 24 hours for predigestion loosely capped in the fume cupboard. Predigestion is essential as the bottles could burst if heated immediately. After that the bottle caps were screwed on tightly, the bottles were placed in plastic trays (twelve on each) having 1 inch depth of water to provide constant heating to each bottle. They were then placed on the water bath in a fume cupboard for 3-3.5 hours at 65°C temperature. After cooling the bottles, 2 cm^3 of deionized water was added, the bottles were recapped, replaced on the water bath and heating continued for a further 2 hours to expel the excess of volatile acid. The bottles were then thoroughly cooled, 15 cm^3 of deionized water was then added to each, and the contents were filtered through Whatman filter paper No 540, size 11.0 cm into 50 cm^3 volumetric flasks.

The volume was made up with deionized water and shaken thoroughly. Some content from the flask was transferred to 4 dram glass bottles, and stored in the refrigerator for analysis. The blanks having no plant samples were run through with the samples under the same procedure. The standards used for Cu, Fe, Mn and Zn determination were also made with the same volume of acids. All reagents used were of analytical grade.

Table 2.1. Textural and physical properties of the four soils study.

Properties	Soils			
	Dreghorn	Caprington	Darleith	Dunlop
% Coarse sand	45.95	28.43	18.48	15.61
% Fine sand	25.09	22.67	22.48	12.16
% Silt	16.15	19.71	25.10	35.15
% Clay	8.81	21.48	27.53	30.92
Textural class	Sandy loam	Sandy clay loam	Clay loam	Clay loam
pH	5.2	5.2	5.4	5.6
% LOI	5.43	7.18	18.39	13.46
% OC	2.15	2.54	8.66	6.38
Total Co	7.57	10.58	16.88	20.85
Total Cu	11.03	22.50	16.71	16.73
Total Fe	17820	23916	46425	59555
Total Mn	539.6	443.5	1349.1	839.0
Total Zn	65.22	146.0	839.02	118.02

Coarse sand = > 0.18 mm, Fine sand = 0.18 - 0.05 mm, Silt = 0.05 - 0.002 mm and Clay = < 0.002 mm.

(1) pH measured in given solution at given ratio using a combination pH electrode.

(2) Loss on ignition, 450°C for 6 hours.

(3) Total Co, Cu, Fe, Mn and Zn in $\mu g\ g^{-1}$ by acid digestion.

(4) % organic carbon by Walkely and Black method.

2.6. Extraction of exchangeable metals from soil by using simple metal salts.

2.6.1. Method

Four soils described in (Table 2.1, section 2.3) were used in this experiment to examine the effect of various extractants. Triplicate 5 gram of each air dried soil was weighed in a 4 ounce thoroughly clean glass bottle and 50 cm^3 of each extractant was added to each bottle, and shaken for 16 hours by using end over end shaker at room temperature. Three blanks having no soil were also run along with samples for each extractant. The suspensions were filtered through Whatman filter paper No 1, size 15.0 cm and filtrates were collected in 100 cm^3 polyethylene bottles to measure Co, Cu, Fe, Mn, Ni and Zn in the extracts.

All the reagents used were of analytical grade and were selected from those cited in the literature as being selective for more or less exchangeable forms of trace metals in soils. The following extractants were used for extracting exchangeable forms of Co, Cu, Fe, Mn, Ni and Zn.

(1) 0.05M and 0.5M $CaCl_2$. 11.099 g and 110.99 g calcium chloride (BDH, chemical Ltd) respectively, were dissolved in deionized water and diluted to 2 litres.

(2) 0.05M and 0.5M $MgCl_2$. 20.331 g and 203.31 g magnesium chloride (BDH, chemical Ltd) were dissolved in deionized water and diluted to 2 litres, respectively.

(3) 0.05M and 0.5M $SrCl_2$. 26.664 g and 266.64 g of strontiuum chloride (H & W) were dissolved in deionized water and diluted to 2 litres.

(4) 0.05M and 0.5M $Pb(NO_3)_2$. 33.123 g and 331.23 g lead nitrate (H & W) were dissolved in deionized water and diluted to 2 litres.

(5) 0.05M and 0.5M $Cu(NO_3)_2$. 14.16 g and 241.60 g of copper nitrate (Riedel de Haen) were dissolved in deionized water and diluted to 2 litres.

(6) 0.05M and 0.5M $Ni(NO_3)_2$. 29.079 g and 290.79 g nickel nitrate (Koch light Laboratories Ltd) were dissolved in deionized water and diluted to 2 litres.

(7) 0.05M and 0.5M $Zn(NO_3)_2$. 29.748 g and 297.48 g zinc nitrate (BDH, Analar) were dissolved in deionized water and diluted to 2 litres.

(8) 0.05M and 0.5M $AgNO_3$. 16.987 g and 169.87 g of silver nitrate (Johnson Matthey, Materials Technology U.K.) were dissolved in deionized water and diluted to 2 litres.

(9) 0.05M and 0.5M NH_4OAC. 7.708 g and 77.08 g of ammonium acetate (BDH chemicals Ltd Poole England) were dissolved separately in deionized water and each were diluted to 2 litres.

Co, Cu, Fe, Mn, Ni and Zn standards were made in each separate extractant to reduce the effect of background electrolytes. The filtrates were used to determine Co, Cu, Fe, Mn, Ni and Zn by atomic absorption spectroscopy and the air acetylene flame was used, with their appropriate wavelength. The objective of this experiment was to select extractants for the removal of exchangeable Co, Cu, Fe, Mn, Ni and Zn.

2.7. Organic matter and oxide bound trace metals.

2.7.1. Method

Four top soils representing a wide range of soil associations were included in this study. The soils were characterized as described in (Table 2.1) with regard to their physical and chemical properties. 1 g of each air dried soil was weighed accurately in 4 ounce glass bottles, using four replicates and 50 cm^3 of each extractant was added to each bottle, and shaken for 16 hours by using end over end shaker at room temperature. Four blanks having no soils were also run along with samples for each extractant. The suspensions were filtered through Whatman filter paper No 1, size 15.0 cm and the filtrates were collected in 100 cm^3 polyethylene bottles.

20 cm^3 of the filtrates of each bottle were passed through a membrane filter, size 0.45 μm. The soil solutions were analysed without filtering and after filtration to 0.45 μm to measure Cu, Fe, Mn and Zn with all the extractants. The following extractants were used for the extraction of organic matter and oxide bound occluded Cu, fe, Mn and Zn.

(1) 0.5M NaOH.

Dissolve 40 g sodium hydroxide pure (Formackem Research International, Ltd) in 1800 cm^3 of deionized water and diluted to 2 litres.

(2) 5% Na_2CO_3.

100 g of sodium carbonate anhydrous (FSA, Laboratory Supplies) was dissolved in 1800 cm^3 deionized water and diluted to 2 litres.

(3) 0.1M $NH_2OH.HCl$ (pH 1).

13.898 g hydroxylamine hydrochloride (Hopkin & Williams) was dissolved in 1800 cm^3 of deionized water and 12.6 cm^3 of HNO_3 was added, dissolved and diluted to 2 litres.

(4) 0.1M $NH_2OH.HCl$ (pH 2).

13.898 g hydroxylamine hydrochloride (Hopkin & Williams) was dissolved in 1800 cm^3 of deionized water, added 1.26 cm^3 of HNO_3 and diluted to 2 litres.

(5) 0.1M $Na_4P_2O_7$ (pH 10).

89.21 g tetra-sodium pyrophosphate anhydrous (Koch-Light Laboratories Ltd) was dissolved in 1800 cm^3 of deionized water and diluted to 2 litres. pH 10.0 was adjusted by the addition of 1M HNO_3 or 1M NH_4OH.

(6) 0.1M $Na_2H_2P_2O_7$ (pH 7).

44.388 g of disodium dihydrogen pyrophosphate was dissolved in 1800 cm^3 of deionized water, pH of the solution was adjusted to 7.00 by the addition of 1M HNO_3 or 1M NH_4OH and diluted to 2 litres.

(7) 0.05M Na_4EDTA (pH 7).

37.224 g $C_{10}H_{14}O_8N_2Na_2$ (ethylenediamine tetra acetic acid disodium salt), (Hopkin & Williams) was dissolved in 1800 cm^3 of deionized water, pH 7.0 was adjusted with the addition of 1M HNO_3 or 1M NH_4OH and diluted to 2 litres.

(8) 0.05M NH_4EDTA (pH 7).

The EDTA solution was made by dissolving 29.20 g ethylenediaminetetraacetic acid (Sigma Chemical Company) in approximately 1900 cm^3 of deionized water containing 16 cm^3 of an ammonia solution (35% m/m NH_3), adjusting the pH to 7.0 by the addition of 1M ammonia (NH_4OH) solution or 1M HNO_3, and finally making upto 2 litres.

(9) 0.1M HCl.

Carefully 17.0 cm^3 concentrated hydrochloric acid (BDH) was added to 1800 cm^3 of deionized water and diluted to 2 litres with water.

(10) Acid oxalate (0.1M oxalic acid/0.175M NH_4oxalate).

25.214 g oxalic acid and 49.74 g ammonium oxalate were dissolved in 1800 cm^3 of deionized water and diluted to 2 litres.

Working standard solutions were prepared for each element covering the anticipated concentrations of each element in each separate extractant to reduce the effect of background electrolyte. These standard solutions were prepared freshly when required. A suitable composite working standard solution was used. The filtrate was used to determine Cu, Fe, Mn and Zn by atomic absorption spectrophotometer using an air acetylene flame and hollow cathode lamp, with their appropriate wavelength for each elements.

2.8. Uptake of heavy metals by rygrass

The present study was under taken to compare soil tests for plant available Cu, Fe, Mn and Zn in different Scottish soils, to relate the extracted amount of these elements to soil Cu, Fe, Mn and Zn fractions and to examine the effect of soil properties on the availability of these elements as measured by plant utilization and by soil tests.

2.8.1. Material and method

A glass house experiment was carried out to see how well plant uptake of Zn correlated with soil Zn extracted by different extractants. The test crop was rye-grass. The five soils namely, (1) Dreghorn (Arkleston) (2) Midelney (top soil) (3) Midelney (sub soil) (4) Dreghorn (Auchincruive) and (5) Zinc contaminated soil were chosen by A. Bakhsh as a part of his Ph.D research programme. Detail description and physical properties of these soils are available in section 2.2 of his thesis. These surplus soils were used for present study (Bakhsh, 1988).

5.0 g air dried soil of each sampling time was weighed accurately in 4 ounce previously clean glass bottles, using three replicates and 50 cm^3 of each extractant was added to each bottle and shaken for 18 hours by using end over end shaker at room temperature. Three blanks having no soils were also run through for analysis with the samples. The suspension was filtered through Whatman filter paper No 40, size 15.0 cm and the filtrates were collected in 100 cm^3 polythelene bottles. Each sampling time soil were analysed for extractable Cu, Fe, Mn and Zn by using four extraction methods, namely (1) 0.05M $CaCl_2$. (2) 2.5% CH_3COOH. (3) 0.05M NH_4EDTA. (4) Acid oxalate.

Extractable Cu, Fe, Mn and Zn were determined by atomic absorption spectrophotometry, using hollow cathode lamp, air acetylene flame and appropriate wavelength for each elements.

2.9. Metal determination by atomic absorption spectroscopy

Atomic absorption spectroscopy (AAS) is a convenient and rapid method of determining trace levels of a large number of metals. The technique is relatively free from inter element interferences and is therefore suitable for the determination of several metals in a single solution. The determination of Co, Cu, Fe, Mn, Ni and Zn by AAS was investigated and generally found to be suitable for the analysis required in this study. All the determinations of these elements were therefore carried out by this technique. Reynolds and Aldous (1970) reported no interference effects from other elements in the determinations of trace metals by AAS, using an air-acetylene flame system.

2.9.1. Method of measurement

Atomic absorption spectrophotometer was set-up as detailed in the manufacturers hand book of Perkin-Elmer, 370A and 1100B. Calibration standards for each element were run with each batch of determinations. For each element the separate calibration standards are required and were freshly prepared by diluting with the appropriate standard solution. Standard atomic absorption conditions are given in table 2.2.

For each element in sequence, water was aspirated and adjusted to zero. One of the calibration standards was aspirated and adjusted the instrument to give a suitable response. Water was aspirated and adjusted the zero if necessary. Samples were aspirated with an aspiration of water after each and measured the instrument response of the sample. The instrument response for each sample was corrected for back ground absorption. To check for any instrument drift both the calibration standards and the blanks were aspirated with an aspiration of water after each and the instrument response was measured.

Table 2.2. Standard atomic absorption conditions for the determination of trace elements.

Elements	Wavelength (nm)	Slit setting (nm)	Linear working range (μg/ml)
Cobalt	240.7	0.2	5
Copper	324.7	0.7	5
Iron	248.6	0.2	5
Manganese	279.5	0.2	3
Nickel	232.0	0.2	5
Zinc	213.9	0.7	1

NB:- Flame type were air acetylene and light source was Hollow cathode lamp for all element studied.

The values are those specified for the instrument by manufacturer and are in general similar to those obtained in practice.

Chapter 3

Extraction of exchangeable metals from soil by using simple metal salts

3.1 Introduction

The extraction of trace elements from soil is widely used as a laboratory tool both for fundamental research and to provide practical advice to farmers. Quantitative relations between such laboratory measurements and the behaviour of soils frequently have only a local validity, however, and are highly dependent upon agricultural practices, soil type and the specific extraction technique used. It may take many years to establish locally reliable quantitative relations for diagnostic use. In general, the chemical aspects of extraction procedure, e.g. reagent type and concentration, can be precisely defined and some physical aspects can also be specified, e.g. extraction time and soil extractant ratio.

The total concentration of an element in soil is only an indication of the amount available. It has become desirable to adopt some arbitrary method of extraction for assessing the availability of micronutrients because of the difficulty of assessing the availability to plants. Many workers have estimated similar fractions usually called: exchangeable, neutral salt exchangeable, water soluble and exchangeable or non-specifically adsorbed ions of trace metals from soil. The selection of reagent for extracting a given form of the metal from the soil, and the sequence of extraction, not only affect the partitioning of metals in different fractions, but also the total amounts of metal in that fraction.

The extractants used for exchangeable microelements have been many and varied, since the amounts in this fraction are usually most closely related to plant uptake. Even those who have gone through entire fraction schemes, not just solubilized plant-available micronutrients, have used different salts and, in some cases, acids for exchangeable forms. Gupta and Chen (1975) used ammonium acetate (NH_4OAC), McLaren and Crawford (1973) used 0.05M $CaCl_2$ and Gibbs (1973) used 1N $MgCl_2$ for exchangeable Zn, Cu and Mn. Magnesium chloride solution has been found suitable as a predictor of Zn uptake (Martens, 1968; Stewart and Berger, 1965).

John (1974) found that the amount of Zn extracted from slightly alkaline soils by different extractants decreased in the order: DTPA > HCl > H_2SO_4 > $MgCl_2$ > NH_4OAC > NaOAC + CH_3COOH > $CaCl_2$. He obtained best correlation with plant uptake with $MgCl_2$ extractable Zn for oats and with NaOAC + CH_3COOH for corn. Sedberry et al. (1979) reported that Zn concentration in rice plants grown on 25 soils was significantly correlated with NH_4OAC (pH 4.8) extractable zinc. According to John (1974) Zn concentrations in corn and oats grown on 27 soils ($pH < 7$) were best correlated with amounts of Zn extracted by 1M $MgCl_2$, acidic NH_4OAC and 1M KCl. Sauerbeck and Styperek (1985); Sanders et al. (1986), (1987) and McGrath and

Cegarra (1992) pointed out that heavy metals extracted with $CaCl_2$ correlate closely with metal uptake by crops.

Work in this chapter concentrates on selective extraction by using different types and different concentrations of simple salts as a means of bringing soil Co, Cu, Fe, Mn, Ni, and Zn into solution before analysis by atomic absorption spectroscopy.

3.2 Materials and Methods

All soil extractions were performed by shaking 5.0 g of air dried soil with 50 cm^3 of either 0.05M or 0.5M reagent concentrations on an end over end shaker for 16 hours. The soil suspensions were filtered and the soil extract was analysed for Co, Cu, Fe, Mn, Ni, and Zn by atomic absorption spectrophotometry (Perkin Elmer 370A and 1100B). All standards had the same bulk composition as the soil extractant to reduce matrix differences. Additional details of these procedures can be found in section 2.6.1 chapter 2.

Not all of the elements could be determined in each extractant. In some cases the element was part of the extracting solution (e.g. Cu in $Cu(NO_3)_2$, Ni in $Ni(NO_3)_2$ and Zn in $Zn(NO_3)_2$. Also for other extractants some elements were present at levels below the detection limit.

3.3 Effect of reagent concentration on amount of exchangeable metals extracted

The amount of Co, Cu and Ni extracted with these reagents was not measurable and therefore the data are not given for these elements. The amount of Fe, Mn and Zn extracted from the four soils with either 0.05M or 0.5M concentrations of different salts are shown in Table 3.1 to 3.3. Student's t test was applied to the data given in Tables 3.1 to 3.3 to compare the two different concentrations of the same extractant for each soil and each element.

This test showed that more iron (Table 3.1) was extracted by the higher concentrations of the extractants in all soils for $CaCl_2$ and $SrCl_2$. However, 0.5M NH_4OAC extracted less Fe than 0.05M NH_4OAC, and the difference between the two concentrations was not clear for $MgCl_2$ and $Ni(NO_3)_2$ extracted iron. Whereas zinc nitrate and lead nitrate did not extract measurable amount of iron at both concentrations.

A clear indication of the differences between the two concentrations may be obtained from table 3.2, which compare the mean Mn values for each extractant and for each soil. Appreciably more manganese was extracted at higher concentrations from all the soils for NH_4OAC, $MgCl_2$, $SrCl_2$ and $Ni(NO_3)_2$. The difference between the two concentrations was not clear for $CaCl_2$, $Zn(NO_3)_2$ and $Pb(NO_3)_2$ extractable manganese.

Table 3.1 Iron extracted by chloride, nitrates and acetates at two concentrations for four soils (μg g-1).

Extractant	Soils			
	(1)	(2)	(3)	(4)
0.05M NH_4OAC	30.13	6.53	10.03	5.80
0.5M NH_4OAC	6.10*	0.00*	5.80*	0.00*
0.05M $CaCl_2$	0.00	0.00	0.00	0.00
0.5M $CaCl_2$	4.63*	1.40*	4.46*	2.50*
0.05M $MgCl_2$	2.40	0.97	1.53	0.77
0.5M $MgCl_2$	2.27ns	0.40*	2.73*	0.20*
0.05M $SrCl_2$	0.00	0.00	0.00	0.00
0.5M $SrCl_2$	1.00*	2.13*	3.60*	2.47*
0.05M $Ni(NO_3)_2$	4.53	1.47	2.50	1.47
0.5M $Ni(NO_3)_2$	5.73*	0.00*	5.13*	0.00*
0.05M $Zn(NO_3)_2$	0.00	0.00	0.00	0.00
0.5M $Zn(NO_3)_2$	0.00	0.00	0.00	0.00
0.05M $Pb(NO_3)_2$	0.00	0.00	0.00	0.00
0.5M $Pb(NO_3)_2$	0.00	0.00	0.00	0.00

(1) = Dreghorn (2) = Caprington
(3) = Darleith (4) = Dunlop
* = Significant at 5% level
ns = Nonsignificant at 5% level.

Table 3.2 Manganese extracted by chloride, nitrates and acetates at two concentrations for four soils (µg g-1).

Extractant	Soils			
	(1)	(2)	(3)	(4)
0.05M NH_4OAC	5.40	0.00	14.00	1.30
0.5M NH_4OAC	9.23*	0.17*	28.83*	2.07*
0.05M $CaCl_2$	12.50	0.00	55.67	4.97
0.5M $CaCl_2$	15.40*	1.80*	53.33*	5.03ns
0.05M $MgCl_2$	16.53	0.70	63.00	4.93
0.5M $MgCl_2$	17.20ns	1.40*	79.67*	5.80*
0.05M $SrCl_2$	13.37	0.00	54.00	4.40
0.5M $SrCl_2$	16.93*	2.13*	62.33*	5.83*
0.05M $Ni(NO_3)_2$	20.47	4.17	65.67	4.93
0.5M $Ni(NO_3)_2$	22.30*	7.93*	69.00*	5.23ns
0.05M $Zn(NO_3)_2$	17.23	5.50	63.67	4.53
0.5M $Zn(NO_3)_2$	18.03*	6.10*	55.00*	3.67*
0.05M $Pb(NO_3)_2$	18.87	9.40	79.33	6.83
0.5M $Pb(NO_3)_2$	18.53ns	12.73*	57.33*	3.87*

(1) = Dreghorn (2) = Caprington
(3) = Darleith (4) = Dunlop
* = Significant at 5% level.
ns = Nonsignificant at 5% level.

Table 3.3 Zinc extracted by chloride, nitrates and acetates at two concentrations for four soils (μg g-1).

Extractant	Soils			
	(1)	(2)	(3)	(4)
0.05M NH_4OAC	0.00	0.00	0.00	0.00
0.5M NH_4OAC	0.00	0.00	0.00	0.00
0.05M $CaCl_2$	0.72	0.00	4.18	0.00
0.5M $CaCl_2$	1.97*	0.78*	6.55*	0.42*
0.05M $MgCl_2$	0.81	0.09	3.46	0.16
0.5M $MgCl_2$	1.69*	0.95*	5.34*	0.63*
0.05M $SrCl_2$	0.00	0.00	2.97	0.00
0.5M $SrCl_2$	1.63*	0.58*	5.37*	0.33*
0.05M $Ni(NO_3)_2$	1.94	1.73	7.12	0.33
0.5M $Ni(NO_3)_2$	2.26*	4.10*	7.70*	0.31ns
0.05M $Pb(NO_3)_2$	1.32	6.62	7.57	0.00
0.5M $Pb(NO_3)_2$	3.51*	8.90*	10.85*	1.55*

(1) = Dreghorn (2) = Caprington
(3) = Darleith (4) = Dunlop
* = Significant at 5% level.
ns = Nonsignificant at 5% level.

The trend for extractable zinc was more clear than for the other elements. Table 3.3 shows that NH_4OAC extracted zinc was not measurable with both the concentrations. While, the zinc extracted with 0.5M concentration of all other extractants were significantly higher than 0.05M concentrations for all soils, except $Ni(NO_3)_2$ extracted Zn for Dunlop soil which shows nonsignificant decrease. The results reported for zinc were also consistant with those of Neilsen et al. (1987) who used different concentrations of $MgCl_2$, $CaCl_2$ and KCl for the removal of exchangeable Mn and Zn and observed that ratio of extracted Zn to Mn decreased much more rapidly for six out of ten soils as the extractant concentration decreased.

It is obvious from this study that the amount of Fe Mn and Zn (Tables 3.1 - 3.3) extracted by various salts of acetate, nitrates and chlorides for most of the soils were increased with the increase of extractant concentrations. Higher concentrations of different salts extract a fraction of trace metals from these soils which was retained by the soil when extracted with lower concentrations of the extractant. Various workers have varied the concentration of the solution, keeping the weight of soil constant. Kelley and Cummins (1921) found upon equilibrating a constant amount of Yolo soil with solution of NaCl, that the replacement of Ca and Mg ions by Na ions increased as the concentration of the solution was increased. Gedroiz as quoted by Kelly (1948) showed the effect of concentration by keeping constant the absolute amounts of both soil and NaCl but varying the concentration of the solution, and found that with constant amounts of both soil and NaCl present, the more concentrated the solution the greater the amount of exchange.

On the other hand NH_4OAC extracted Fe from all the soils and $MgCl_2$ and $Ni(NO_3)_2$ extracted Fe for Caprington and Dunlop soil (Table 3.1), $Zn(NO_3)_2$ extracted Mn for Darleith and Dunlop soils and $Pb(NO_3)_2$ extracted Mn for Dreghorn, Darleith and Dunlop soils (Table 3.2) and $Ni(NO_3)_2$ extracted Zn (Table 3.3) for Dunlop soil was not increased with the higher concentrations of the solutions. Which indicates that not only the concentration of the reagent affects the exchange of micronutrients but the kinds of the cations, type of soil and the nature of the replacing ion also plays an important role on the exchange of micronutrients. NH_4OAC did not extract measurable Zn and very small amounts of Mn with both the concentrations from these soils but it extracted more Fe than the other reagents for all the soils. Lower amounts of Mn and no measurable Zn in NH_4OAC extract might be due to the divalent cations of Zn^{++} and Mn^{++} which can not be easily replaced by the monovalent cation of NH_4^+ in NH_4OAC

3.4 Comparison of reagents as extractants exchangeable metals.

Mean values of exchangeable Co, Cu, and Ni extracted with 0.05M and 0.5M concentrations of different acetate, nitrates and chlorides were very low or below the detection limit. Therefore, the data obtained for these elements are not given here.

Many investigators reported very small amounts of Co, Cu and Ni in exchangeable fractions. Very little cobalt (0.10 to 0.20 $\mu g\ g^{-1}$) was extracted with 2.5% (W/V) acetic acid and 2% (W/V) dithiazone from the agricultural soils of Valderas area, Spain (Aller and Deban, 1989). Mclaren and Crawford (1973) used 0.05M $CaCl_2$ for the extraction of exchangeable Cu and found very little (< 0.010 to 0.503 $\mu g\ g^{-1}$) of exchangeable Cu in soils. Sposito et al. (1982) reported very small amount of exchangeable Ni, Cu, Zn, Cd and Pb in the field soils which was extracted with KNO_3 and H_2O. Jopony (1985) used KNO_3 for the removal of exchangeable Cu, Zn, Ni and Co in soils and sediment of Ranu, Sabah and found very small amount of these metals in exchangeable fractions. Shuman (1985) used $Mg(NO_3)_2$ and found very small amount (0.01 - 0.22 $\mu g\ g^{-1}$) of Cu in exchangeable fraction of the soils. While, Sims (1986) reported little Cu (0.1 mg kg^{-1}) in exchangeable fraction by using the same extractant $Mg(NO_3)_2$. Miller et al. (1986) used $Pb(NO_3)_2$ and also reported less than 0.1 mg kg^{-1} of exchangeable Cu in the soils. More recently, Liang et al. (1991) used 0.5M $Ca(NO_3)_2$ and 0.05M $Pb(NO_3)_2$ + 0.1M $Ca(NO_3)_2$ for the extraction of Cu and found very little Cu (0.3 - 0.8 and 0.1 - 0.5 µg g^{-1}) in $Ca(NO_3)_2$ and Pb-displaceable fractions in Saskatchewan soils. McGrath and Cegarra (1992) also reported that $CaCl_2$ solubilized undetectable quantities of Cr (< 0.01 µg g^{-1}) from the soils. There are various reason for the non removal of Co, Cu and Ni with exchangeable salts. The lack of greater effect of either salts on the extraction of exchangeable Co, Cu and Ni indicates the fact that extractant concentration is not good indicator for the measurement of exchangeable metals but soil solution ratio also plays an important role for the measurement of exchangeable metals. In this study the soil solution ratio was 1:10, which is insufficient for the measurement of Co, Cu and Ni. Many investigators pointed out that cation exchange is influenced by the ratio of the weight of soil to the volume of solution used. McLaren and Crawford (1973) noticed this point and reported that although the amount of Cu slightly increased with reducing the amount of soil to 5 gram, there was insufficient copper for reliable analysis. The lower amounts of Co, Cu and Ni present in exchangeable fraction might be due to the smaller weight of soil analysed. Neilsen et. al. (1987) also observed that soil extractant ratio affects the amount of Zn extracted. They compared 1:2 and 1:5 ratio of soil and $MgCl_2$ solution and found that 1:5 ratio removed more Zn than 1:2 ratio of the soil solution. The amount of exchangeable Co, Cu, and Ni in these soils is very low or below the detection limit, which indicates that bulk of the content of cobalt, copper, and nickel in these soils is strongly bound with the organic matter and oxides bound or residual fractions. Tiller (1979) pointed out that isotopic exchange of minor nutrients is slow and erratic when a large quantity of Mn oxide is present in the soil.

Mean values of iron, manganese and zinc extracted with 0.05M concentrations of different reagents are given in Tables 3.4 - 3.6. Data given in these Tables were analysed by an F test to compare the difference between the extractable amounts of Fe, Mn and Zn by different extractants. These F values were also used to obtained the Sheffe least significant difference (LSD) between the mean values of each extractant for each soil.

Table 3.4 shows that $Zn(NO_3)_2$, $Pb(NO_3)_2$, $SrCl_2$ and $CaCl_2$ extracted Fe was not measurable. NH_4OAC extracted the higher amounts of Fe than the different salts of nitrates and chlorides. Although, nitrate salts extracted less Fe than NH_4OAC, they extracted more Fe than the salts of chlorides. From the chlorides only $MgCl_2$ extracted a little Fe, which shows nonsignificant difference with $Ag(NO_3)_2$ for all the soils.

Table 3.4 Mean values of iron extracted with 0.05M concentrations of different extractant (µg g-1)

Extractant	Soil			
	(1)	(2)	(3)	(4)
NH_4OAC	30.13c	6.53d	10.03d	5.80c
$Ni(NO_3)_2$	4.53b	1.47b	2.50b	1.47b
$Zn(NO_3)_2$	0.00	0.00	0.00	0.00
$Pb(NO_3)_2$	0.00	0.00	0.00	0.00
$Ag(NO_3)_2$	2.03a	1.33ab	1.20a	1.00a
$Cu(NO_3)_2$	5.23b	2.70c	8.17c	8.43d
$SrCl_2$	0.00	0.00	0.00	0.00
$CaCl_2$	0.00	0.00	0.00	0.00
$MgCl_2$	2.40a	0.96a	1.53a	0.77a

(1) = Dreghorn (2) Caprington
(3) = Darleith (4) Dunlop

The figures in a column with the same following letter are not significantly different at 5 % level.

Table 3.5 Mean values of manganese extracted with 0.05M concentrations of different extractant (μg g-1)

Extractant	Soil			
	(1)	(2)	(3)	(4)
NH_4OAC	5.40b	0.00a	14.00a	1.30a
$Ni(NO_3)_2$	20.47g	4.17c	65.67e	4.93d
$Zn(NO_3)_2$	17.23e	5.50d	63.67d	4.53c
$Pb(NO_3)_2$	18.87f	9.40e	79.33f	6.83f
$Ag(NO_3)_2$	1.91a	4.33c	62.67d	3.57b
$Cu(NO_3)_2$	23.83h	28.70f	84.33g	6.00e
$SrCl_2$	13.37d	0.00a	54.00b	4.40c
$CaCl_2$	12.50c	0.00a	55.67c	4.97d
$MgCl_2$	16.53e	0.70b	63.00d	4.93d

(1) = Dreghorn (2) Caprington
(3) = Darleith (4) Dunlop

The figures in a column with the same following letter are not significantly different at 5 % level.

Concentrations of soil manganese extracted by using 0.05M acetate, nitrates and chlorides are shown in Table 3.5. On the whole, the amount of manganese extracted by NH_4OAC was less than for the other extractants, while $Cu(NO_3)_2$ extracted the most manganese from Dreghorn, Caprington and Darleith soils, and $Pb(NO_3)_2$ extracted the most Mn from Dunlop soil. It is clear from the Table 3.5 that appreciable amounts of Mn were removed by various salts of nitrates and chlorides for all the soils, except Caprington soil Mn extracted with chlorides. Which shows no measurable Mn or extracted very small amounts of Mn by chlorides.

Table 3.6 Mean values of zinc extracted with 0.05M concentrations of different extractant (μg g-1)

Extractant	Soil			
	(1)	(2)	(3)	(4)
NH_4OAC	0.00	0.00	0.00	0.00
$Ni(NO_3)_2$	1.94d	1.73c	7.12e	0.33c
$Pb(NO_3)_2$	1.32c	6.62d	7.57f	0.00a
$Ag(NO_3)_2$	1.28c	1.32b	4.57d	0.20b
$Cu(NO_3)_2$	2.37e	7.26e	9.04g	1.22d
$SrCl_2$	0.00a	0.00a	2.97a	0.00a
$CaCl_2$	0.72b	0.00a	4.18c	0.00a
$MgCl_2$	0.81b	0.09a	3.46b	0.16b

(1) = Dreghorn (2) Caprington
(3) = Darleith (4) Dunlop

The figures in a column with the same following letter are not significantly different at 5 % level.

Table 3.6 shows that NH_4OAC extracted Zn was not measurable from all the soils. $SrCl_2$ extracted Zn from Dreghorn, Caprington and Dunlop soil and $CaCl_2$ extracted Zn from Caprington and Dunlop soil were also below the detection limit. Calcium chloride extracted zinc from Dreghorn soil and magnesium chloride extracted zinc from three soils were very low. Whereas, Darleith soil extracted zinc with all the chloride were considerable.It is clear from Table 3.6 that different salt of nitrate removed appreciable amount of Zn from all the soils, except Dunlop soil extracted Zn with $Pb(NO_3)_2$ which shows no measurable Zn.

Mean values of Fe, Mn and Zn extracted with 0.5M concentrations of acetates, nitrates and chlorides are given in Tables 3.7 to 3.9. Data given in these Tables were analysed by an F test to see the difference between the extractable amounts of Fe, Mn and Zn for four soils. This test showed that Fe, Mn and Zn extracted by 0.5M concentrations of acetates, chlorides and nitrates were highly significant at 5% level for each soil. These F values were also used to obtain the Sheffe least significant difference (LSD) between the mean values of each extractant for each soil.

Data given in Table 3.7 shows that Fe extracted with 0.5M concentrations of $Zn(NO_3)_2$ and $Pb(NO_3)_2$ for four soils, $Ni(NO_3)_2$ and NH_4OAC extracted Fe for Caprington and

Dunlop soil was not measurable. NH_4OAC extracted Fe for Dreghorn and Darleith soil was higher than of the all other reagents. Iron extracted with salts of different chlorides was less than NH_4OAC and $Ni(NO_3)_2$ for Dreghorn and Darleith soil but the Fe extracted with chlorides for Caprington and Dunlop soil was higher than NH_4OAC and $Ni(NO_3)_2$, as NH_4OAC and $NI(NO_3)_2$ did not extract any measurable Fe from these two soils.

Table 3.7 Mean values of iron extracted with 0.5M concentrations of different extractant (µg g-1)

Extractant	Soil			
	(1)	(2)	(3)	(4)
NH_4OAC	6.10d	0.00a	5.80e	0.00a
$Ni(NO_3)_2$	5.73d	0.00a	5.13d	0.00a
$Zn(NO_3)_2$	0.00	0.00	0.00	0.00
$Pb(NO_3)_2$	0.00	0.00	0.00	0.00
$SrCl_2$	1.00a	2.13d	3.60b	2.47b
$CaCl_2$	4.63c	1.40c	4.46c	2.50b
$MgCl_2$	2.27b	0.40b	2.73a	0.20a

(1) = Dreghorn (2) Caprington
(3) = Darleith (4) Dunlop

The figures in a column with the same following letter are not significantly different at 5 % level.

The concentrations of Fe in the exchangeable fraction were found to be very small, which indicates that the majority of soil Fe was associated with crystalline Fe oxides and residual fractions. These results agree with the others (Sims and Patrick, 1978 and Shuman, 1985), who found that the bulk of the soil Fe was associated with the residual and crystalline Fe oxide fractions. Kuo et al. (1983) used $MgCl_2$ for the extraction of exchangeable metals and found that Fe extracted by $MgCl_2$ was below the detection limit (< 0.1 ppm). Menzel and Smith (1984) reported that most of the iron contained in igneous rocks is in the ferrous (Fe^{++}) form and much of the iron in well-drained soils is in the ferric form. Many compounds of ferrous iron have low solubilities, but ferric iron compounds are even less soluble. The lower amount of Fe obtained with exchangeable salt may be due to the insolubility of these forms of Fe in these soils and is mostly unavailable to plants. Shuman (1985) also reported very small amounts of Fe by using $Mg(NO_3)_2$ solution. Same results have been obtained by Singh et al. (1988), who used $Mg(NO_3)_2$ for the removal of exchangeable Fe and found a very small (< 0.1%) of Fe in this fraction. They also pointed out that majority of the soil Fe was found to be associated with crystalline and residual fraction.

Table 3.8 Mean values of Mn extracted with 0.5M concentrations of different extractant (µg g-1)

Extractant	Soil			
	(1)	(2)	(3)	(4)
NH_4OAC	6.53a	0.00a	17.70a	1.20a
$Ni(NO_3)_2$	22.30f	7.93e	69.00f	5.23c
$Zn(NO_3)_2$	18.03d	6.10d	55.00c	3.67b
$Pb(NO_3)_2$	18.53e	12.73f	57.33d	3.87b
$SrCl_2$	16.93c	2.13c	62.33e	5.83d
$CaCl_2$	15.40b	1.80c	53.33b	5.03c
$MgCl_2$	17.20c	1.40b	79.67g	5.80d

(1) = Dreghorn (2) Caprington
(3) = Darleith (4) Dunlop

The figures in a column with the same following letter are not significantly different at 5 % level.

A comparison of extractants for manganese (Table 3.8) showed that 0.5M concentrations of different nitrates and chlorides were more effective than NH_4OAC. Extractable amount of Mn with NH_4OAC was not measurable for Caprington soil and very small amount of Mn was extracted with NH_4OAC for other three soils. Considerable amounts of manganese were extracted with nitrates and chlorides for all the soils, except Caprington soil extracted Mn with chlorides. As it is clear from the Table 3.8 that different salts of nitrates and chlorides removed significant amounts of Mn from the soils. Which indicates large proportion of Mn in these soils were present in exchangeable and soluble forms. Same results were obtained by the other workers. Sims et al. (1979) found relatively high exchangeable Mn, especially at low soil pH values. While, Jarvis (1984) found that only small proportion of the total manganese were exchangeable with either NH_4^+, Ca^{2+}, Mg^{2+} or Zn^{2+} and only relatively low concentrations were present as exchangeable or mobile Mn^{2+}. Whereas, Shuman (1985) used $Mg(NO_3)_2$ for the extraction of exchangeable metals and found 0.2 to 145 µg g^{-1} of manganese in exchangeable fraction. He also obtained higher Mn values than that of the Cu, Fe and Zn in exchangeable fraction. Sims (1986) used $Mg(NO_3)_2$ for the removal of exchangeable Mn and found 5.1 to 13.6 mg kg^{-1} of Mn in this fraction. He claimed that soil Mn was primarily found in the exchangeable and organic fractions.

Table 3.9 Mean values of zinc extracted with 0.5M concentrations of different extractant (µg g-1)

Extractant	Soil			
	(1)	(2)	(3)	(4)
NH_4OAC	0.00	0.00	0.00	0.00
$Ni(NO_3)_2$	2.26c	4.10c	7.70c	0.31a
$Pb(NO_3)_2$	3.51d	8.90d	10.85d	1.55c
$SrCl_2$	1.63a	0.58a	5.37a	0.33a
$CaCl_2$	1.97b	0.78b	6.55b	0.42a
$MgCl_2$	1.69a	0.95b	5.34a	0.63b

(1) = Dreghorn (2) Caprington
(3) = Darleith (4) Dunlop

The figures in a column with the same following letter are not significantly different at 5 % level.

There is a large variation in the amounts of zinc extracted from the soils by NH_4OAC, nitrates and chlorides (Table 3.9). On the whole, NH_4OAC extracted zinc was not measurable from all the soils, whereas, $Pb(NO_3)_2$ extracted the most zinc and $SrCl_2$ extracted the least zinc. Considerable amounts of zinc were extracted with all the nitrates and chlorides from all the soils. Iyengar et al. (1981) found that zinc in exchangeable, specifically adsorbed, organically bound and residual fraction was more available than Zn in Fe, Al and Mn oxides associated forms. Orabi (1981) reported 0.3 to 1.5 ppm of zinc extracted with $MgCl_2$ from the soils. Shuman (1985) used $Mg(NO_3)_2$ for the extraction of exchangeable Zn and found 0.02 to 1.47 $\mu g\ g^{-1}$ of zinc in this fraction. While, Sims (1986) used the same extractant $Mg(NO_3)_2$ for the removal of exchangeable Zn and found 0.6 to 1.8 mg kg^{-1} of exchangeable zinc. Singh et al. (1988) reported 1.0 to 1.5 $\mu g\ g^{-1}$ of Zn by using $Mg(NO_3)_2$ solution for the removal of exchangeable fraction. Results reported here were also with the agreement of Bakhsh (1988) who used $CaCl_2$ and ammonium acetate for the removal of exchangeable Zn and found very small amounts of Zn ranging from 0.00 to 4.65 and 0.00 to 1.25 mg kg^{-1} of Zn extracted with $CaCl_2$ and ammonium acetates. He also claimed that $CaCl_2$ extracted more Zn than ammonium acetate. It is well known that soil prefers to hold divalent cations than monovalent cations. The amount of Zn extracted with NH_4OAC was not measurable in these soils. Non detectable values of Zn in NH_4OAC extract might be due to the monovalent ions of NH_4^+ in NH_4OAC solution. John (1974) reported that the presence of chloride ions in the solution, which would promote formation of soluble $ZnCl_2$, might be the other possible reason for getting higher values of extractable Zn with chlorides than acetates. The importance of exchangeable and specifically adsorbed fractions of zinc was pointed out by Liang et al, (1991) when they found the positive correlation between amount of zinc in exchangeable, lead displaceable and acetic acid soluble fractions and plant uptake.

It is clear from the results and from the above discussions that appreciable amounts of Mn and Zn but very small amounts of Co, Cu, Fe and Ni in these soils were present in the exchangeble form. The exchangeable metals are held by electrostatic forces on colloid surfaces and a majority are held in specific sites with high affinity for the metals. The conventional methods for measuring exchangeable nutrients required their displacement with concentrated salt solutions. It is interesting that exchange of Mn and Zn with NH_4OAC was lower than the salts of chlorides and nitrates but it extracted more Fe than other reagents. Shuman (1991) reported that divalent ions should have greater replacing power than monovalent ion and the lower amount of Mn and Zn with NH_4OAC may be due to the monovalent ions of NH_4^+ in NH_4OAC. As reported earlier that most of the total Fe was associated with Fe oxide fraction. The higher amounts of Fe in NH_4OAC extracts may be due to acidic nature of NH_4OAC, which might dissolve some oxide fraction of Fe.

Chapter 4

Organic matter and oxide bound trace metals

4.1 Introduction

Chemical analysis of soils is conducted for different purposes, such as pedogenic studies, trace metal chemistry, diagnosis of nutrient deficiencies, soil fertility evaluation, determination of the concentration of potential pollutants and study of plant nutrient uptake. Total content of trace elements in soil is of great importance in geochemical studies, whilst knowledge of soluble forms can provide basic information for agronomic practices. The available level of trace elements is most certainly of greater environmental significance than the total level. However, as weathering and microbial action supply additional available concentrations of these elements, the ultimate theoretical limit for the capacity of any soil for farming is the total content of the trace element. Although the amounts of micronutrients removed in crops seem insignificant compared with those of macronutrients, a deficiency of one or more of these micronutrients can have as devastating an effect on crop growth as that of a major nutrient.

Separation of various forms of elements in soils has been useful in studying the retention and release of elements by the soil to the plant. In determining the distribution of an element in soils, one can find better extractants that remove elements in proportion to that removed by plants. Also, the dynamics of how elements move from one chemical form to another, in response to changing soil conditions, can be studied using fractionation techniques. In studying soils and sediments, researchers have used slightly different fractionation schemes. Some have emphasized fractionating the soil itself, while others have sought the forms of the elements. The fractions of soils usually separated are the organic matter, hydrous oxides of Fe, Al and Mn fractions. In determining chemical forms, researchers have often used some, or all of the above fractions as parts of the nonexchangeable forms and residual solid materials but they usually also include soluble and exchangeable forms.

Organic matter in soils is usually extracted by using DTPA or EDTA and some workers have used these methods for determining the micronutrients associated with the organic matter fraction (Lindsay and Norvell, 1978; Archer and Hodgson, 1987 and Bakhsh, 1988). Archer and Hodgson (1987) used 0.05M EDTA for the extraction of Cd, Co, Cu, NI, Pb, Se, Zn and Mn, and Bakhsh (1988) used 0.05M NH_4EDTA for the removal of organically bound Zn of the soil. Other methods proposed have been pyrophosphate (McLaren and Crawford, 1973) and NaOH (Lund et al., 1985). Lund et al. (1985) used 0.05M NaOH to mobilize organically bound micronutrients and found that NaOH also dissolved Pb, Zn carbonate and small proportion of Cd, Cu and Ni carbonates. Bascomb (1968) suggested the use of pyrophosphate ($K_4P_2O_7$) to extract the organically bound micronutrients. McLaren and Crawford (1973) found that bulk of the available Cu reserves in the organic fraction were extracted by 0.1M $K_4P_2O_7$. But Bakhsh (1988) observed that pyrophosphate also produce a dissolution of some oxide fraction. Thus altering the extraction from the oxide fraction several extractants have been used in this study.

In extracting the micronutrients associated with the oxides of Mn and Fe, three extractants are in common use. The first is $NH_2OH.HCl$ method of Chao (1972), which has been employed by Jarvis (1984), Singh et al. (1988) and Liang et al. (1991). The other extractants are pyrophosphate and acid ammonium oxalate (McKeague and Day, 1966). These extractants dissolve Fe and Al oxide, whereas, the former method mostly dissolves Mn oxide fraction of the soil. McLaren and Crawford (1973), Jarvis (1984) and Liang et al. (1991a) have used acid oxalate for the removal of Mn and Fe oxide fraction of the soil. In this comparative study, an attempt was made to evaluate several extracting solutions as to their effeciency and specificity to dissolve organic matter and oxide bound fraction of the soil.

The extractants used for soluble and exchangeable microelements have been many and varied, these have already been described in a previous chapter. Therefore, the work in this chapter was undertaken to discuss the organic matter and oxide bound fraction of the micronutrients.

In soil extracts metals are not exclusively in dissolved forms but are also present in suspended material, which is difficult to remove. Bascomb (1968) and McKeague and Schuppli (1982) showed that this may be fine particulate amorphous material, or it may have been formed by coagulation of solutes in the pyrophosphate extracts. The methods used for clarifying of pyrophosphate extracts are high-speed centrifugation (McKeague, 1967) and the addition of Superfloc, a flocculating agent, followed by centrifugation at low speed (Sheldrick and McKeague, 1975). The study of Schuppli et al. (1983) shows that centrifugation at high speed or at low speed with Superfloc is inadequate to sediment suspended material completly. Adding a salt, such as Na_2SO_4, is not effective at low concentration, and at high concentration it appears to precipitate dissolved metals. Ultrafiltration of the centrifugates through 0.025-μm filters removes particulate material in a simple and effective way. In this study, soil extracts were cleared by precipitation of suspended materials and organic matter with filtration through 0.45-μm filters. Since the study of microelements in soil fractions can answer many questions about how microelements are retained and released to plants, an experiment was undertaken to fractionate soils to investigate the amounts of Cu, Fe, Mn and Zn in organic matter and oxide bound forms.

4.2 Material and methods

Two experiments were conducted with four top soil and ten extractants for their extractable contents of trace elements.

A) Comparison between the pair of extractants

One gram of each air dried soil and 50cm^3 of each extractant (already described in section 2.7 chapter 2) were shaken for 16 hours on end over end shaker in a room temperature and filter through Whatman no 1 filter paper.

B) Comparison between the filter papers

20 cm^3 of the filtrate of each extract was also passed through a 0.45μm membrane filter. Membrane filter were never folded, handled, or damaged in any way. The effect of extractant and filter type on the extractability of Cu, Fe, Mn, and Zn were measured on atomic absorption spectrophotometer using an air acetylene flame and matrix matched standards. The extractants were chosen because they represent a wide range of different kinds of chemical attack and are commonly used by soil scientists to investigate the availability of elements to plants. The detail of these procedure and reagents used can be found in section 2.7 chapter 2.

4.3 Results and discussion

4.3.1 Comparison between different pairs of extractants

Mean values of extractable and percentage of total Cu, Fe, Mn and Zn for four soils are given in tables 4.1 - 4.4. Statistical analysis of the data by an F test showed that there was a significant difference between the various extractants in all the soils studied for each elements.

The values of copper, iron, manganese and zinc (Tables 4.1 - 4.4) indicate some differences within the same pool extracted by two different types of extractant. A Sheffe least significant difference (LSD) test was applied to the data given in Tables 4.1 - 4.4 to compare two different extractants used for the same pool of copper, iron, manganese and zinc; ie. sodium hydroxide (NaOH) versus sodium carbonate (Na_2CO_3); hydroxylamine hydrochloride (pH 1) ($NH_2OH.HCl$ pH 1) versus hydroxylamine hydrochloride (pH 2) ($NH_2OH.HCl$ pH 2); tetra sodium pyrophosphate ($Na_4P_2O_7$) versus sodium dihydrogen pyrophosphate ($Na_2H_2P_2O_7$) and ammonium ethylenediaminetetraacetic acid (NH_4EDTA) versus sodium ethylenediaminetetraacetic acid (Na_4EDTA).

The trends in changes in the extractable amounts of copper, iron, manganese and zinc were generally not similar for the four soils studied, and therefore the results for each element are discussed separately.

4.3.1.1 Soil copper

The results obtained (Table 4.1) from the average of the four soils show that the extraction power of different reagents for copper was in the following order: acid oxalate > NaOH > HCl > Na_2CO_3 > Na_4EDTA > NH_4EDTA > Na_4P2O_7 > $Na_2H_2P_2O_7$ > $NH_2OH.HCl$ (pH 1 and 2).

Although hydroxylamine hydrochloride is a mild reducing agent commonly used to mobilize trace metals adsorbed on, or occluded in manganese oxides, the extractable amounts of Cu with this reagent in these soils were not measurable. This indicates that Cu associated with Mn oxide fraction was very low. Sims (1986) also used $NH_2OH.HCl$ (pH 2) to dissolved Mn oxide fraction of the soils and found little copper in this fraction. Singh et al. (1988) reported very small amount of copper ranging from 0.00 to 0.66 mg kg^{-1} by using $NH_2OH.HCl$ in Calcarious soils of India. While, Liang et al. (1991) reported 0.6 to 1.7 mg kg^{-1} of Cu extracted with hydroxylamine hydrochloride in Saskatchewan soils.

Although there were considerable variations in the proportions of total copper present in the various fractions, on an average 50 percent of the total Cu was brought into solution by NaOH, Na_2CO_3 and strong acid (HCl). Miller et al. (1986) reported that a large proportion of Cu was extracted with 0.5M NaOH and 0.5M HCl from freeze-dried and oven dried samples of copper-enriched Swine manure. They claimed that strong acid (HCl) completely dissolved solid phase Cu from both freeze-dried and oven dried manure and the basic reagent NaOH was nearly as effective, due to their ability to peptize humic materials and solubilize them along with associated metals. Louma and Jenne (1976) stated that 0.5M HCl has been shown to dissolve copper oxide and basic copper carbonate, but only a little copper sulfide. Dolar and Keeney (1971) reported that 0.1N HCl extracted more Cu than did the 0.1M EDTA-NH_4OAC extractant.

Pyrophosphate extracted only about 30 percent of the total copper, similar to the value found by Mclaren and Crawford (1973) for the extraction of copper in this fraction. They recommended potassium pyrophosphate for removing predominantly organically bound copper because it gives better extraction of colloidal organic matter and also less solution of oxide materials. Hydroxylamine hydrochloride and pyrophosphate reagents were used by a number of workers: ie. (McLaren and Crawford, 1973; Miller et al., 1986 and Liang et al., 1991) for the extraction of Mn oxide and organic matter fraction.

Miller et al. (1986) recognized that pyrophosphate solubilized oxides and that hydroxylamine hydrochloride did not. As the Cu extracted with hydroxylamine hydrochloride was not measurable and appreciable amounts of Cu were detected by pyrophosphate, it is probable that a complexing agent such as pyrophosphate solubilized oxides. Pyrophosphate reagent has been shown to be capable of extracting most of the Cu from Mn oxides (Miller et al., 1986).

Table 4.1 Mean values of extractable Cu (µg g^{-1}) with various extractants and % of total copper in different soils.

	Soils				
Extractant	1	2	3	4	Mean
NaOH	7.03ab 63.74%	11.20bc 49.78%	9.23ab 55.24%	6.81b 40.71%	8.57 51.19%
Na_2CO_3	6.27bc 56.84%	7.31cd 32.49%	10.45ab 62.54%	5.48bc 32.76%	7.38 44.09%
$NH_2OH.HCl$ (pH-2)	0.00f 0.00%	0.00e 0.00%	0.00c 0.00%	0.00d 0.00%	0.00 0.00%
$NH_2OH.HCl$ (pH-1)	0.00f 0.00%	0.00e 0.00%	0.00c 0.00%	0.00d 0.00%	0.00 0.00%
$Na_4P_2O_7$	3.75de 34.00%	7.13d 31.69%	6.25b 37.40%	4.25c 25.40%	5.34 31.90%
$Na_2H_2P_2O_7$	2.12e 19.22%	6.25d 27.78%	5.63b 33.69%	3.87c 23.13%	4.47 26.70%
NH_4EDTA	3.92de 35.54%	9.39bcd 41.73%	7.75ab 46.38%	3.12c 18.65%	6.04 36.08%
Na_4EDTA	4.68cd 42.43%	11.83bc 52.58%	8.07ab 48.29%	3.34c 19.96%	6.98 41.70%
0.1M HCl	5.75bcd 52.13%	13.44ab 59.73%	6.79b 40.63%	4.93bc 29.47%	7.72 46.12%
A.oxalate	8.51a 77.15%	16.50a 73.33%	12.77a 76.42%	11.71a 69.99%	12.37 73.89%
Total	11.03	22.50	16.71	16.73	16.74

1 = Dreghorn 2 = Caprington 3 = Darleith 4 = Dunlop

Figures in a column with the same letter following are not significantly different at the 5 % level using a Scheffe LSD test.

The chelating agent EDTA was more effective in Cu removal than pyrophosphate from these soils. About 40 percent of total soil Cu was removed by this reagent, which indicates that this ligand is able to compete successfully for Cu with organic ligands in the soil. Bloomfield and Pruden (1975) found that much of the metal from sludges was extracted by EDTA. The results obtained here were also within the range reported by Archer and Hodgson (1987). They reported 0.5 - 75 mg dm^{-3} of Cu extracted with 0.05M EDTA and concluded that on an average EDTA extracted almost five times as much Cu as acetic acid. While, Burridge and Hewitt (1987) found 0.5 to 75 mg kg^{-1} of Cu extracted by 0.05M EDTA when they were comparing two soil-extraction procedures.

More than 70 percent of the total Cu was extracted with acid oxalate. As this reagent dissolves the amorphous Fe and Al oxides. So, the higher amount of Cu in this fraction may be due to dissolution of this fraction. LeRiche and Wier (1963); McLaren and Crawford (1973) and Liang et al. (1991) have used acid ammonium oxalate for Cu extraction from the Fe oxide fraction. Copper extracted with this reagent was much more than reported by McLaren and Crawford (1973) and Liang et al. (1991) but LeRiche and Weir (1963) foud very high amounts of Cu in the Fe Oxide fraction. Baker (1974) concluded from the literature that Cu resides mainly with inorganic fraction or in precipitated forms, as opposed to organic complexes or in primary minerals. This conclusion seem to agree with the data presented here. Gupta and Mackay (1966) reported that

0.2M ammonium oxalate (pH 3.0) solution seemed to be the most appropriate reagent for determining copper, because it gave more complete removal. Moreover, this solution has a strong buffering capacity, thus preventing any substantial fluctuations to the reaction of extracts obtained from soils of different pH values.

Total soil copper in the four soils varied from 11.03 to 22.50 mg kg^{-1}. Which falls within the range reported by others (Mclaren and Crawford, 1973; Miller et al., 1986; Archer and Hodgson, 1987; Singh et al., 1988 and Liang et al., 1991). McLaren and Crawford (1973) reported the range of 2.0 to 37.9 mg kg^{-1} of total Cu from 24 English soils. Miller et al. (1986) reported 26.9 and 18.9 mg kg^{-1} of total Cu from slurry-dried and freeze-dried samples of Swine manures. Singh et al. (1988) found 11.9 to 48.1 mg kg^{-1} of total Cu in calcarious soils of India. While Liang et al. (1991) dissolved the soil with aqua regia-HF-HNO_3 and HCl and found 6.5 to 39.0 mg kg^{-1} of total Cu from Saskatchewan soils.

4.3.1.2 Soil iron

The mean and percentage of total iron extracted by various extractants are presented in Table 4.2. It is clear from Table 4.2 that iron values in the sodium carbonate fraction are very small, ranging 31 - 140 mg kg^{-1} and in the acid oxalate fraction are very high ranging from 4450 - 18926 mg kg^{-1}.

Data given in Table 4.2 shows that genrally, NaOH, $NH_2OH.HCl$ (pH 1), $Na_2H_2P_2O_7$ and Na_4EDTA extracted more iron than Na_2CO_3, $NH_2OH.HCl$ (pH 2), $Na_4P_2O_7$ and NH_4EDTA, respectively.

On an average, 2 % and less than 1 % of the total iron was extracted with NaOH and Na_2CO_3 respectively. As the NaOH digestion does not attack the iron oxide minerals and large amounts of iron were associated with this fraction, very low quantity of Fe extracted by NaOH may be attributed to this reason. Similar observations have been obtained by Norrish and Taylor (1961) they reported that only 2% of the total iron was removed from Tasmania soil goethites with this reagent. They observed that it dissolved clays to leave iron oxides. Sodium carbonate has been used as a weaker and better buffered alkali than sodium hydroxide for mobilizing organic matter by many workers. Farmer et al. (1977) reported that cold 5% Na_2CO_3 was a partial solvent and it does not alter the Allophane and Imogolite. Sodium hydroxide extractable Fe contents were much lower (mean 0.44%) than the dithionite-citrate bicarbonate (DCB), acid ammonium oxalate and pyrophosphate (Evans and Wilson, 1985). The concentration of iron extracted by hydroxylamine hydrochloride (pH 2) ranged from 1.10 to 4.09% and by hydroxylamine hydrochloride (pH 1) ranged from 5.07 to 12.25% of the total soil Fe, which is with in the range of 2.8 to 15.3% of total soil Fe extracted with 0.25M $NH_2OH.HCl$ + 0.25M HCl reported by Singh et al. (1988). They also found very little Fe extracted with 0.1M $NH_2OH.HCl$ (pH 2, HNO_3).

Table 4.2 Mean values of extractable Fe (μg g^{-1}) with various extractants and % of total iron in different soils.

	Soils				
Extractant	1	2	3	4	Mean
NaOH	292fg 1.63%	245c 1.02%	1329fg 2.86%	420gh 0.71%	571 1.55%
Na_2CO_3	75g 0.42%	92c 0.38%	31h 0.07%	140h 0.24%	84 0.23%
$NH_2OH.HCl$ (pH-2)	497f 2.79%	979c 4.09%	829g 1.79%	657fgh 1.10%	741 2.01%
$NH_2OH.HCl$ (pH-1)	1411d 7.92%	2930b 12.25%	3192d 6.88%	3017d 5.07%	2638 7.14%
$Na_4P_2O_7$	3494c 19.61%	2233b 9.34%	10177c 21.92%	8284c 13.91%	6047 16.37%
$Na_2H_2P_2O_7$	5380a 30.19%	4218a 17.64%	12139b 26.15%	9598b 16.12%	7834 21.21%
NH_4EDTA	301fg 1.69%	760c 3.18%	1544ef 3.33%	670fg 1.13%	819 2.22%
Na_4EDTA	408f 2.29%	997c 4.17%	1903ef 4.10%	1001f 1.68%	1077 2.92%
0.1M HCl	1006e 5.65%	2299b 9.61%	2019e 4.35%	2448e 4.11%	1943 5.26%
A.oxalate	4450b 24.97%	4606a 19.26%	15918a 34.29%	18926a 31.78%	10975 29.72%
Total	17920	23916	46425	59555	36929

1 = Dreghorn 2 = Caprington 3 = Darleith 4 = Dunlop

Figures in a column with the same letter following are not significantly different at the 5 % level using a Scheffe LSD test.

Chao and Zhou (1983) found that acid hydroxylamine hydrochloride was the best extractant for amorphous iron oxides and recommended to test this reagent for use with soil samples. One obvious reservation regarding its use on soil samples is the strong acidity of this solution (pH 1), which could cause significant dissolution of other crystalline species, especially labile trioctahedral clay minerals, such as Fe-containing chlorites. And the difference between the hydroxylamine hydrochloride (pH 1 and 2) was considered to be the acidity of the extracting solution. Chao (1972) observed that increased acidity of hydroxylamine hydrochloride tended to increase dissolution of iron oxides. Ross et al. (1985) compared hydroxylamine hydrochloride with commonly used oxalate method for the extraction of amorphous or poorly crystalline Fe oxide in soil studies and claimed that amounts of hydroxylamine extracted Fe were closest to the amounts extracted with the oxalate method. They also pointed out that hydroxylamine solutions are more easily analyzed by atomic absorption than are oxalate solutions, which tend to clog the burner. In this study the amount of hydroxylamine hydrochloride extracted Fe is lower than the oxalate and pyrophosphate. A number of workers have used $NH_2OH.HCl$ for the removal of Mn-oxide fraction. As the amount of Fe extracted with $NH_2OH.HCl$ reagent was very low which indicates that Fe occluded with Mn oxide fraction was very small.

From the results it would appear that pyrophosphate gave good iron removal, extracting 9.34 to 30.19% of the total soil Fe. Although pyrophosphate extracted less Fe than acid oxalate, the quantity of Fe extracted with this reagent was much more than any other extractant. Higher amounts of Fe in the pyrophosphate fraction were also supported by Shuman (1982) who reported that $Na_4P_2O_7$ gave the same Fe values averaged over the three soils as the oxalate solution, but in two out of three soils, the $Na_4P_2O_7$ extracted more Fe than the oxalate. Organic matter is an important factor in the movement of Fe within the soil profile and the relative proportions of the two are therefore of intrest. Fe will be most intimately associated with colloidal organic matter. Pyrophosphate is effective in dispersing colloidal sesquioxide-organic matter complexes present in soils. Such complexes might have a wide range of particle sizes. This hypothesis would account for the fact that pyrophosphate extracts appreciable Fe only from soil samples containing organic matter (McKeague and Schuppli (1982). While, Bascomb (1968) reported that potassium pyrophosphate (pH 10) extracts peptizable organic matter and separates amorphous hydrous forms from the less active crystalline Fe oxides. Miller et al. (1986) used $K_4P_2O_7$ for the organic fraction of trace elements and found 0.7 to 17.6 mg kg^{-1} of Fe in this fraction. Although pyrophosphate extractable Fe content were always lower than acid oxalate contents, Kassim et al. (1984) have shown that pyrophasphate can also extract ferrihydrite from podzolic B horizons in addition to organically bound Fe.

There was no significant difference between the amounts of iron extracted with NH_4EDTA and Na_4EDTA. The amount of Fe extracted by the EDTA solutions from the various soils ranged from 1.13 to 4.17 % of the total soil Fe. Iron extracted with this reagent were much lower than reported by others: ie. Borggaard (1976), (1979) and (1981). It has been shown that EDTA can selectively extract amorphous iron oxides from soils (Borggaard, 1979, 1981) and a synthetic mixture of amorphous iron oxide, goethite and hematite (Borggaard, 1976). As pointed out previously (Borggaard, 1979), the EDTA method should also be tested on selected minerals to decide if it can serve as a reference method against which other less time-consuming methods may be tested. The same author, Borggaard (1982), used EDTA for selective extraction of amorphous iron oxides from selected silicates and found only a very small amount of total iron in this fraction. Results reported here were also in agreement with Farmer et al. (1983), they reported that 0.05M EDTA at pH 7.0 removed relatively small amounts of Fe from the soil. This reagent proved a poor extractant for the organic matter in the BS horizon soil.

Hydrochloric acid has been used to dissolve acid-soluble metals from soil. It removes less Fe from the soils than both pyrophosphate and acid oxalate. Also the amounts obtained by 0.1M HCl was less than the hydroxylamine hydrochloride (pH 1) but it extracted more

Fe than hydroxylamine hydrochloride (pH 2). The ability of hydrochloric acid to dissolve iron oxides may be attributable to the formation on the oxide surface of Fe-Cl complexes that tend to decrease the surface positive charge and thus to increase the dissolution (Sidhu et al., 1981). Chao and Zhou (1983) observed that extraction with 1M HCl would seem to provide a fairly good estimate of amorphous iron oxide in hydrous iron oxide and synthetic hydrous iron oxide. However, the extraction with 1M HCl may partially attack other components of the soils and sediments such as clays and amorphous allophanic materials (Birrell and Fields, 1952). The smaller amount of iron extracted with 0.1M HCl in these soils might be due to lower concentration of acid used. As reported by Chao and Zhou (1983), the extent of dissolution generally increased with the concentration of acid. Singh et al. (1988) also found very small amounts of iron extracted with this reagent.

The majority of the soil Fe was found to be associated with acid oxalate fraction. The percentage of total soil Fe ranged from 19.26 to 34.29% for this fraction. The acid ammonium oxalate solution has also been used in geochemical studies to dissolve amorphous iron oxides (Hoffman and Fletcher, 1981 and Sondag, 1981). The results reported here indicate that acid oxalate extracted the maximum and considerably higher percentage of Fe from all the soils than all other extractants. These results are consistent with Ross et al. (1985), who compared hydroxylamine and ammonium oxalate for the extraction of Fe from soil and found that oxalate extracted relatively larger amounts of Fe than hydroxylamine. They thought that the higher oxalate extractable iron values could be due to the presence of magnetite. But Wang et al.(1987) observed that the large differences between oxalate and hydroxylamine extractable Fe are not caused by the presence of magnetite. They thought the higher amount of Fe extracted with oxalate should be attributed to the more effective extraction of pedogenic amorphous Fe hydrous oxides by oxalate than hydroxylamine solution. Farmer et al. (1983) reported acid oxalate (pH 3) to be the most effective single extractant for the inorganic forms of translocated Fe in podzol B horizons. A slight attack by oxalate on freshly ground Fe-bearing silicates has also been demonstrated by (Arshad et al., 1972 and Borggaard, 1982) but whether such an attack also takes place in soils is still unknown. Taylor and Schwertmann (1974) considered that the high solubility in ammonium oxalate of maghemite examined by them was related to its high Fe(11) content. Borggaard (1982) observed that ammonium oxalate also attacks the crystalline iron oxides. The higher amounts of Fe extracted with oxalate reagent is possibly due to the dissolution of crystalline Fe oxides fractions.

Total iron concentrations of the soils studied were very high. The average concentrations of the total Fe dissolved by H_2SO_4-HNO_3 mixture was 17920 - 59555 mg kg^{-1}, which is within the range reported by others; Lyon et al. (1952) reported 0.5 to 5.0% of total Fe and Shuman

(1982) found 0.24 to 3.19% of total Fe in soil. Singh et al. (1988) found 1.7 to 4.8% of total Fe in soils. Levesque and Mathur (1988) reported 0.154 to 3.575% of total Fe from different organic soils.

4.3.1.3 Soil manganese

Manganese concentrations associated with each soil varied widely. The average amount of Mn and range of Mn extracted from each soil are shown in Table 4.3. The difference in the amount of Mn extracted from each soil is probably due to differences in the native levels of Mn as well as extracting procedures.

Table 4.3 shows small difference between the same pool of Mn extracted by different pairs of extractant. Although Na_2CO_3 extracted less Mn than NaOH, the difference between these two were not significant at 5% level. Hydroxylamine hydrochloride (pH 1) and $Na_2H_2P_2O_7$ extracted more manganese than $NH_2OH.HCl$ (pH 2) and $Na_4P_2O_7$ respectively, from all the soils studied. The difference between the NH_4EDTA and Na_4EDTA was not quite clear.

Soil Mn as extracted by the ten extractants also varied widely (Table 4.3). The Na_2CO_3 and NaOH removed the least manganese and the acid oxalate and $NH_2OH.HCl$ removed the most Mn from the soil. In contrast, solutions of NaOH and Na_2CO_3 were used for the removal of organic bound trace elements. The amount of Mn extracted with the

NaOH and Na_2CO_3 were very low or below the detection limit, which indicates that these extractants are less effective for the removal of Mn from soil. The rationale for alkali dissolution is that poorly-ordered inorganic materials in soil usually have larger specific surface areas than their crystalline counterparts and would have a higher dissolution rate. But in this study the dissolution of Mn is very low because most of the manganese was associated with Mn oxide and Fe oxide fractions, which were not dissolved by the alkali solutions. Heintze and Mann (1949) also observed that hydrated Mn oxides are poorly soluble in alkaline extractants.

Manganese data in Table 4.3 show that $NH_2OH.HCl$ (pH 1) extracted more managnese than the $NH_2OH.HCl$ (pH 2) and it solubilized nearly as much Mn as did the oxalate. Hydroxylamine hydrochloride extractant has been found suitable for solubilizing the Mn oxides, while leaving the Fe oxides (Chao, 1972). The percentage of Mn extracted with both $NH_2OH.HCl$ (pH 1) and $NH_2OH.HCl$ (pH 2) was much more than all other extractant, except acid oxalate, which indicates that in these soils a large proportion of Mn were associated with Mn oxide fraction. Dhangpatra et al. (1979) compared seven extractants for the estimation of plant available Mn in selected Kentucky soils and found that large amounts of Mn were extracted with hydroxylamine hydrochloride.

Table 4.3 Mean values of extractable Mn (µg g^{-1}) with various extractant and % of total manganese in different soils.

	Soils				
Extractant	1	2	3	4	Mean
NaOH	7.8f 1.45%	3.0d 0.68%	25.3e 1.85%	6.3f 0.75%	10.6 1.34%
Na_2CO_3	0.0f 0.00%	0.0d 0.00%	0.0e 0.00%	0.0f 0.00%	0.0 0.00%
$NH_2OH.HCl$ (pH-2)	274.4b 50.85%	137.2c 30.89%	518.2c 38.40%	121.9cd 14.53%	262.9 33.16%
$NH_2OH.HCl$ (pH-1)	277.7b 51.46%	233.4ab 52.53%	728.3b 53.96%	225.3b 26.85%	366.2 46.19%
$Na_4P_2O_7$	134.3d 24.87%	54.4d 12.17%	387.5d 28.69%	96.0cde 11.44%	168.0 21.19%
$Na_2H_2P_2O_7$	171.1c 31.71%	171.0bc 38.55%	405.6d 30.09%	139.4c 16.41%	221.8 27.98%
NH_4EDTA	129.0d 23.90%	158.8bc 35.85%	375.4d 27.80%	66.7de 7.95%	182.5 23.02%
Na_4EDTA	89.6e 16.60%	136.8c 30.89%	382.5d 28.39%	65.8e 7.84%	168.7 21.28%
0.1M HCl	139.3cd 25.81%	157.3bc 35.40%	350.6d 26.02%	138.5c 16.51%	196.4 24.77%
A.oxalate	393.1a 72.84%	259.4a 58.39%	1012.6a 75.09%	448.1a 53.41%	528.3 66.64%
Total	539.6	443.5	1349.1	839.0	792.8

1 = Dreghorn 2 = Caprington 3 = Darleith 4 = Dunlop

Figures in a column with the same letter following are not significantly different at the 5 % level using a Scheffe LSD test.

Same observation has been found by Shuman (1982), he also pointed out that $NH_2OH.HCl$ solubilized as much Mn as most of the other extractants indicating that it is specific for Mn oxides. The results obtained here were within the range reported by Jarvis (1984), who found 12 to 901 mg kg^{-1} of Mn extracted with $NH_2OH.HCl$ (pH 2).

The metals extracted by pyrophosphate, considered to describe the fraction bound to organic matter in soil (McKeague et al., 1971), were on the average 11.44 to 38.55% of the total soil Mn (Table 4.3). The manganese extracted by pyrophosphate is mainly divalent and complexed by organic matter (Heinze and Mann, 1949). They also reported that hydrated manganese oxides are fully soluble in neutral pyrophosphate. Pyrophosphate extracts can also contain manganese derived from oxides of higher oxidation states reduced by organic matter (Heintze, 1957). Considerable amounts of the manganese were extracted from the present soils by pyrophosphate (Table 4.3). Pyrophosphate has been used in studies of the movement of iron and associated organic matter between soil horizons (McKeague, 1967; Bascomb, 1968) and also been used to estimate organically bound Cu (McLaren and Crawford, 1973) and Mn by Iu et al. (1981) who reported high contents of organically bound manganese. They found that pyrophosphate extracted 28% of the manganese removed by a single oxalate extraction in an aerobic soil at 60% field capacity. The results reported in my study were higher than that reported by Jarvis (1984) who found 24 to 159 mg kg^{-1} of Mn extracted with 0.1M Potassium pyrophosphate. Miller et al. (1986) used 0.1M $K_4P_2O_7$ for the extraction of organically bound fraction of trace elements and found 0.3 to 11.1% of the total Mn in this fraction. Niskanen (1989) found 120 to 1910 μmol/kg of extractable Mn with 0.05M $K_4P_2O_7$ from clay and silt soils.

From the present study it appeared that EDTA as NH_4EDTA and Na_4EDTA solutions extracted nearly the same amount of Mn from the soil. Extractable Mn ranged from 7.84 to 35.85% of the total soil Mn. These results are in consistance with Levesque and Mathur (1988) who found that on an average 26.8% of the total soil Mn was extracted with 0.1M EDTA. While McNeal et al. (1985) reported very small amounts (4.9 to 44 mg kg^{-1}) of Mn in this fraction. EDTA is also used to assess organically bound Mn. The extractable contents of sodium pyrophosphate and EDTA were similar but $Na_2H_2P_2O_7$ extracted somewhat more Mn than did EDTA from the present soils. Jarvis (1984) obtained 3 to 97 mg kg^{-1} of Mn from the soil with this reagent and observed that EDTA extracted less Mn than that of the pyrophosphate. Niskanen (1989) reported 130 - 3720 μmol/kg of soil Mn extracted with 0.02M Na_2-EDTA.

Hydrochloric acid has been used to dissolve acid soluble metals from the soil and sediments. On average about 25% of the total soil Mn was dissolved by 0.1M HCl. The percentage of Mn in HCl fraction was appreciable. Hydrochloric acid extractant dissolves some of the soluble minerals by acid attack, has a strong cation-exchange potential and keeps the cations in solution by forming complexes with chloride. McNeal et al. (1985) suggested from their study that higher amount of clay present in the soil tends to have low amounts of the trace elements dissolved by HCl. This hypothesis seems to be true, because the extractable content of Dunlop soil Mn is lower than the Caprington and Dreghorn soils. Although Dunlop soil has higher total manganese than both the other two soils. The decrease in HCl extractable Mn in Dunlop soil may be due to the higher percentage of clay content in this soil. Levesque and Mathur (1988) used 0.1M HCl for extraction of Mn from soils and found Mn in the range of 2 to 222 mg kg^{-1}.

In all soils much more manganese was extracted with acid oxalate than the other extractants, the largest proportional increases were in the soils with the lowest total contents (Table 4.3). The higher Mn released must result from either reduction of highly crystalline oxides or from forms released with the dissolution of Fe oxides. As the amount of Mn extracted with $NH_2OH.HCl$ was also higher, which is more specific for dissolution of Mn oxide fraction (Chao, 1972). It is concluded from this study that additional Mn removed by acid oxalate was present in Fe oxide or crystalline Fe oxide fraction. Similar results have been obtained by Jarvis (1984), who used acid oxalate to estimate total Mn oxides and found a large proportion (115 to 2057 mg kg^{-1}) of Mn in acid oxalate fraction.

The total Mn contents of most soils range between 200 to 5000 mg kg^{-1} (Goldschmidt, 1958) and the present ranges was from 443 to 1349 mg kg^{-1} in these soils. However, Shuman (1982) found 30 to 1250 ppm of total Mn in soil. While, Jarvis (1984) found a range of 304 to 2041 mg kg^{-1} in the surface soils and from 241 to 2298 mg kg^{-1} in the sub soils.

4.3.1.4 Soil zinc

There was a large variation in the amounts of zinc extracted from soils by various extracting solutions (Table 4.4). On the whole, acid oxalate extracted the most Zn (12.86 to 61.43 mg kg^{-1}) and alkaline solutions of Na_2CO_3 and NaOH extracted the least Zn (0.00 to 5.10 mg kg^{-1}) from the soils.

The values of zinc (Table 4.4) shows some differences within the same pool of zinc extracted by two different types of extractant. Table 4.4 shows that Na_2CO_3 extractable zinc was not measurable in any soil and NaOH extracted very small amount of Zn from all the soils. While $NH_2OH.HCl$ (pH 1), $Na_4P_2O_7$ and NH_4EDTA extracted higher amounts of zinc than that of the $NH_2OH.HCl$ (pH 2), $Na_2H_2P_2O_7$ and Na_4EDTA.

On an average very small amounts (2.4%) of the total soil zinc were dissolved with alkali solution of NaOH. Which suggests that the alkaline solutions are not effective for the removal of Zn in these soils. These results are in agreement with McGrath and Cegarra (1992) who reported small proportion of Zn extracted with this solution. However, Sposito et al. (1982) found a large proportion of Zn extracted with NaOH from soils amended with sludge.

Hydroxylamine hydrochloride extracted 9.63 to 19.41% of the total Zn from the soil. Which fell within the range reported by Sims (1986) of 2 to 16% of the total zinc in this fraction. It is well known and already described that $NH_2OH.HCl$ has been used to dissolve Mn oxide fraction of the trace element, and a number of workers have used this reagent to remove Mn oxide fraction of the Zn. Singh et al. (1988) used $NH_2OH.HCl$ (pH 2) and found 0.1 to 2.6 mg kg^{-1} of zinc. Liang et al. (1991b) reported very small amount (1.9 to 2.6%) of the total Zn in Mn oxide fraction from the Sasketchewan soils.

About 10% of the total soil Zn was extracted with $Na_4P_2O_7$ and nearly 6% of the total Zn extracted with EDTA. Many workers have used pyrophosphate and EDTA to dissolve organic fraction of the soil. The $Na_4P_2O_7$ extractable Zn for all the soils studied was much higher than EDTA extractable Zn. This might be due to the dissolution of some oxide bound Zn, as the amount of acid oxalate extractable Zn for all the soils was higher. This increase in $Na_4P_2O_7$ extractable Zn may relate to the high amount of free oxide bound Zn in these soils. The partial dissolution of Mn oxides by $K_4P_2O_7$ has been reported by Iyengar et al. (1981). Shuman (1982) reported that

$Na_4P_2O_7$ also dissolves some of the Fe oxide fraction. Bakhsh (1988) compared EDTA with pyrophosphate and found that pyrophosphate extracted more Zn than EDTA. He also observed that the increase in pyrophosphate extractable Zn might be due to the dissolution of free oxide bound Zn. Haq et al. (1980) reported greater mean values for extractable Zn (57 mg kg^{-1}) with EDTA than DTPA (48 mg kg^{-1}). EDTA was reported as good chelator extractant for metals (Norvell, 1984). Fogbani, Ajayi and Ali (1985) used 0.05M EDTA and observed that it extracted more zinc ranging from 3.9 to 9.3% of the total Zn from the surface and the entire soil profile. Zinc extracted from these soils was within the range reported by McNeal et al. (1985) who found 0.2 to 14 mg kg^{-1} of Zn extracted with EDTA. Archer and Hodgson (1987) stated that the normal range of EDTA extractable zinc was 1.3 to 28 mg dm^{-3}. Zinc values extracted with NH_4EDTA were also within the range reported by Bakhsh (1988) who used 0.05M NH_4EDTA and obtained 1.85 to 16.4 mg kg^{-1} of Zn from different soils. While Levesque and Mathur (1988) reported greater mean values (17.6 mg kg^{-1}) of zinc extracted with 0.1M EDTA. More recently McGrath and Cegarra (1992) used 0.05M Na_2EDTA and found large proportion of Zn in this fraction. More than 40% of the zinc was recovered in the EDTA fraction in sludge-treated soils study by these authers.

Table 4.4 Mean values of extractable Zn (µg g^{-1}) with various extractant and % of total zinc in different soils.

			Soils		
Extractant	1	2	3	4	Mean
NaOH	1.13e 1.73%	5.10f 3.49%	3.82e 2.86%	1.08de 0.92%	2.78 2.40%
Na_2CO_3	0.00e 0.00%	0.00g 0.00%	0.00f 0.00%	0.00f 0.00%	0.00 0.00%
$NH_2OH.HCl$ (pH-2)	4.07cd 6.24%	15.22d 10.43%	12.05d 9.02%	3.17d 2.69%	8.62 7.45%
$NH_2OH.HCl$ (pH-1)	6.76b 10.36%	24.17c 16.56%	20.92b 15.66%	10.55c 8.94%	15.60 13.48%
$Na_4P_2O_7$	5.38bc 8.23%	13.25d 9.08%	17.63c 13.20%	8.38c 7.09%	11.16 9.65%
$Na_2H_2P_2O_7$	0.00e 0.00%	5.38f 3.68%	5.38e 4.02%	0.00e 0.00%	2.69 2.33%
NH_4EDTA	4.07cd 6.24%	10.21e 6.99%	10.54d 7.89%	2.32de 1.97%	6.79 5.87%
Na_4EDTA	2.87d 4.40%	9.79e 6.70%	9.16d 6.86%	1.77de 1.50%	5.90 5.10%
0.1M HCl	6.28b 9.63%	28.34b 19.41%	22.51b 16.86%	14.29b 12.11%	17.85 15.43%
A.oxalate	12.86a 19.72%	35.65a 24.42%	61.43a 46.00%	38.71a 32.80%	37.16 32.12%
Total	65.22	145.98	133.55	118.02	115.69

1 = Dreghorn 2 = Caprington 3 = Darleith 4 = Dunlop

Figures in a column with the same letter following are not significantly different at the 5 % level using a Scheffe LSD test.

The 0.1M HCl-extractable zinc ranged from 6.28 to 28.34 mg kg^{-1} with an average of 17.85 mg kg^{-1}. 0.1M HCl extracted higher amounts of zinc than all other extractants, except acid oxalate. These results are in consistance with Ponnamperuma et al. (1981), who compared HCl with EDTA and DTPA and found a large amount of Zn extracted with hydrochloric acid. They prefer HCl method because dilute hydrochloric acid is inexpensive, is readily available in developing country and is not toxic. It gives clear odorless filtrates that are easy to use in atomic absorption spectrophotometers. Greater amounts of zinc were also reported by Neilsen et al. (1987) who obtained 6.8 to 161 mg kg^{-1} zinc extracted with HCl. Saeed and Fox (1978) found that 0.1M HCl extraction of zinc to be an adequate indication of plant available Zn for highly weathered soils such as those found in many areas of the humid tropics. The picture that emerges from the above discussion and from Table 4.4 is that HCl extracts more zinc than EDTA, pyrophosphate and alkaline solution, which suggests that HCl is a slightly stronger extractant of Zn than these extractants. It could also be true that HCl not only extracts Zn from the pH-dependent sites on the organic colloids but also from Zn containing minerals, which behaviour was strongly suggested by Lauer's (1971) findings. The disadvantage of this method is that it may dissolve some oxide fraction as well.

The mean results in Table 4.4 for extractable Zn with acid oxalate ranged from 12.86 to 61.43 mg kg^{-1}, indicating a large amount of zinc is present in this fraction. Most workers used acid oxalate (Tamm's solution) for the extraction of free oxide bound metals. Shuman (1979) reported that more than 40% of the total zinc was extracted with acid oxalate. Elsokkary (1979) reported the value of zinc in this fraction ranged from 10.00 to 21.75 mg kg^{-1} in alluvial soils. Haq et al. (1980) reported 6 - 191 mg kg^{-1} of Zn extracted with acid oxalate in contaminated soils. Bakhsh (1988) reported 10.9 to 49.4 mg kg^{-1} of zinc extracted with acid oxalate.

Total soil trace elements are not directly related to plant availability, but their determination can provide a knowledge about soil development, and also useful information for the distribution of soil micronutrients within different soil pools on a percentage basis. The results given in table 4.4 show that total concentrations of Zn in four soils, dissolved by using H_2SO_4-HNO_3 mixture, ranged from 65.22 to 145.98 mg kg^{-1}. Similar amounts of total Zn were obtained by Bakhsh (1988), he found 77.05 to 127.68 mg kg^{-1} of zinc when he dissolved the soil with H_2SO_4-HNO_3 mixture. Jopony (1985) digested the soil with 4.0M HNO_3 (80°C) and found 400 µg g^{-1} of total Zn. Archer and Hodgson (1987) used the HNO_3-$HClO_4$ acid mixture for total trace metal determination of soils in England and Wales, and reported 3.9 to 975.0 mg kg^{-1} of zinc. Singh et al (1988) used HF-$HClO_4$ and HCl mixture and found 33.2 to 104.2 mg kg^{-1} of total Zn in 11 calcareous soils of India.

It is obvious from this study that every pair of extractants used for Cu, Fe, Mn and Zn extraction from the same soil pool either had different extracting ability or affected the other pools as well as their specific pool. The major reason for the extraction of organic matter and oxide bound fractions in this study was to determine their microelement contents. The Cu and Zn data are shown in Table 4.1 and 4.4. The picture that emerges from these Tables is that alkaline solution (NaOH and Na_2CO_3) extracted more Cu and 0.1M HCl removed more Zn than all other extractants, except acid oxalate from all the soils. The pyrophosphate and EDTA extractants solubilized appreciable amounts of Cu and Zn. It is intresting that $NH_2OH.HCl$ extracted considerable amounts of Zn but did not removed measurable Cu in these soils, which indicates that in these soils copper is strongly associated with organic matter and Fe oxide fraction. The amounts of Cu and Zn extracted with acid oxalate were higher than all other extractants.

The results in Table 4.2 and 4.3 indicate that pyrophosphate may extract more Fe than just that associated with the organic matter and hydroxylamine hydrochloride extracted more Mn than all other extractants, except acid oxalate but it extracted less Fe than pyrophosphate. Manganese extracted with EDTA and 0.1M HCl was reasonable, but the percentage of Fe extracted with these reagents was very small. Pyrophosphate extracted higher amounts of Fe from all the soils except acid oxalate. Acid oxalate also extracted much more Fe and Mn than all other extractants, which indicates that acid oxalate is a successful reagent for the removal of Fe oxide fraction of the microelements.

Some workers have reported that certain metals are associated with amorphous Fe oxide or crystalline Fe oxide. It is still not understood if the amount of microelements present in oxalate fraction is due to the dissolution of Fe oxide fraction. Therefore for further study on the distribution of microelements among fraction needs to include some other reagents to be studied in detail to expand the understanding of microelement soil chemistry and to assist in the search for better microelement soil extractant methods.

4.3.2 Comparison between metal concentrations in extracts following filtration through paper or membrane filters.

The concentrations of copper, iron, manganese and zinc in the four soils studied as affected by extraction with different extractants and the filtration of the solution through membrane filter are shown in Table 4.5 to 4.8. The average values of these elements obtained from all the soil extracts by filtration through 0.45 μm pore diameter were considerably lower than those obtained by the filtration through Whatman No 1 filter. Student t test was applied to the data given in Tables 4.5 to 4.8 to compare the difference between the two filter papers. A common effect resulting from the filtration of soil extracts containing Cu, Fe, Mn or Zn is that the concentrations of these elements in the filtrates were reduced for all the soils. The small increase with filtration through membrane filter in copper values with acid oxalate solution of Dreghorn and Dunlop soil (Table 4.5), Mn values with NH_4EDTA solution of Caprington soil, $Na_2H_2P_2O_7$ and $NH_2OH.HCl$ (pH 1) solutions of Dreghorn soils (Table 4.7) and also Zn values in NH_4EDTA solution of Dreghorn soil (Table 4.8) is possibly due to contamination. Similar results were obtained by Frink and Peech (1962) when they compared cellophane membranes and Whatman nos 32, 42 or ss602 for the measurement of Al. They concluded that large amounts of aluminium were lost on filtration through cellophane membrane.

There are various reasons for the reduction of microelements with filtration through a membrane filter. As many types of filter materials contain different levels of anions. Jay (1985) reported that all the filters contain leachable anion concentrations sufficient to cause contamination of environmental water samples. He further reported that all millipore filters show unacceptable levels of leachable anions with some values as high as 31 µg of NO_3^- per disk and 8.7 µg of SO_4^{2-} per disk. Elkhatib et al. (1987) conducted a study of filter materials on the anionic content of soil solution and found that millipore (HAWP 0.45 µm) filter produced unacceptable increases in the F^- and Cl^- contents of the soil solution. These anions can possibly contaminate the soil solutions during the filtration process. It is considered that this small reduction in the concentration of Cu, Fe, Mn and Zn is attributable to adsorption of these elements by the membrane filters.

John and VanLaerhoven (1976) observed that fibrous cellulose filters adsorb significant quantities of Cd^{2+}. Jardine et al. (1986) compared the commercially available and membrane filter paper varying compositions and found that many of the filter materials removed a significant portion of Al from solution (0 - 87% of added Al). The same authors, Jardine et al. (1986), reported that several filter discs when equilibrated with unneutralized and partially neutralized $AlCl_3$ removed a significant portion of Al from solution. They observed that significant quantities of F^-, PO_4^{3-} and SO_4^{2-} were present in the 0.45 µm filter disc, which removed appreciable quantities of Al from solution. They also noted that several filter materials also contained high concentrations of Cl^- and NO_3^-, though these anions are the serious contaminants of the filter disc.

Another cause of reduction on the amount of Cu, Fe, Mn and Zn in soil solution might be due to the interaction between the solutes and the membrane filters. Goenaga et al. (1987) reported that concentration of humic acid and Al were reduced with filtration through membrane. They thought that the losses of Al and humic acids from solutions resulting from filtration of synthetic solution apear to be due to interactions between the solutions and the membrane filters.

Table 4.5 Copper concentration ($\mu g\ g^{-1}$) of different extracting solution before and after filtration through 0.45µm.

		Soils			
Extractants		1	2	3	4
NaOH	A)	7.03	11.20	9.23	6.81
	B)	5.49*	9.89ns	10.33ns	10.33ns
Na_2CO_3	A)	6.27	7.31	10.45	5.48
	B)	5.49ns	4.96ns	7.84ns	3.92ns
$NH_2OH.HCl$ (pH-2)	A)	0.00	0.00	0.00	0.00
	B)	0.00	0.00	0.00	0.00
$NH_2OH.HCl$ (pH-1)	A)	0.00	0.00	0.00	0.00
	B)	0.00	0.00	0.00	0.00
$Na_4P_2O_7$	A)	3.75	7.13	6.25	4.25
	B)	3.38ns	6.63ns	6.25ns	4.25ns
$Na_2H_2P_2O_7$	A)	2.12	6.25	5.63	3.87
	B)	1.38ns	5.75ns	5.00ns	3.63ns
NH_4EDTA	A)	3.92	9.39	7.75	3.12
	B)	2.25ns	8.50ns	4.48ns	1.95ns
Na_4EDTA	A)	4.68	11.83	8.07	3.34
	B)	4.68ns	10.94ns	6.05ns	2.44ns
0.1M HCl	A)	5.75	13.44	6.79	4.93
	B)	5.08*	12.60*	6.13*	3.94*
Oxalate	A)	8.51	16.50	12.77	11.71
	B)	10.64*	16.23ns	10.11ns	13.83ns

1 = Dreghorn 2 = Caprington 3 = Darleith 4 = Dunlop

A) = Filter through Whatman no 1
B) = Filter through 0.45µm
* = Significant difference at 5% level
ns = Nonsignificant difference at 5% level

Table 4.6 Iron concentration (µg g^{-1}) of different extracting solution before and after filtration through 0.45µm.

		Soils			
Extractants		1	2	3	4
NaOH	A)	292	245	1329	420
	B)	105*	82*	1107*	268*
Na_2CO_3	A)	75	92	31	140
	B)	45*	56*	29ns	100*
$NH_2OH.HCl$	A)	497	979	829	657
(pH-2)	B)	483ns	968ns	774*	594ns
$NH_2OH.HCl$	A)	1411	2930	3192	3017
(pH-1)	B)	1392ns	2852ns	3085ns	2871ns
$Na_4P_2O_7$	A)	3494	2233	10177	8284
	B)	3239ns	1943*	9051*	7517*
$Na_2H_2P_2O_7$	A)	5380	4218	12139	9598
	B)	4467*	3304ns	10246*	8236*
NH_4EDTA	A)	301	760	1544	670
	B)	290ns	749ns	1514ns	665ns
Na_4EDTA	A)	408	997	1903	1001
	B)	369ns	862*	1823ns	923*
0.1M HCl	A)	1006	2299	2019	2448
	B)	981*	2296ns	2018ns	2422*
Oxalate	A)	4450	4606	15918	18926
	B)	4199*	4575ns	14602*	17422*

1 = Dreghorn 2 = Caprington 3 = Darleith 4 = Dunlop

A) = Filter through Whatman no 1
B) = Filter through 0.45µm
* = Significant difference at 5% level
ns = Nonsignificant difference at 5% level

Table 4.7 Manganese concentration ($\mu g\ g^{-1}$) of different extracting solution before and after filtration through 0.45µm.

Extractants		Soils 1	2	3	4
NaOH	A)	7.8	3.0	25.3	6.3
	B)	6.9ns	1.9*	24.1ns	6.3ns
Na_2CO_3	A)	0.0	0.0	0.0	0.0
	B)	0.0	0.0	0.0	0.0
$NH_2OH.HCl$	A)	274.4	137.2	518.2	121.9
(pH-2)	B)	259.1*	99.1*	480.1ns	109.8*
$NH_2OH.HCl$	A)	277.7	233.4	728.3	225.3
(pH-1)	B)	285.7ns	221.3ns	696.2ns	193.2
$Na_4P_2O_7$	A)	134.3	54.4	387.5	96.0
	B)	129.8ns	49.1*	250.0*	95.9ns
$Na_2H_2P_2O_7$	A)	171.1	171.0	405.6	139.4
	B)	259.9*	152.1ns	418.3	171.1
NH_4EDTA	A)	129.0	158.8	375.4	66.7
	B)	121.6ns	161.3ns	372.5	66.5
Na_4EDTA	A)	89.6	136.8	382.5	65.8
	B)	80.9ns	133.1ns	380.0ns	58.3ns
0.1M HCl	A)	139.3	157.3	350.6	138.5
	B)	137.8*	156.8ns	349.4ns	135.5
Oxalate	A)	393.1	259.4	1012.6	448.1
	B)	352.2ns	250.0ns	901.0*	441.8

1 = Dreghorn 2 = Caprington 3 = Darleith 4 = Dunlop

A) = Filter through Whatman no 1
B) = Filter through 0.45µm
* = Significant difference at 5% level
ns = Nonsignificant difference at 5% level

Table 4.8 Zinc concentration ($\mu g\ g^{-1}$) of different extracting solution before and after filtration through 0.45μm.

Extractants		Soils 1	 2	 3	 4
NaOH	A)	1.13	5.10	3.82	1.08
	B)	0.00*	2.58*	2.74*	0.00*
Na_2CO_3	A)	0.00	0.00	0.00	0.00
	B)	0.00	0.00	0.00	0.00
$NH_2OH.HCl$	A)	4.07	15.22	12.05	3.17
(pH-2)	B)	4.19ns	14.63ns	11.00*	3.12ns
$NH_2OH.HCl$	A)	6.76	24.17	20.92	10.55
(pH-1)	B)	6.28ns	22.87*	19.85ns	9.66ns
$Na_4P_2O_7$	A)	5.38	13.25	17.63	8.38
	B)	5.00ns	11.38*	16.63*	7.00ns
$Na_2H_2P_2O_7$	A)	0.00	5.38	5.38	0.00
	B)	0.00	0.38*	2.25*	0.00
NH_4EDTA	A)	4.07	10.21	10.54	2.32
	B)	4.31ns	9.93ns	10.33ns	1.85ns
Na_4EDTA	A)	2.87	9.79	9.16	1.77
	B)	1.76*	8.42ns	8.01ns	1.02ns
0.1M HCl	A)	6.28	28.34	22.51	14.29
	B)	5.40*	26.88*	21.60*	13.41*
Oxalate	A)	12.86	35.65	61.43	38.71
	B)	12.86ns	34.12ns	43.11*	36.05ns

1 = Dreghorn 2 = Caprington 3 = Darleith 4 = Dunlop
A) = Filter through Whatman no 1
B) = Filter through 0.45μm
* = Significant difference at 5% level
ns = Nonsignificant difference at 5% level

The term particulate describes the matter retained by the membrane filter. Removal of micro-particulates by the filter elements may have been due to mechanical retention of perticles greater than 0.45 µm in diameter. Metal associated closely with these particles could not pass through membrane filter and the reduction in the concentration of these elements might be due to this reason. This result was consistent with the hypothesis that the reduction in Al concentration was due to the removal of micro-particulate, retained by 0.025 µm in diameter (Menzies et al., 1991). Sharp (1973) reported that 0.3 to 3.1 % of the total carbon retained by 0.8 µm pore size membrane filter. He claimed that retention of organic carbon by this membrane filter was the particulate class of the matter. Ultra filtration of the centrifugates through 0.025µm filters removes particulate material in a simple and effective way from the precipitated iron of $K_4P_2O_7$ and $Na_4P_2O_7$ extractants (Neskenan 1989).

Another possible cause of reduction of microelement with the filtration through membrane filter might be due to the complexation of metals with colloidal particles. Vold and Vold (1966) defined that colloidal particles are those between about 1 µm and 1 nm in their smallest dimensions. Sharp (1973) reported that colloidal matter consists of fine particles in the approximate range of 0.001 to 1.0 µm. He used 0.025 µm and 0.003 µm membrane filter for the removal of colloidal class 1 and colloidal class 2 and found that 10 and 20 % of the total organic carbon, respectively were retained by these membrane filters. Bascomb (1968) reported that as much as half of the Fe extracted in 0.1M potassium pyrophosphate at pH 10 was unable to pass through 10 μm millipore filter and suggested that this is due to the precipitation of organically bound iron.

As filtration of soil solution through pore diameters of less than 0.45 μm resulted the reduction of total Cu, Fe, Mn and Zn concentration in most of the samples, ultra-filtration should be considered in further study of these elements. Although, the amount of microelements reduced considerably with the filtration through membrane filters, the difference between the two filtrates were not significant statistically at 5% level for most of the solutes. Therefore it is also suggested to include smaller size pore diameters membrane filters for further study of these elements.

Chapter 5

Uptake of heavy metals by ryegrass

5.1 Introduction

The micronutrient cations required by field crops include copper, iron, manganese and zinc. The distribution of these cations in different fractions varies considerably in soils. Consequently, the effects of soil properties on the distribution and uptake of Cu, Fe, Mn and Zn have been reported by various workers (Sims and Patrick, 1978; Iyengar et al., 1981; Shuman, 1985; Neilsen et al., 1986 and Sims, 1986).

Micronutrient deficiencies often develop on certain crops growing in areas where modern agricultural technology is used for increasing food production per unit area. Imbalance of nutrients in soils may cause yield reduction due to their antagonistic effect, while balanced application of micronutrients has a marked influence on the crop yields. Soils deficient in micronutrients may respond to the application of Cu, Fe, Mn and Zn individually or in combination. Continuous cultivation, leaching and NPK fertilizer application may bring about deficiency symptoms.

Soil tests employing chemical extractants are generally preferred because of their convenience and despite the difficulty of selecting a reagent that removes similar proportions of an element from the soil as does the plant root. A wide range of extractant solutions has been employed to extract the different forms of heavy metal ions from the soil, but in the absence of knowledge of the forms taken up by plant roots, such studies have been largely empirical and based on a trial and error approach.

The treatment of soils with solutions of neutral salts removes metal ions that are present in soil solution and adsorbed on exchange sites, though the recognition of exchange sites for heavy metals is difficult. Reagents used for this purpose include dilute solutions of ammonium acetate, ammonium nitrate, potassium chloride, magnesium chloride and calcium chloride. Dilute acid extractants remove a greater proportion of the less-readily exchangeable ions such as those held on specific sorption sites. The extent to which the more strongly bound of these ions are displaced depends upon the strength of the acid used. Acid solutions may also dissolve soil carbonates and sesquioxides to release occluded cations that would not normally be available to plants.

Dilute acids frequently used as soil extractants include acetic, hydrochloric, nitric and sulphuric acid, both individually and in mixtures. Large quantities of trace elements are dissolved from soils by concentrated acids through destruction of soil minerals and organic matter, releasing firmly bound and structural ions that would not be available to plants. A number of workers, however, have found that values for heavy metals extracted in this way, and also total soil contents of these elements, may be useful indicators of plant uptake in some situations.

Information on the relative demand of particular crop rotations for micronutrients is needed. The deficiency of scientific information on the effect of continuous cropping on the availability of Cu, Fe, Mn and Zn in soils prompted this study.

Soil and grass samples were analysed to measure the soluble Cu, Fe, Mn and Zn extrcated by different extractants in the hope that this might reveal factors influencing the availability of these metals to plants.

The main objective of present study was to assess the effect of continuous cropping on the distribution of Cu, Fe, Mn and Zn in soils and the relationship between the amount of these elements in the soil with plant uptake.

5.2 Materials and methods

The soils and plant tissue analysed in the present study were from the experiment conducted by A. Bakhsh (1988). The five soils were (1) Dreghorn (Arkleston) (2) Dreghorn (Auchincruive) (3) Midelney (Top soil) (4) Midelney (Sub soil) and (5) Zinc contaminated soils. Fresh samples of the five soils in the pot experiment collected in Spring 1986. Four soils were sampled approximately 0 - 20 cm depth, and Midelney (subsoil) was taken from below

20 cm depth. All fresh soils were passed through a 2 mm stainless steel sieve.

Pot culture technique

400 g (on oven dry basis) of each fresh soil was weighed 10.0 cm internal-diameter plastic pots fitted with saucers. For all soils there were 17 replicates in which grass was grown, and 3 replicates having no grass, in a latin square design having four blocks (see next page). 0.5 g perennial ryegrass (Lolium perenne) was sown in each pot. Deionized water was supplied via the saucers, and this watering procedure was adopted throughout the experiment, except in some cases when the surfaces of Midelney (topsoil and subsoil) pots remained dry, then additional water was added to the top of the pot.

After the germination of grass, fertilizer was applied as NPK and Mg in solution, at the rate of 100, 50, 100 and 5 mg per pot respectively. The source of nutrients were NH_4NO_3 for N, KH_2PO_4 and KCl for P and K, and $MgSO_4.7H_2O$ for Mg.

After each harvest the same dose of nutrients was applied to the cropped pots in solution from the top using a 10 cm^3 pipette. During the winter, supplementary lighting was provided by large fluorescent tubes held 0.75 m above the pots. It was observed that the lighting allowed the grass to grow at half the mid-summer rate. Yield was measured from block 1 up to the 4th harvest by cutting the grass 3.0 cm above the soil for all except Midelney (subsoil) which was cut 4.0 cm above the soil. In order to enhance the vegetative growth of grass, this height was increased by 1.0 cm from the 5th harvest for each soil. This initial difference of one cm in height for Midelney (subsoil) was due to its compaction with watering and so the soil level was below the edge of the pots. The block of 25 pots used for yield measurement and grass zinc analysis was discarded after the 4th cutting as growth had ceased, and later three replicates (one for each of blocks 2, 3 and 4) were used for yield and grass zinc measurements. Three pots for all soils, one from each block (2, 3 and 4) were sacrificed at the ist, 3rd, 6th and 8th harvest to measure zinc in soil, while subsamples from control pots were also taken at the same time. Grass was dried in an oven at 80°C for 48 hours before measuring the yield. Detailed description of these soils are available in his thesis (Bakhsh, 1988). 5.0 g of air dried soil was extracted with 50 cm^3 of each extractant, ie. 0.05M $CaCl_2$, 2.5% acetic acid, 0.05M NH_4EDTA and acid oxalate (Tamm's solution), for exchangeable, specific adsorbed, organic bound and free oxide fraction of Cu, Fe, Mn and Zn. Plant material was extracted by the wet pressure digestion method adopted by Adrian and Stevens (1973) and used for calcium determinationn by Adrian and Stevens (1977). The detailed descriptions of these procedures are available in section 2.5.2 and 2.8.1.

Determination of Cu, Fe, Mn and Zn of all the soil samples and grass tops were made with a flame Atomic Absorption Spectrophotometer using appropriate standard solutions.

Layout for pot experiment

Design = latin square

Block 1

D4	C3	A1	E5	B2
E2	D1	B4	A3	C5
A5	E4	C2	B1	D3
C1	B5	E3	D2	A4
B3	A2	D5	C4	E1

Block 2

C5	E2	D1	B4	A3
D3	A5	E4	C2	B1
B2	D4	C3	A1	E5
A4	C1	B5	E3	D2
E1	B3	A2	D5	C4

Block 3

A1	D4	E5	B2	C3
B4	E2	A3	C5	D1
C2	A5	B1	D3	E4
E3	C1	D2	A4	B5
D5	B3	C4	E1	A2

Block 4

B1	A5	C2	D3	E4
E5	D4	A1	B2	C3
C4	B3	D5	E1	A2
D2	C1	E3	A4	B5
A3	E2	B4	C5	D1

A = Dreghorn (Earliest)

B = Midelney (topsoil)

C = Midelney (subsoil)

D = Dreghorn (Auchincruive)

E = Zinc contaminated soil

Table 5.1 Properties of test soils (Bakhsh, 1988)

Soil Series	pH water[1]	pH $CaCl_2$[1]	% LOI[2]	% Clay[3]	% iron oxide[4]
1. Dreghorn (Auchincruive)	5.8	4.8	5.0	8.1	0.40
2. Dreghorn (Arkleston)	5.6	4.8	5.0	15.7	0.39
3. Midelney (topsoil)	7.4	7.0	8.4	40.4	0.59
4. Midelney (subsoil)	8.0	7.5	3.2	53.0	0.41
5. Zn contaminated soil	6.3	5.6	10.1	24.4	0.82

1. pH measured in given solution at given ratio using a combination pH electrode.
2. Loss on ignition, 450°C for 6h.
3. Clay content measured by pipette method.
4. Oxalate extractable iron oxide, measured by oxalate extracttion.

5.3 Results and Discussion

Mean values of Cu, Fe, Mn and Zn extracted by the different extractants from the soils in the grass and control pot samples at different sampling times are presented in Figures 5.1ab to 5.20ab.

The trends in changes in the extractable amounts of metals by $CaCl_2$, acetic acid, EDTA and acid oxalate were generally not similar for the four elements studied and therefore, the results of the soil analysis were described separately for each element.

5.3.1 Soil Copper, iron, manganese and zinc

Figures 5.1ab - 5.3ab show that no measurable copper was extracted with $CaCl_2$ and acetic acid from both grass and control pots soil of Dreghorn (Arkleston), Dreghorn (Auchincruive) and Midelney (Top soil). EDTA and acid oxalate extractable Cu show a large decrease in the first 10 weeks and there was no difference between the later samplings for both grass and control pot samples of these soils. Midelney (Sub soil) (Figures 5.4ab) also showed no measurable Cu with $CaCl_2$, acetic acid and EDTA for each time of sampling, except EDTA extracted Cu initialy, while acid oxalate extracted Cu showed a large decrease in the first 10 weeks and did not show any difference between the other times of sampling for both the pots. Figures 5.5ab show that copper in the Zinc contaminated soil extracted with $CaCl_2$ was also below the detection limit. Acetic acid extracted Cu remained unchanged with time, EDTA extracted Cu showed a small increase in the initial sampling period and then remained unchanged over the later sampling, while acid oxalate extracted Cu decreased rapidly from over the first 10 weeks and did not show any distinction after that for both the pots.

Figure 5.1 to 5.5.

Represent the extractable Cu in grass pot and control pot samples of Dreghorn (Arkleston), Dreghorn (Auchincruive), Midelney (Top soil), Midelney (Sub soil) and Zinc contaminated soils.

Error bars

Represent the 95% confidence interval for three(3) replicates measurements

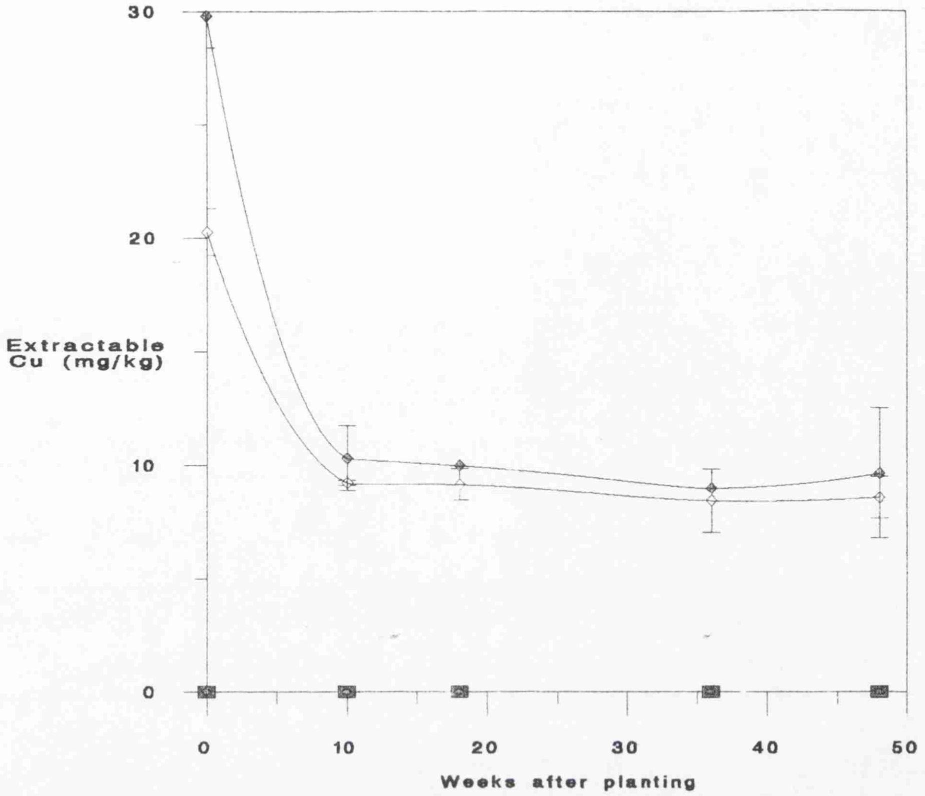


Fig 5.1a Grass pot samples of Dreghorn (Arkleston) soil

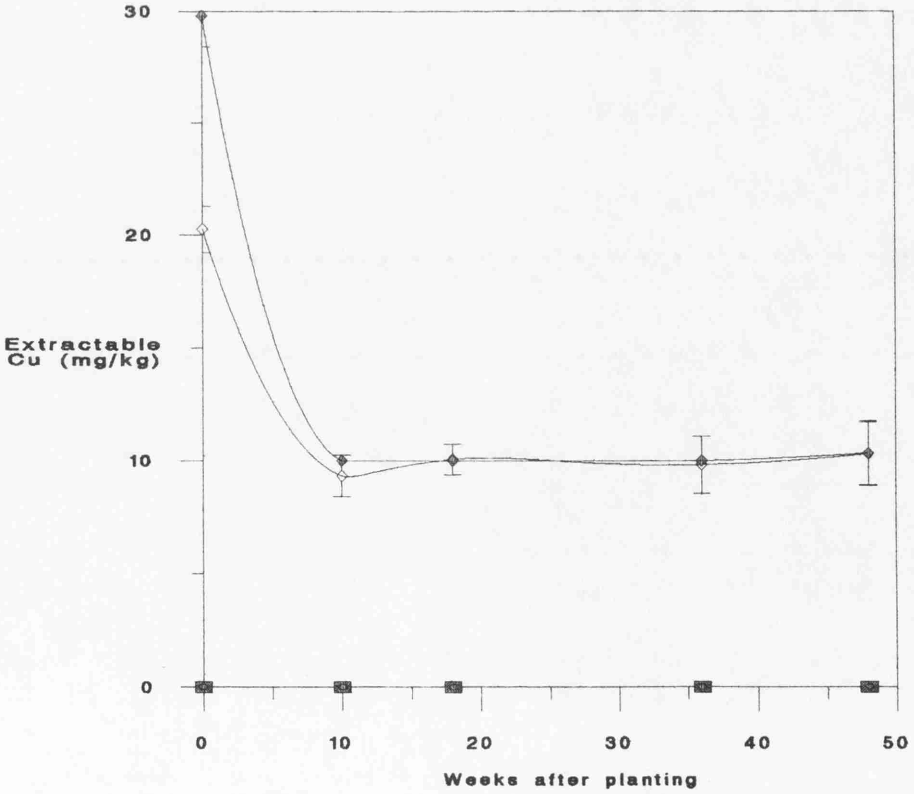


Fig 5.1b Control pot samples of Dreghorn (Arkleston) soil

⊡ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

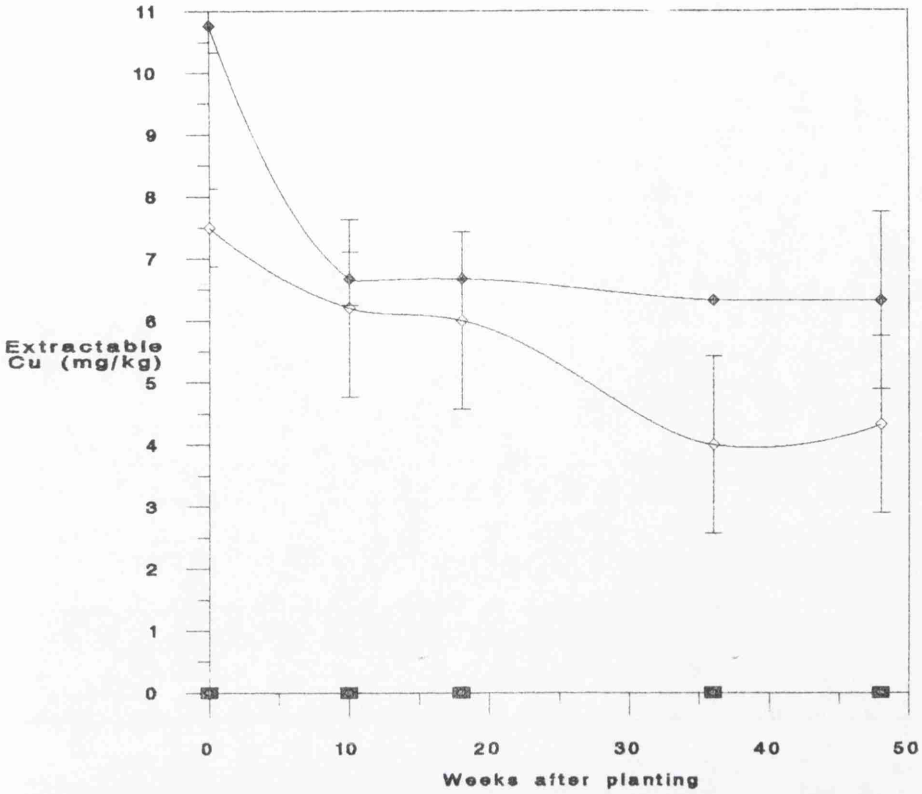


Fig 5.2a Grass pot samples of Dreghorn (Auchincruive) soil

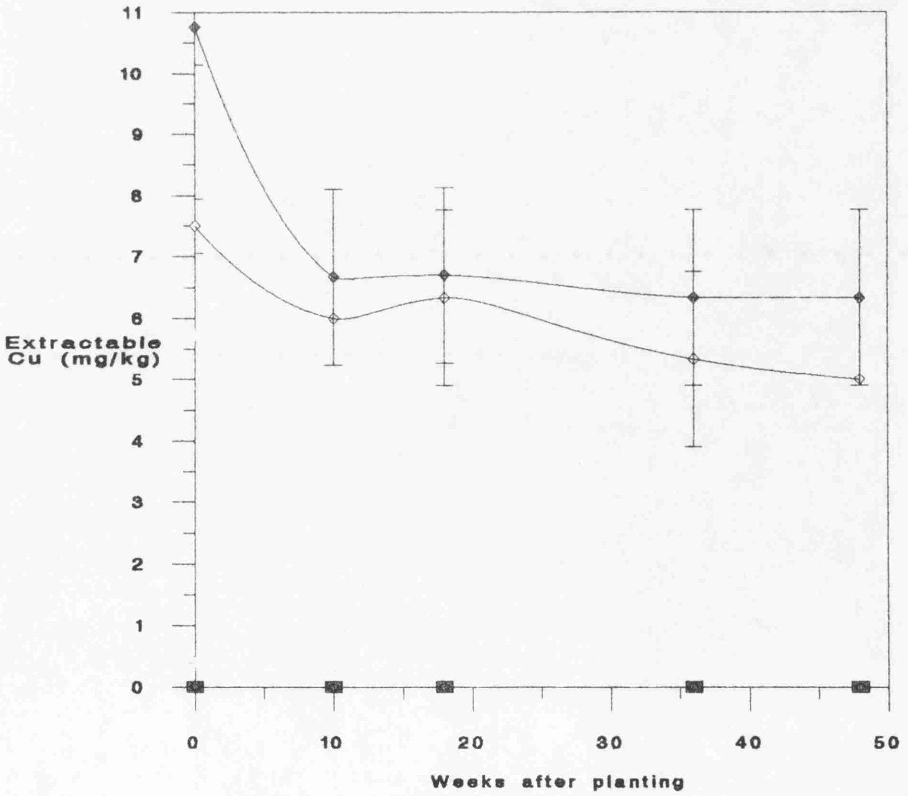


Fig 5.2b Control pot samples of Dreghorn (Auchincruive) soil

⊠ Calcium chloride
▦ Acetic acid
◇ EDTA
◈ Acid oxalate

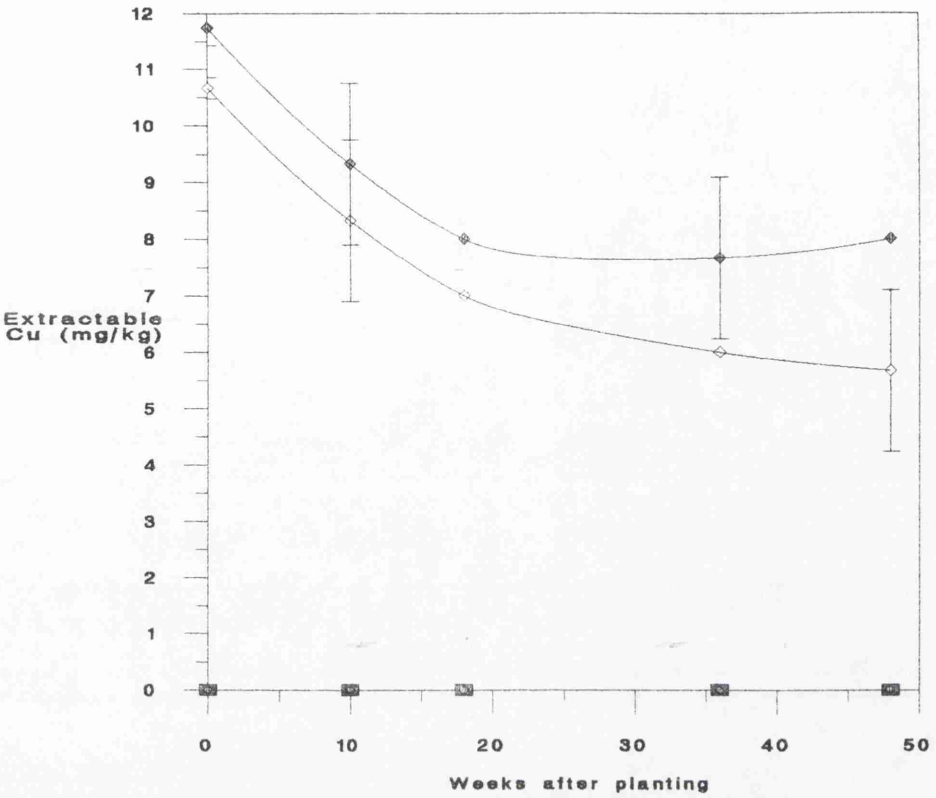


Fig 5.3a Grass pot samples of Midelney (Top soil)

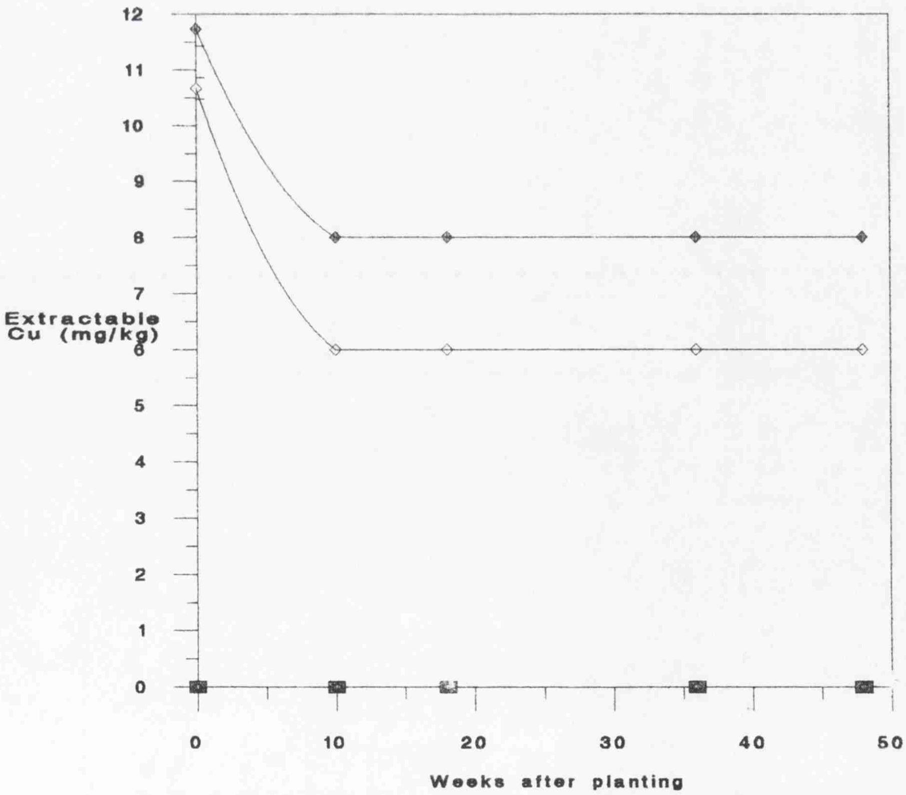


Fig 5.3b Control pot samples of Midelney (Top soil)

▣ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

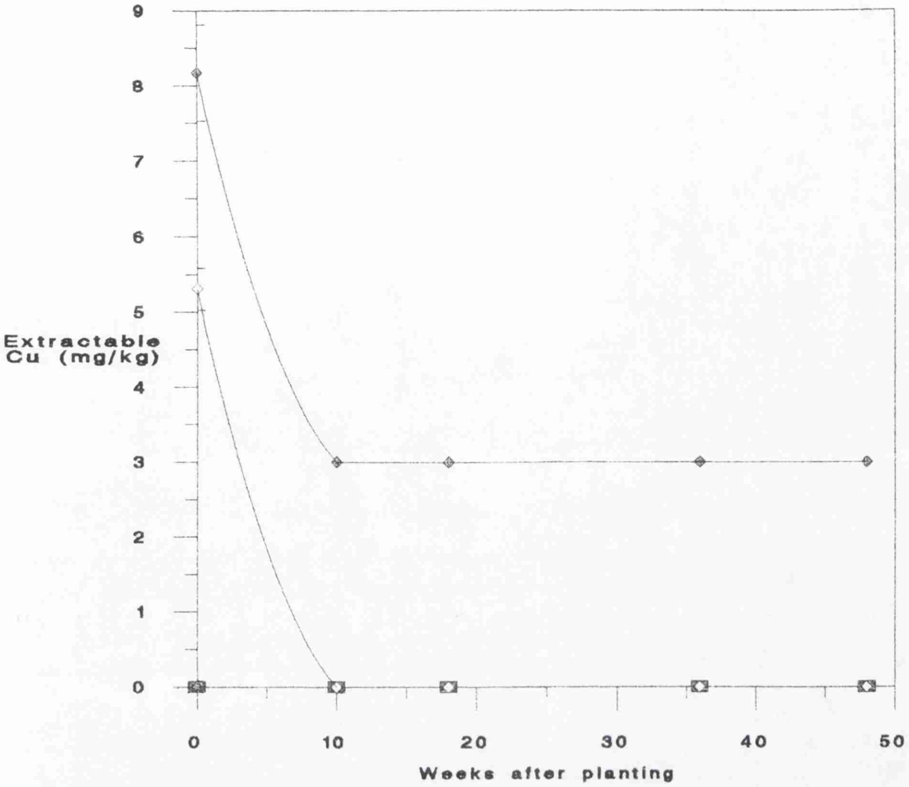


Fig 5.4a Grass pot samples of Midelney (Sub soil)

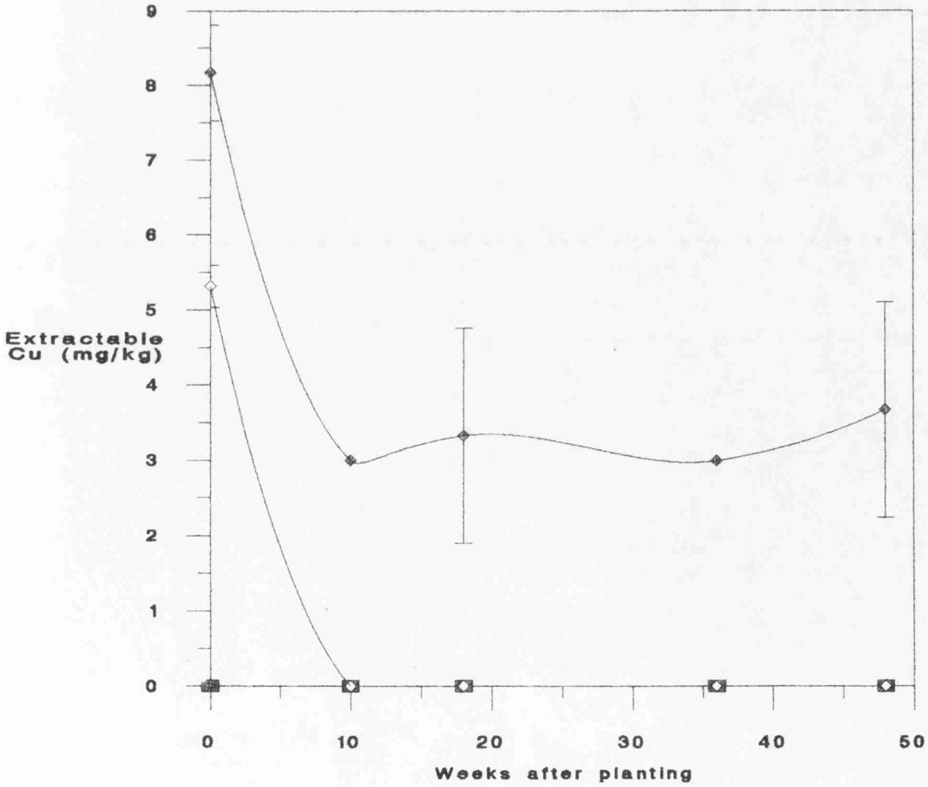


Fig 5.4b Control pot samples of Midelney (Sub soil)

▣ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

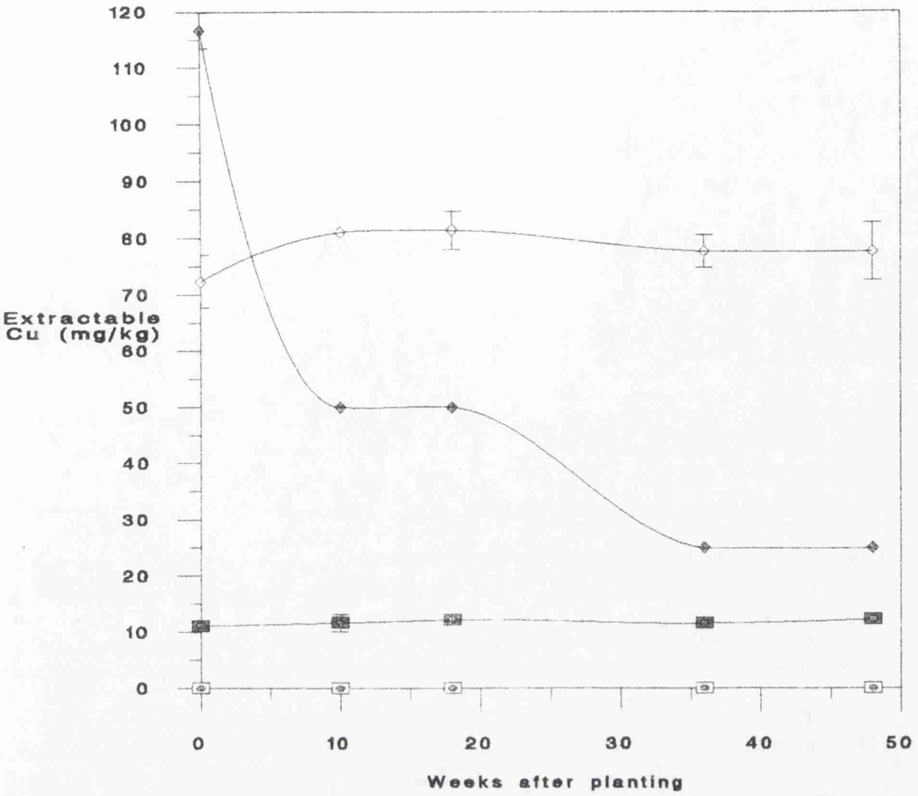


Fig 5.5a Grass pot samples of Zinc contaminated soil

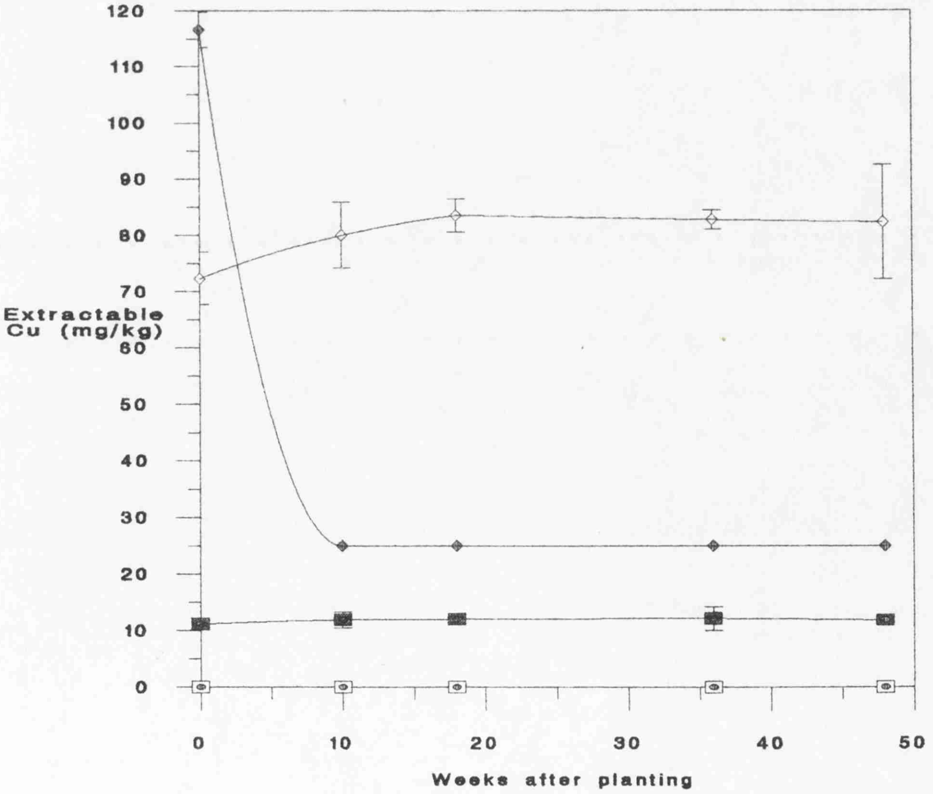


Fig 5.5b Control pot samples of zinc contaminated soil

⊡ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

Figures 5.6ab to 5.10ab shows that $CaCl_2$ and acetic acid extractable Fe was not measurable from both grass and control pots for all the soils. Dreghorn (Arkleston) soil Fe extracted with EDTA showed a little increase with time for both the pots, and acid oxalate extracted Fe in this soil increased over the first 10 weeks and did not show any clear difference at the later sampling times for both pots. Dreghorn (Aucincruive) and Zinc contaminated soil Fe extracted with EDTA was not affected with time, while acid oxalate extracted Fe increased in the earlier sampling times, and thereafter it remained unchanged. Midelney (Top soil) and Midelney (Sub soil) Fe extracted with EDTA and acid oxalate also increased from over the first 10 weeks and did not shows any difference for both grass and control pots at the later sampling times.

Figure 5.6 to 5.10.

Represent the extractable Fe in grass pot and control pot samples of Dreghorn (Arkleston), Dreghorn (Auchincruive), Midelney (Top soil), Midelney (Sub soil) and Zinc contaminated soils.

Error bars

Represent the 95% confidence interval for three(3) replicates measurements

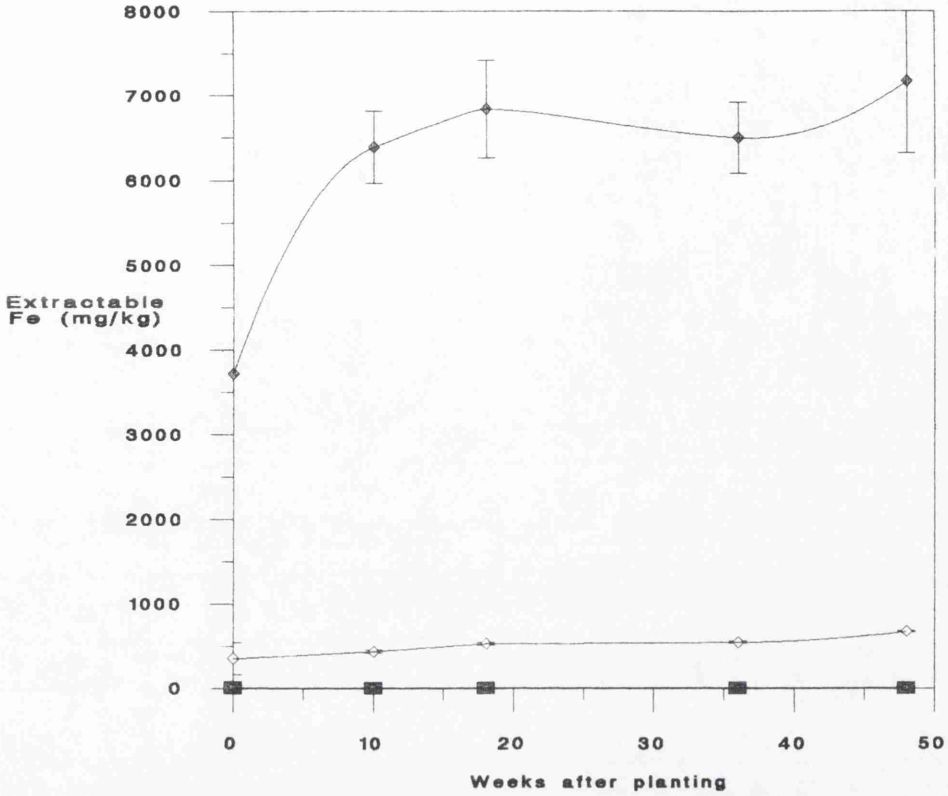


Fig 5.6a Grass pot samples of Dreghorn (Arkleston) soil

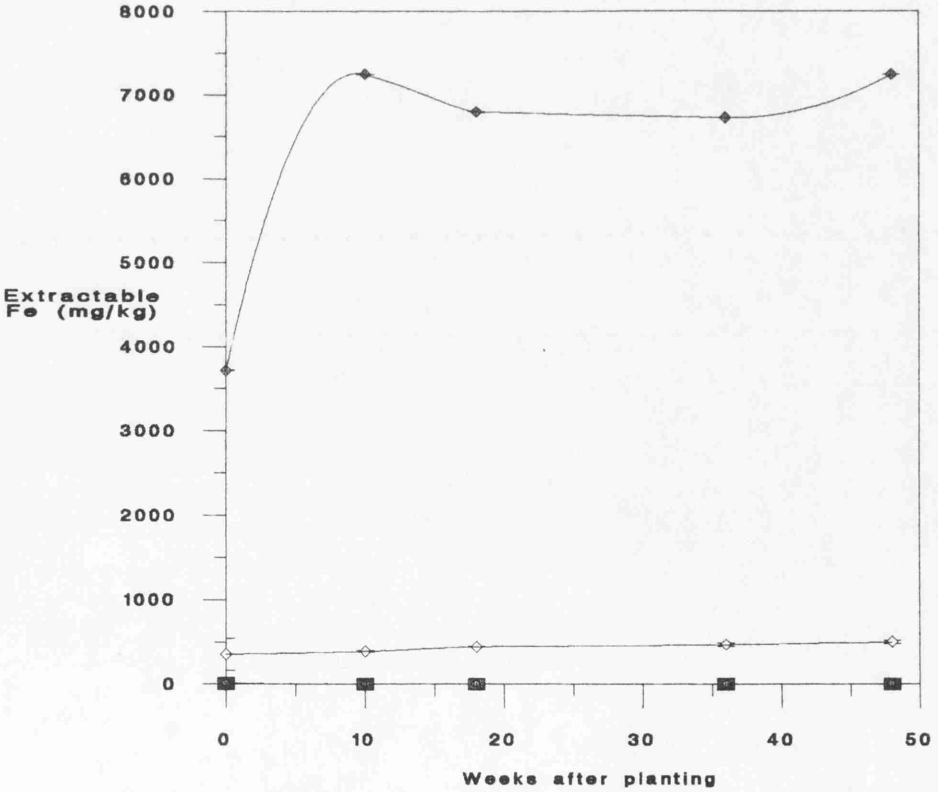


Fig 5.6b Control pot samples of Dreghorn (Arkleston) soil

▣ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

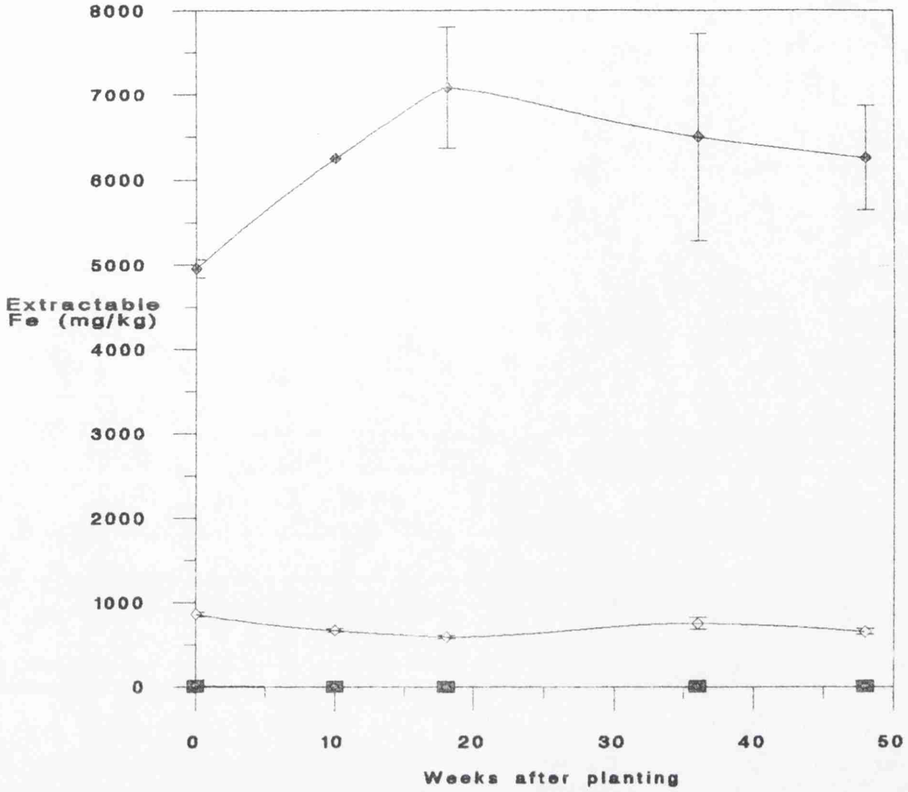


Fig 5.7a Grass pot samples of Dreghorn (Auchincruive) soil

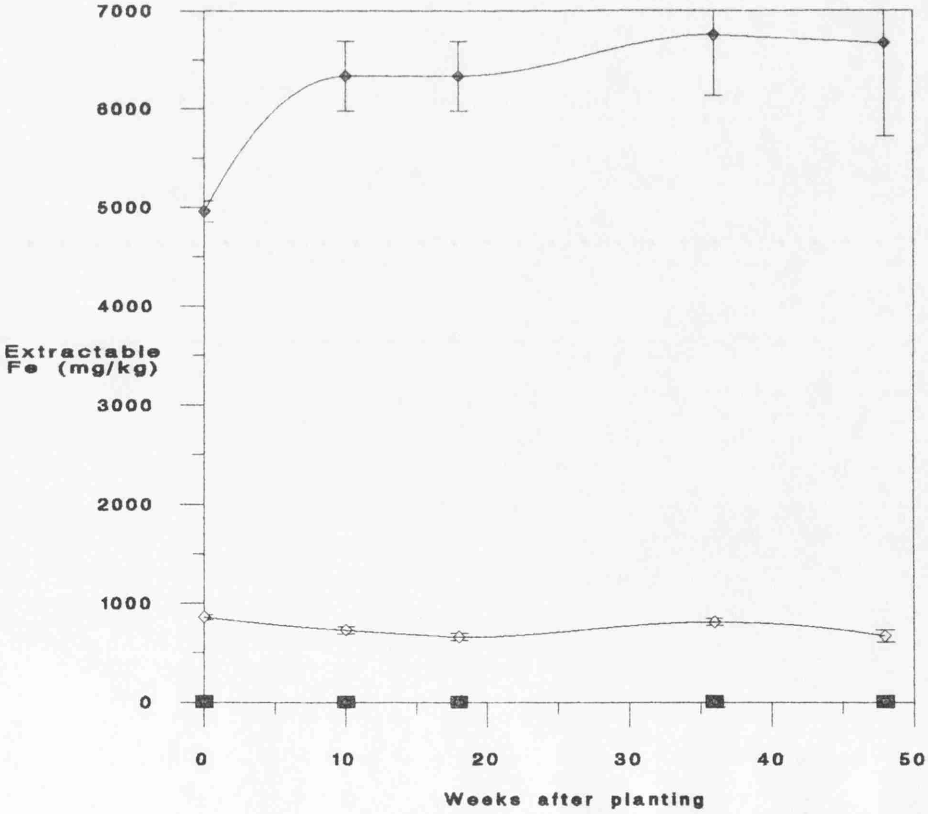


Fig 5.7b Control pot samples of Dreghorn (Auchincruive) soil

▣ Calcium chloride
■ Acetic acid
◇ EDTA
◈ Acid oxalate

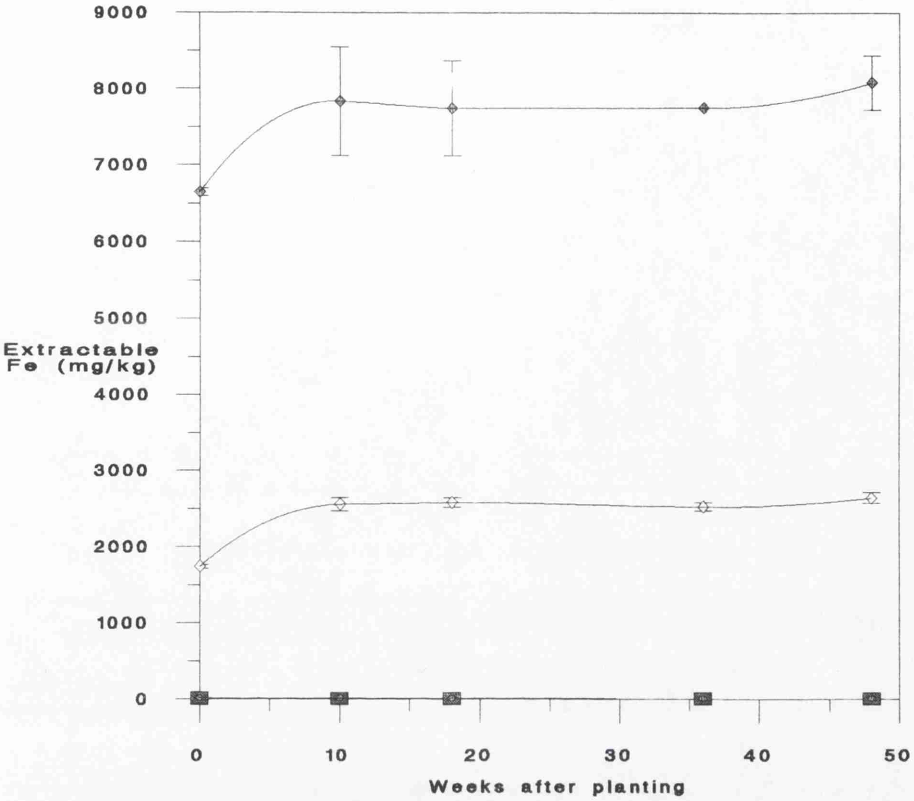


Fig 5.8a Grass pot samples of Mideiney (Top soil)

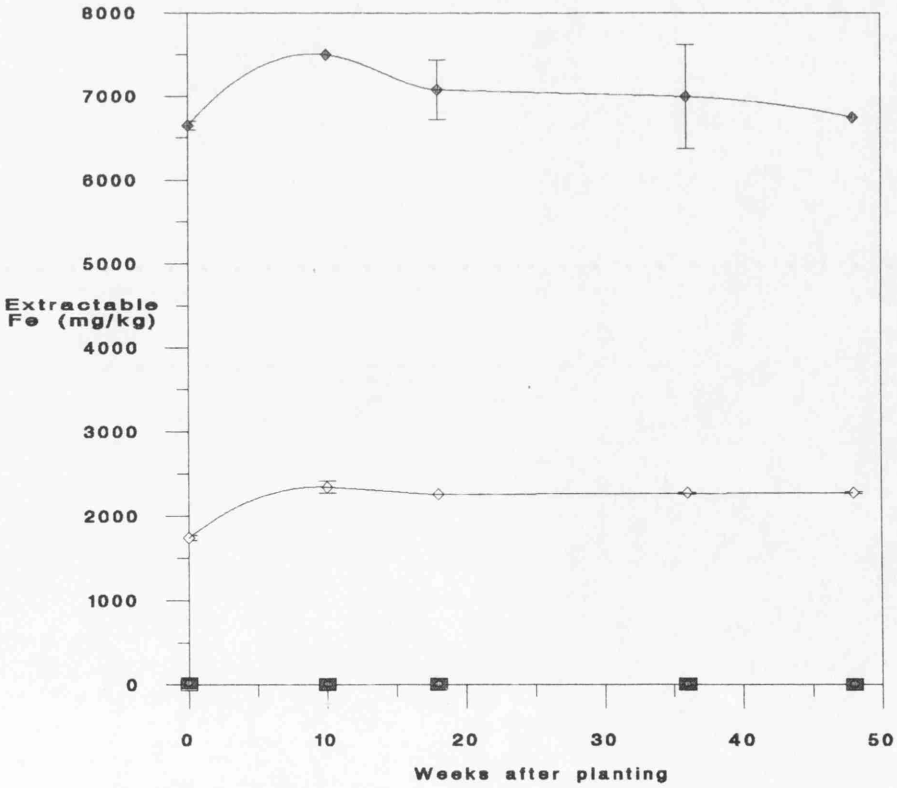


Fig 5.8bControl pot samples of Mideiney (Top soil)

▣ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

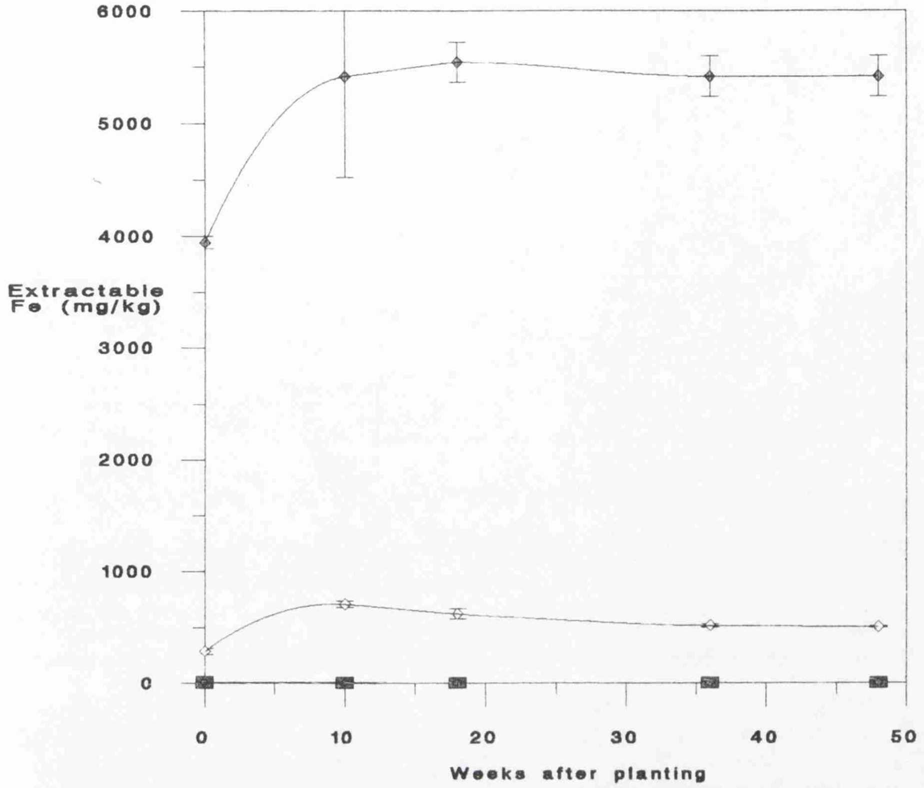


Fig 5.9a Grass pot samples of Midelney (Sub soil)

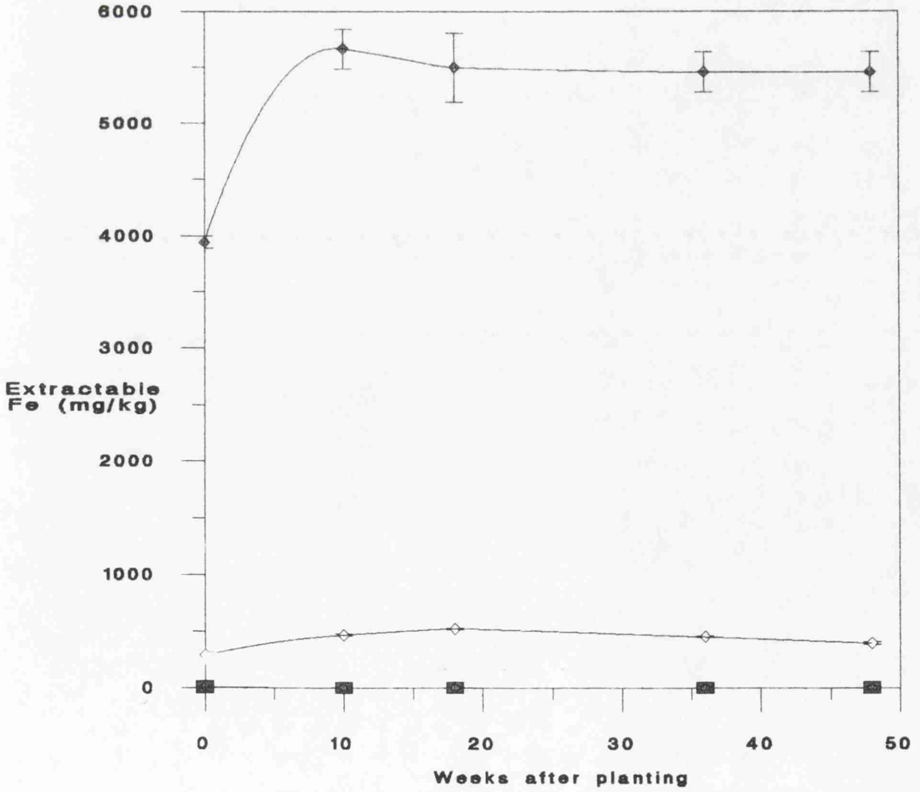


Fig 5.9b Control pot samples of Midelney (Sub soil)

▣ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

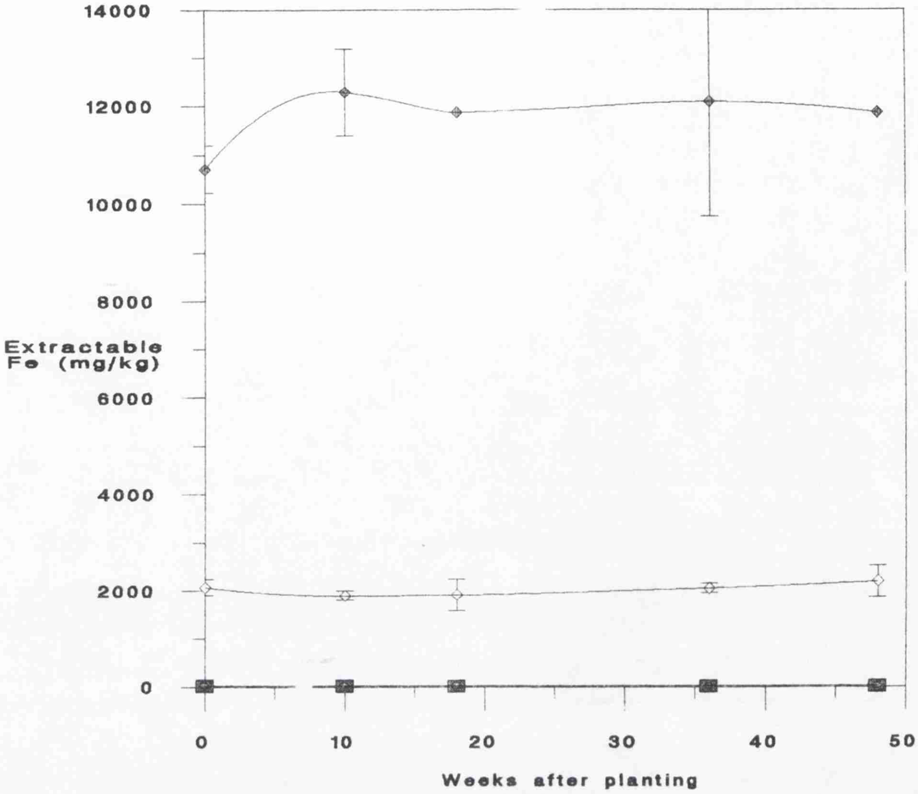


Fig 5.10a Grass pot samples of zinc contaminated soil

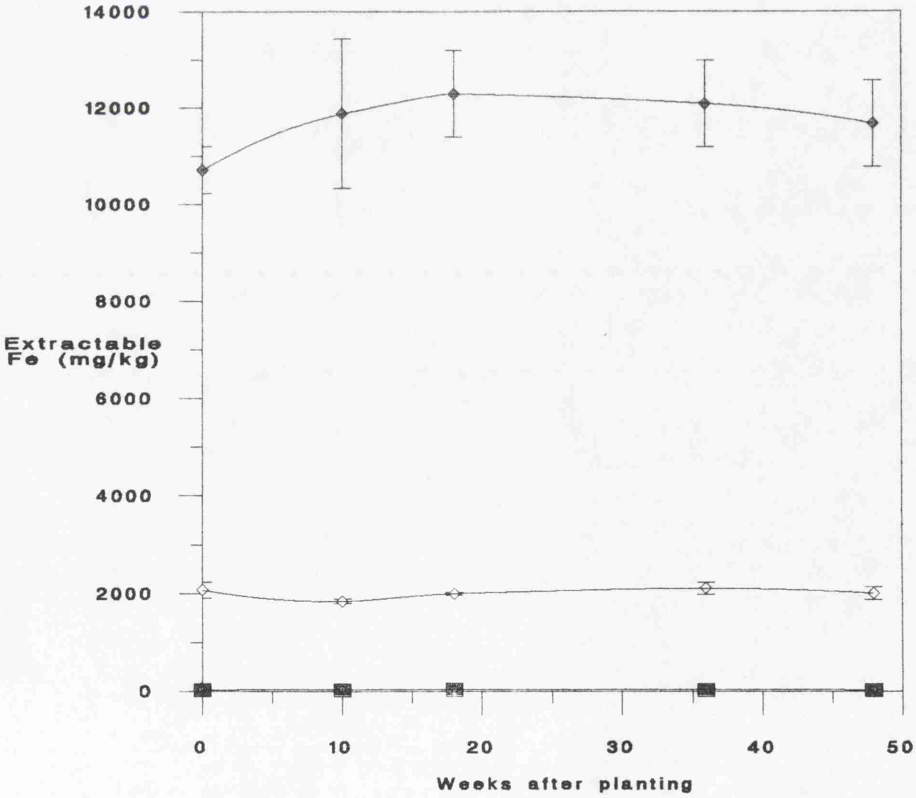


Fig 5.10b Control pot samples of zinc contaminated soil

▣ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

Figures 5.11ab to 5.15ab shows that Dreghorn (Arkleston) soil Mn extracted with $CaCl_2$ and acetic acid remained unchanged with time, whereas EDTA and acid oxalate extracted Mn increased over the first 10 weeks and did not show any differences at the other date of sampling for both grass and control pots. Dreghorn (Auchincruive) soil Mn extracted with $CaCl_2$, acetic acid and EDTA increased initially and remained unchanged with the later times of sampling, while acid oxalate extracted Mn in this soil showed a little increase with time for both grass and control pots. Extractable amounts of Mn in Midelney (Top soil) with all the extractants increased over the first 10 weeks and did not show any difference at the later times of sampling for grass pots. Whereas, manganese extracted with $CaCl_2$ and acetic acid from control pots remained unchanged with time, and the trend for EDTA and acid oxalate remained the same as for grass pots. Midelney (Sub soil) Mn extracted with $CaCl_2$ and acetic acid did not show any clear differences with the time of sampling, while EDTA and acid oxalate extracted Mn decreased over the first 10 weeks and remain unchanged thereafter the grass and control pots. Zinc contaminated soil Mn extracted with $CaCl_2$, acetic acid and EDTA showed a little increase in earlier sampling period and no change in the later time of sampling, whereas acid oxalate extracted Mn showed a little decrease initilly and thereafter it remained unchanged.

Figure 5.11 to 5.15.

Represent the extractable Mn in grass pot and control pot samples of Dreghorn (Arkleston), Dreghorn (Auchincruive), Midelney (Top soil), Midelney (Sub soil) and Zinc contaminated soils.

Error bars

Represent the 95% confidence interval for three(3) replicates measurements

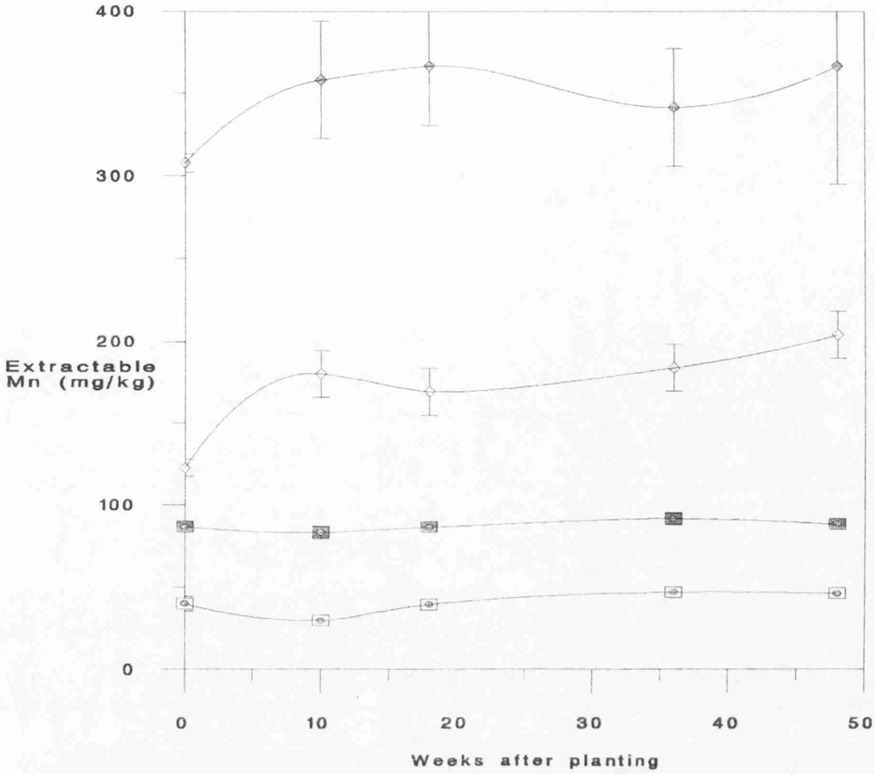


Fig 5.11a Grass pot samples of Dreghorn (Arekleston) soil

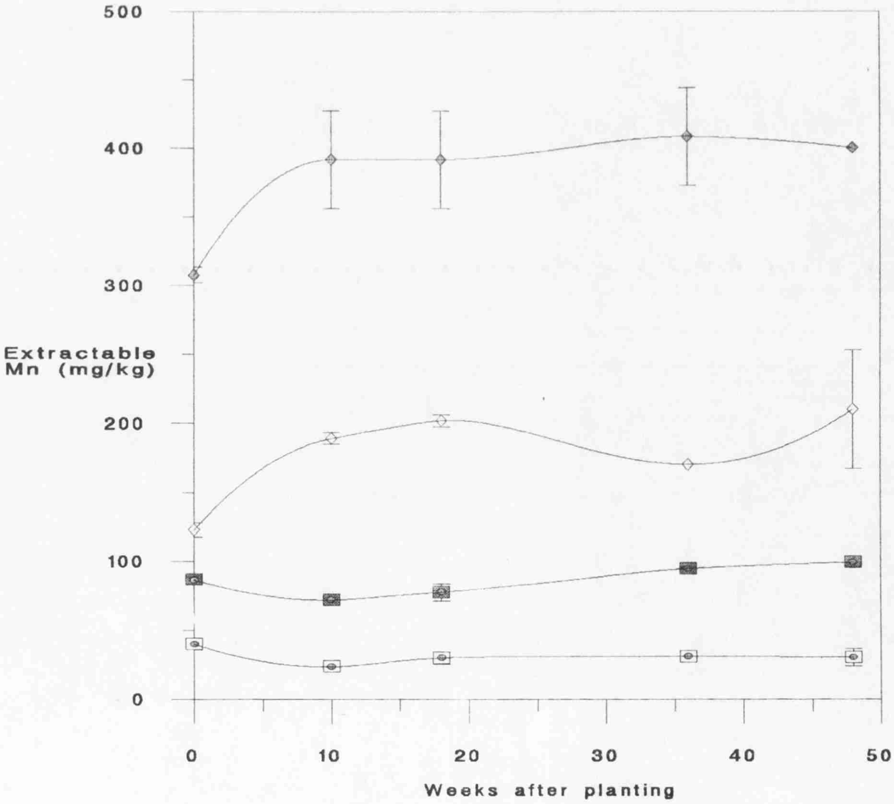


Fig 5.11b Control pot samples of Dreghorn (Arkleston) soil

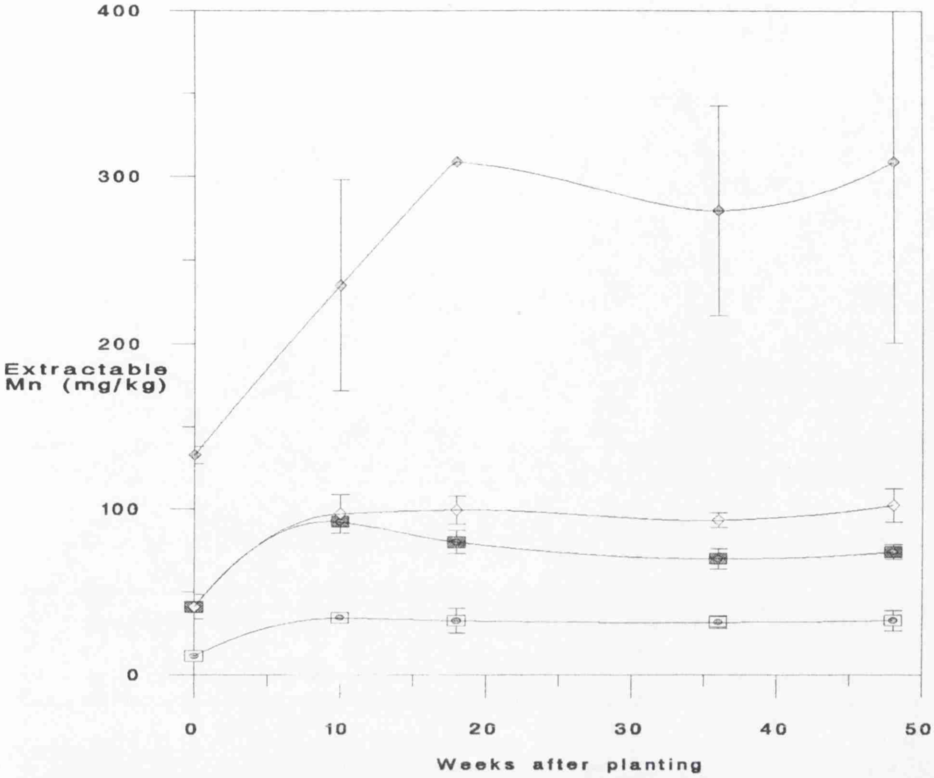


Fig 5.12a Grass pot samples of Dreghorn (Auchincruive) soil

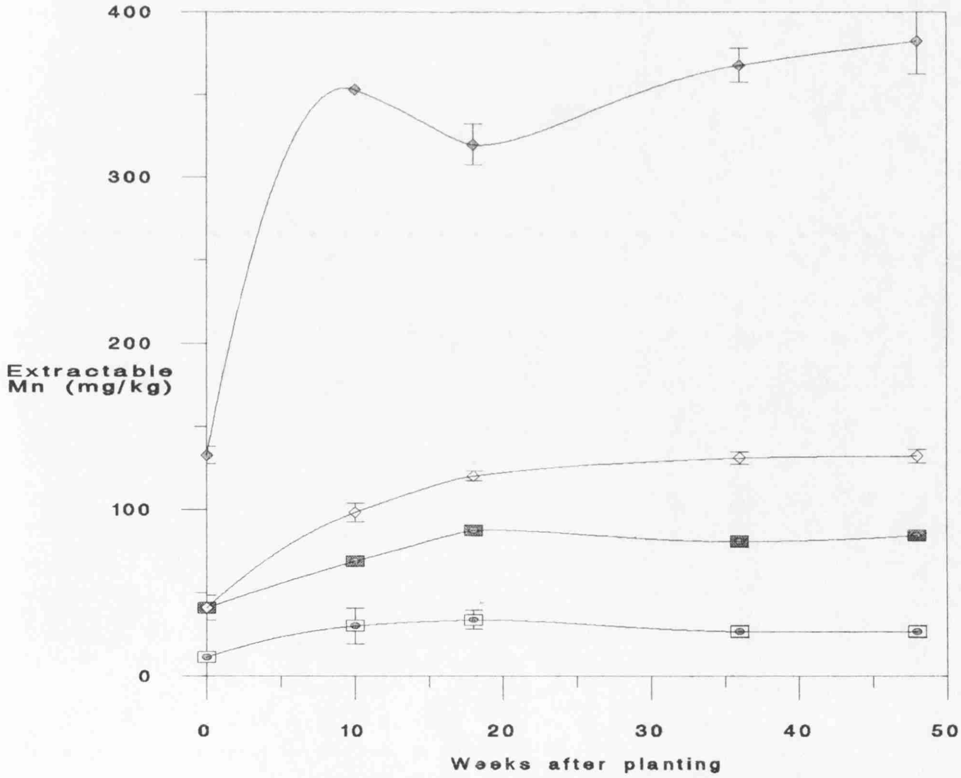


Fig 5.12b Control pot samples of Dreghorn (Auchincruive) soil

⊡ Calcium chloride
▣ Acetic acid
◇ EDTA
◈ Acid oxalate

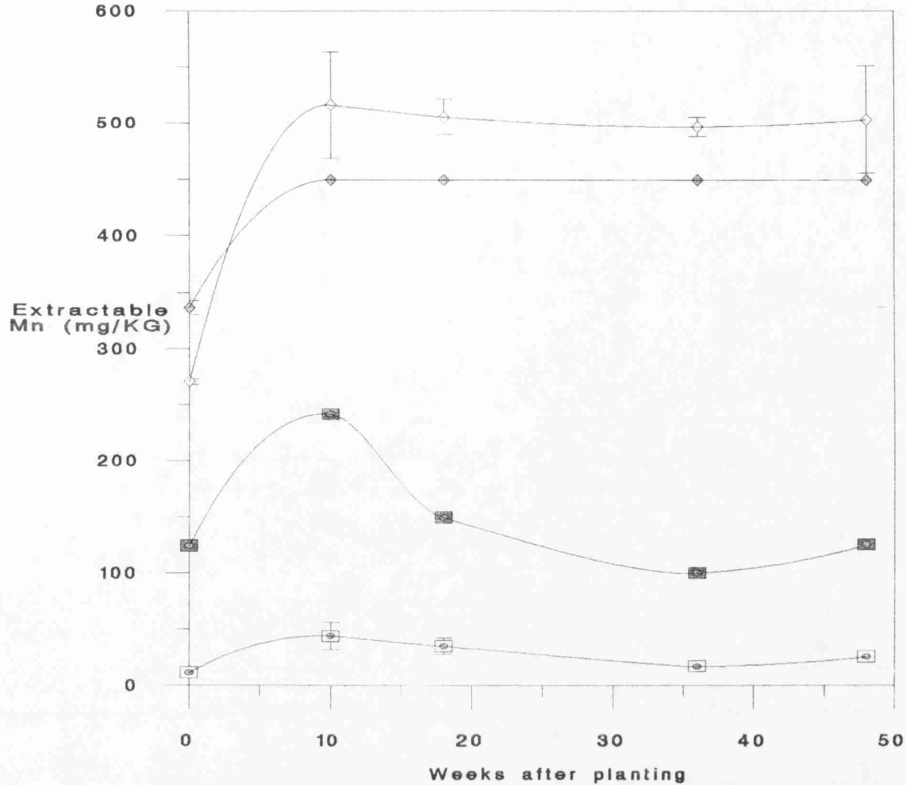


Fig 5.13a Grass pot samples of Midelney (Top soil)

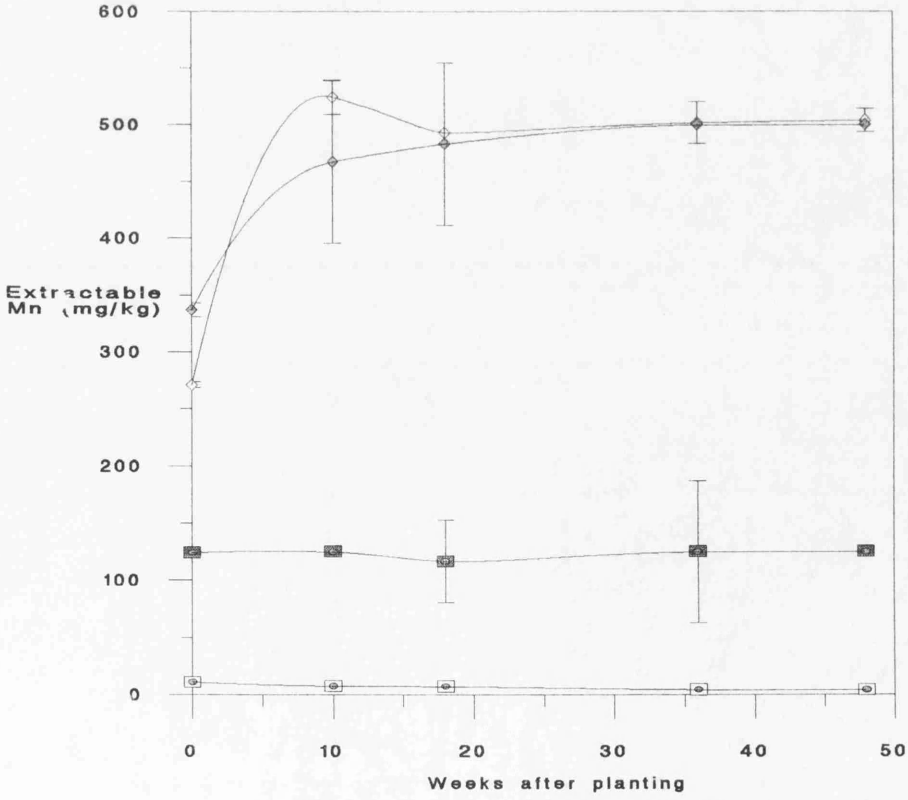


Fig 5.13b Control pot samples of Midelney (Top soil)

⊡ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

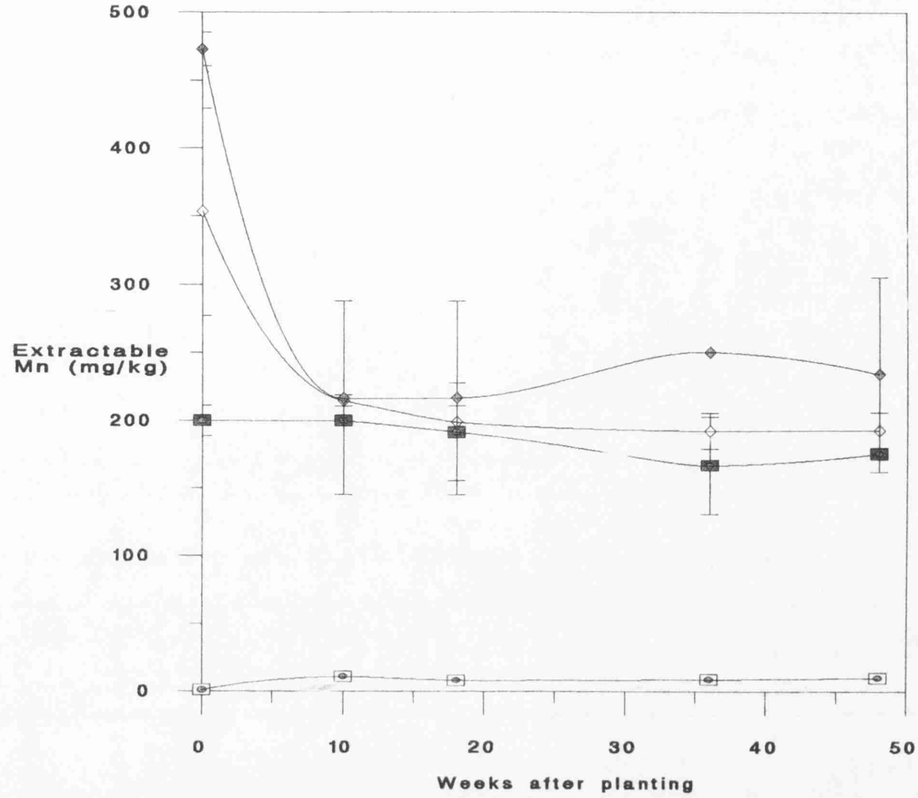


Fig 5.14a Grass pot samples of Midelney (Sub soil)

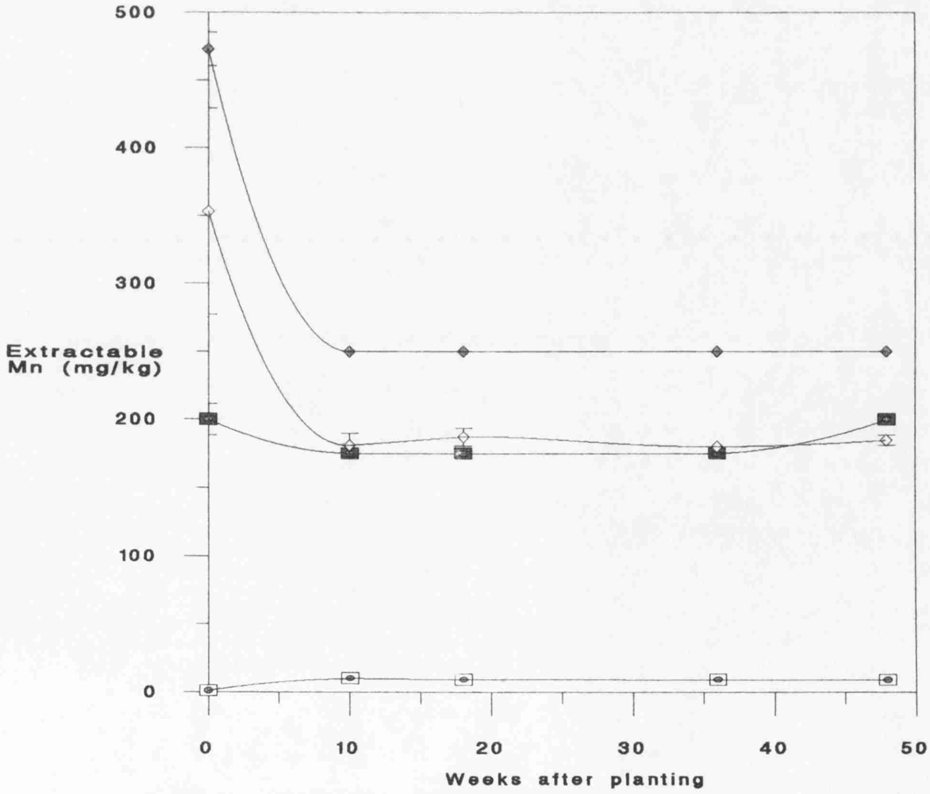


Fig 5.14b Control pot samples of Midelney (Sub soil)

⊡ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

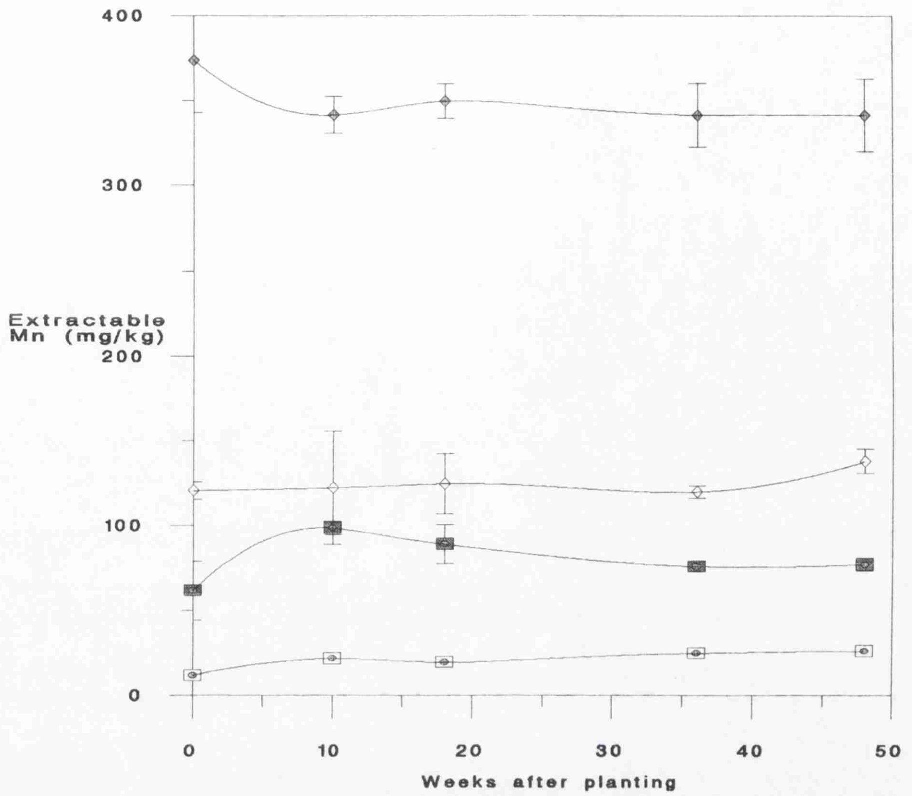


Fig 5.15a Grass pot samples of Zinc contaminated soil

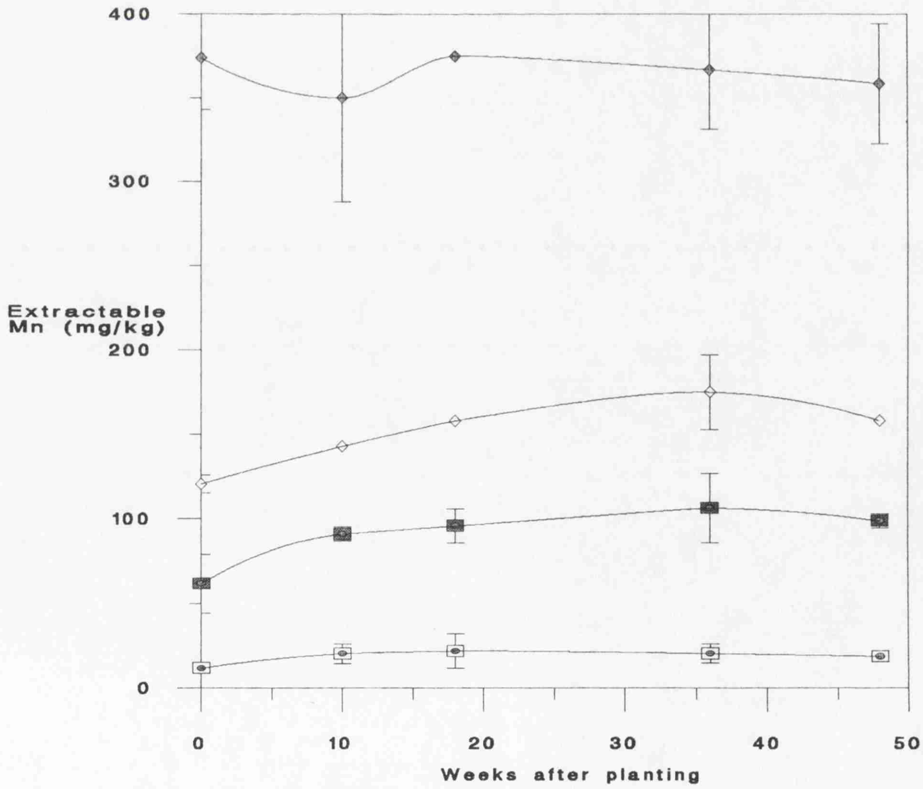


Fig 5.15b Control pot samples of Zinc contaminated soil

Figures 5.16ab to 5.20ab shows that $CaCl_2$ extractable Zn of Dreghorn (Arkleston) soil increased with time, whereas acetic acid, EDTA and acid oxalate extractable Zn in this soil decreased initially and thereafter it remained unchanged for both grass and control pots. Dreghorn (Aucincruive) soil Zn extracted with $CaCl_2$ showed a gradual increase with time for both pots, acetic acid extracted Zn of grass pots decreased gradually with time and remain unchanged for control pots, while EDTA and acid oxalate extracted Zn decreased initially to week 10 and did not show any differences between the other date of samplings for both the pots. Midelney (Top soil) Zn extracted with $CaCl_2$ was not measurable in both the grass and control pots, acetic acid and acid oxalate extractable Zn decreased initially and thereafter it remained constant, whereas EDTA extractable Zn in Midelney (Top soil) showed no difference between the different dates of sampling for both sets of pots. Midelney (Sub soil) extractable Zn was not measurable at any date of sampling with all the extractants, except a small proportion of acetic acid, EDTA and acid oxalate extracted Zn in the initial week. Zinc contaminated soil Zn extracted with $CaCl_2$ remained unchanged with time for control pots and increased gradually with time in grass pots, acetic acid and acid oxalate extractable Zn decreased initially and remained unchanged in the later period of sampling, whereas time of sampling did not show any effects on the extractability of Zn with EDTA.

Figure 5.16 to 5.20.

Represent the extractable Zn in grass pot and control pot samples of Dreghorn (Arkleston), Dreghorn (Auchincruive), Midelney (Top soil), Midelney (Sub soil) and Zinc contaminated soils.

Error bars

Represent the 95% confidence interval for three(3) replicates measurements

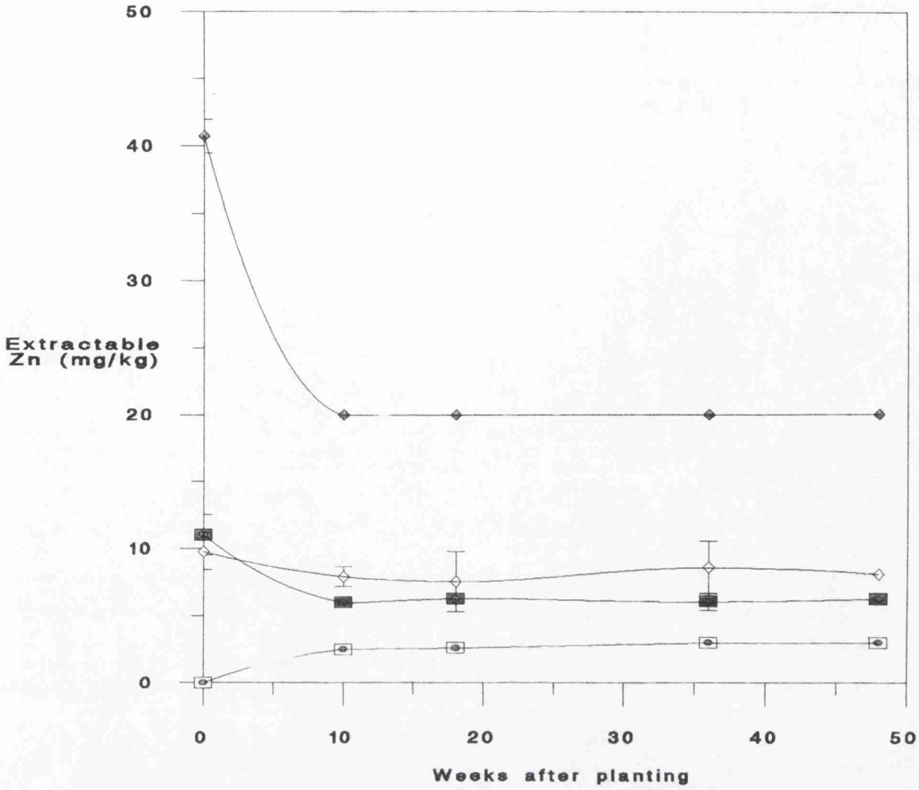


Fig 5.16a Grass pot samples of Dreghorn (Arkleston) soil

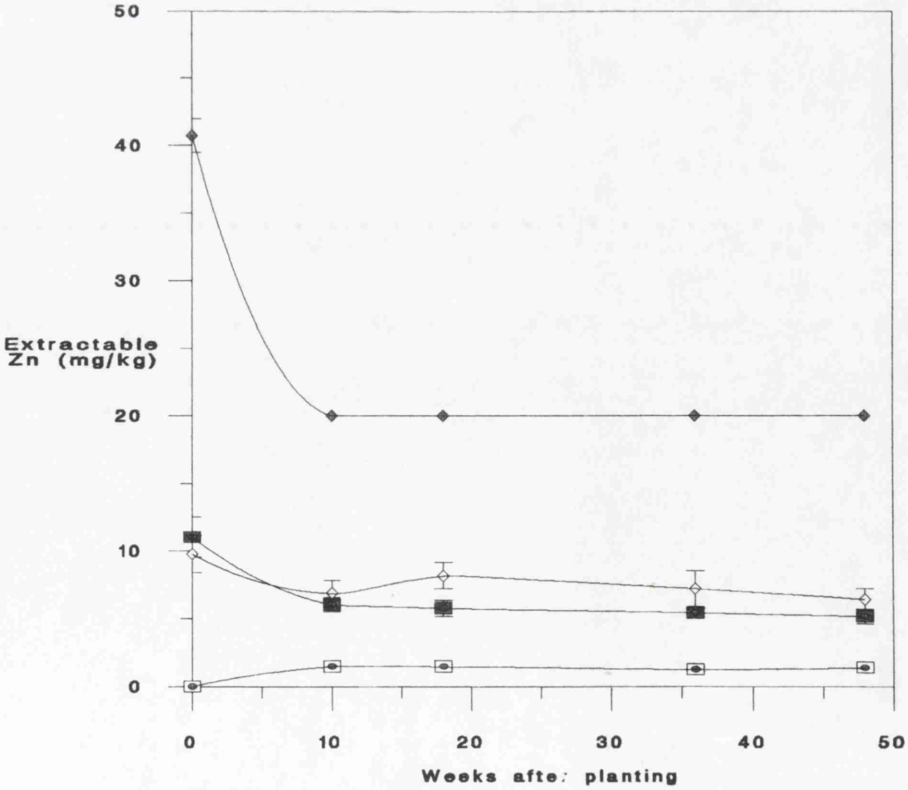


Fig 5.16b Control pot samples of Dreghorn (Arkleston) soil

⊡ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

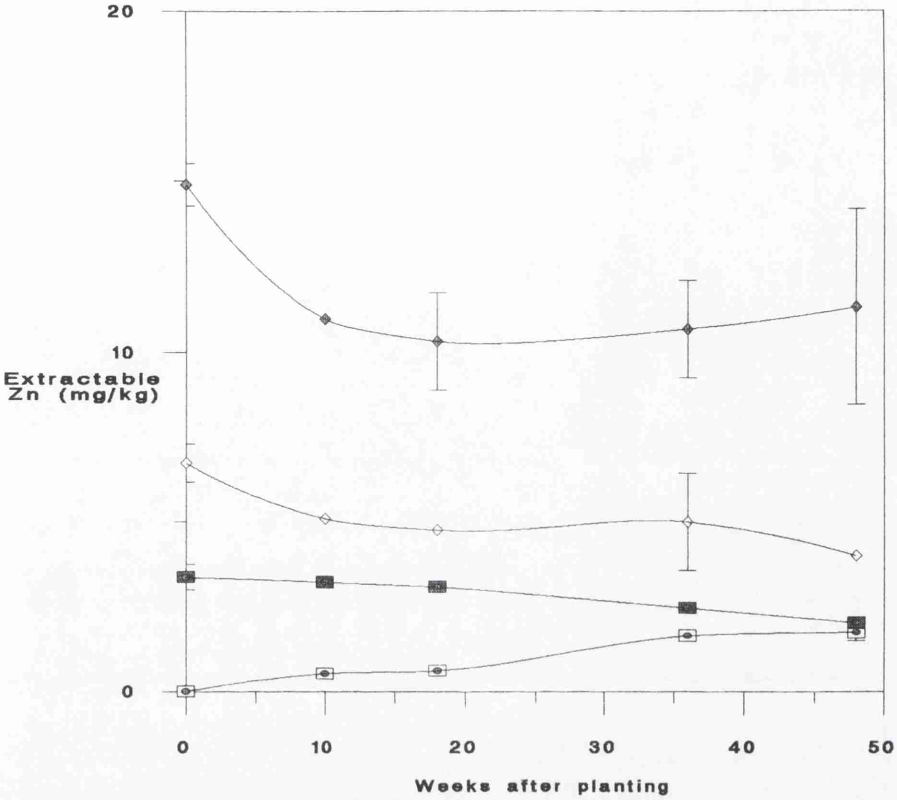


Fig 5.17a Grass pot samples of Dreghorn (Auchincruive) soil

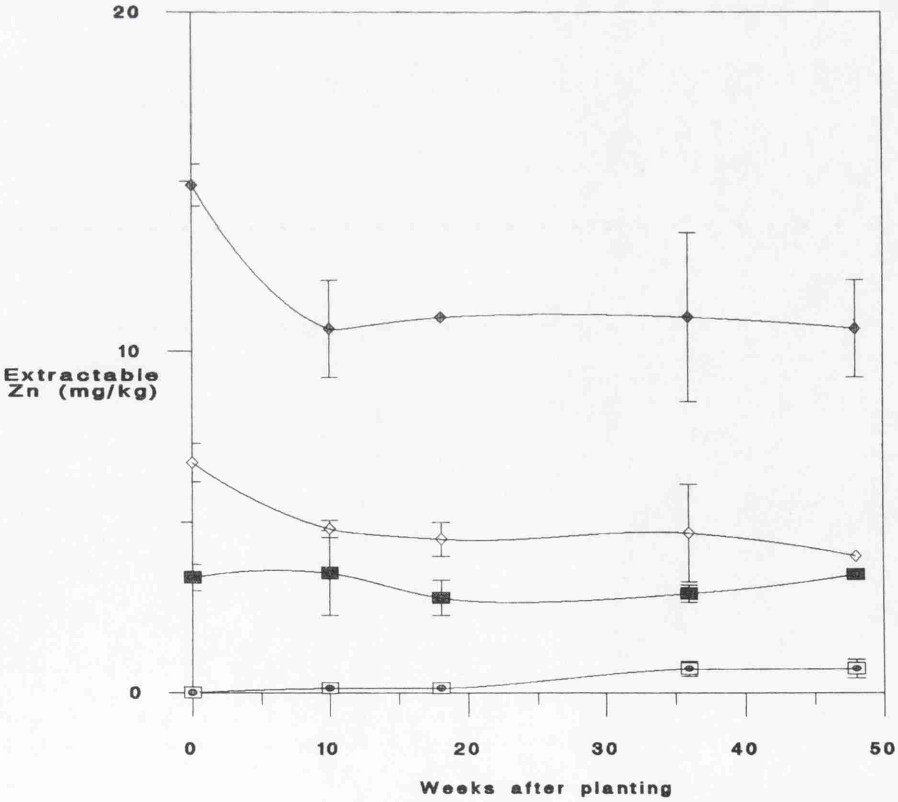


Fig 17b Control pot samples of Dreghorn (Auchincruive) soil

⊡ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

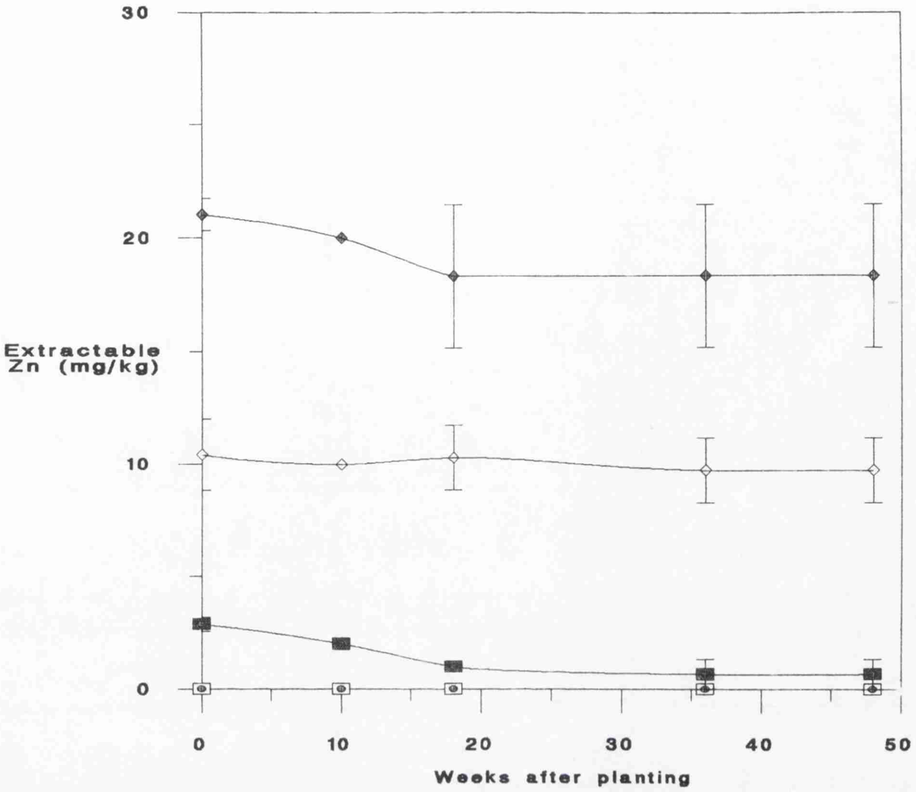


Fig 5.18a Grass pot samples of Mideiney (Top soil)

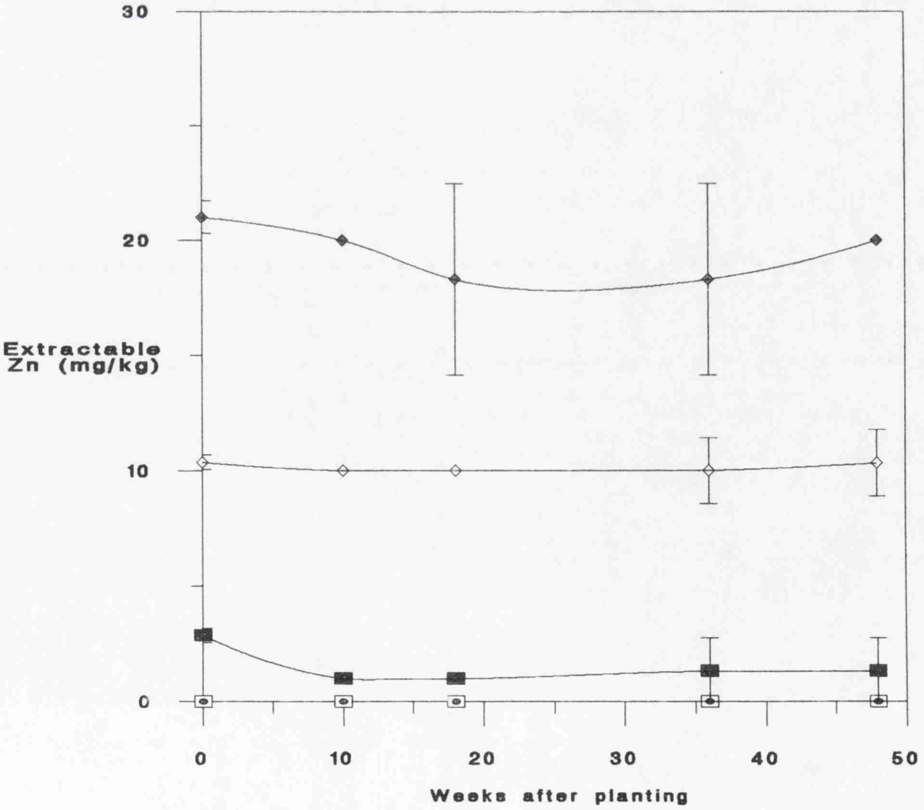


Fig 5.18b Control pot samples of Mideiney (Top soil)

- ⊡ Calcium chloride
- ■ Acetic acid
- ◇ EDTA
- ◆ Acid oxalate

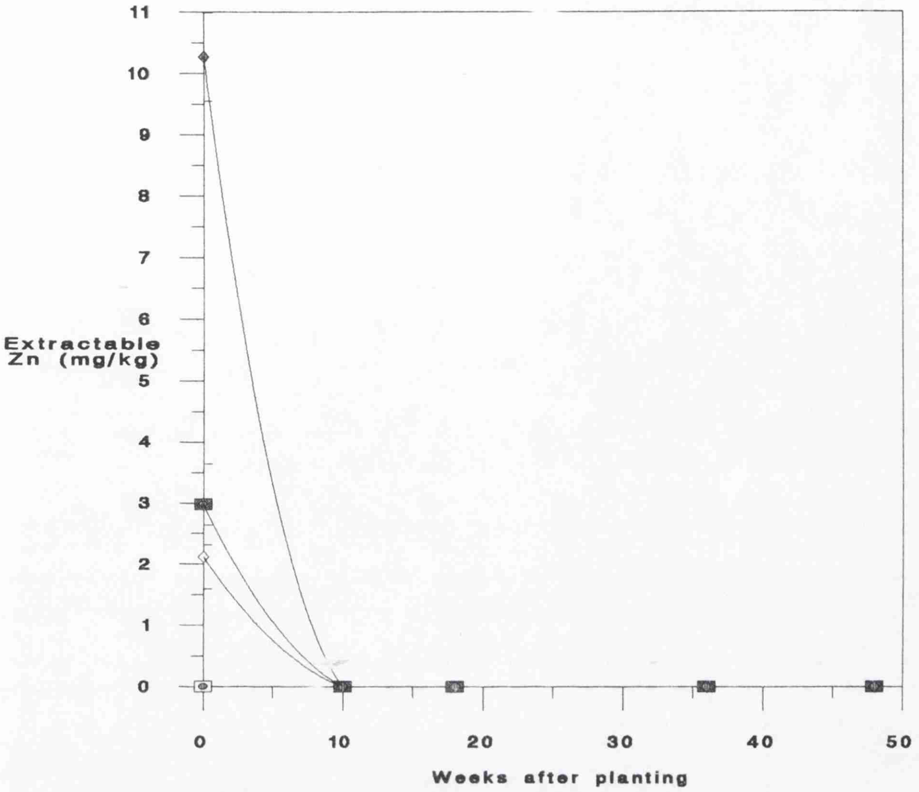


Fig 5.19a Grass pot samples of Midelney (Sub soil)

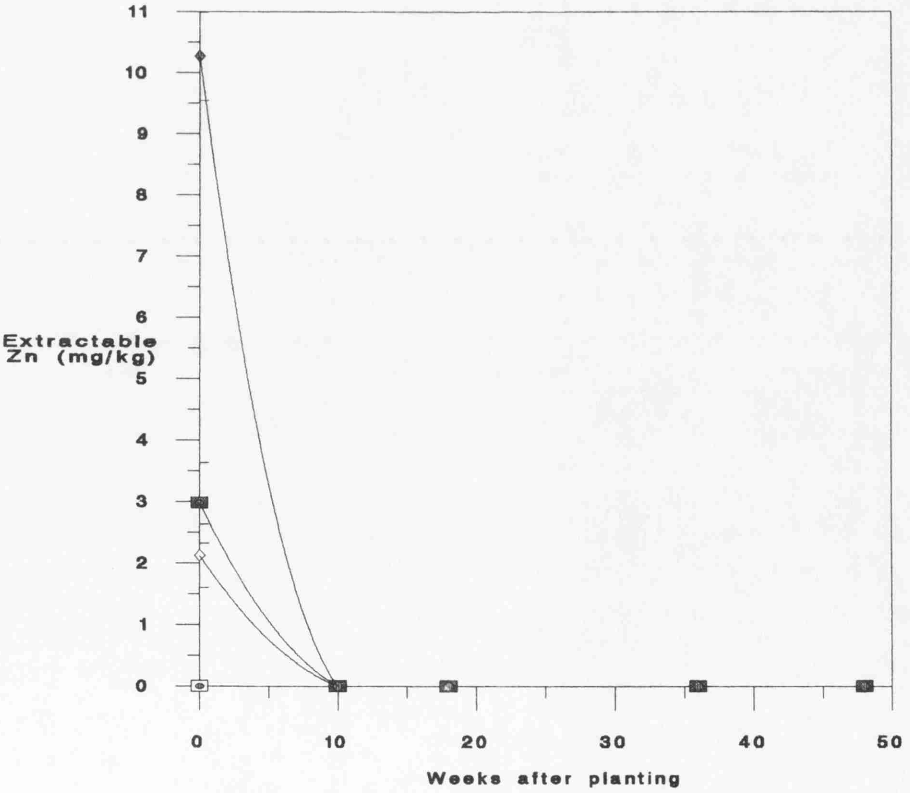


Fig 5.19b Control pot samples of Midelney (Sub soil)

⊡ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

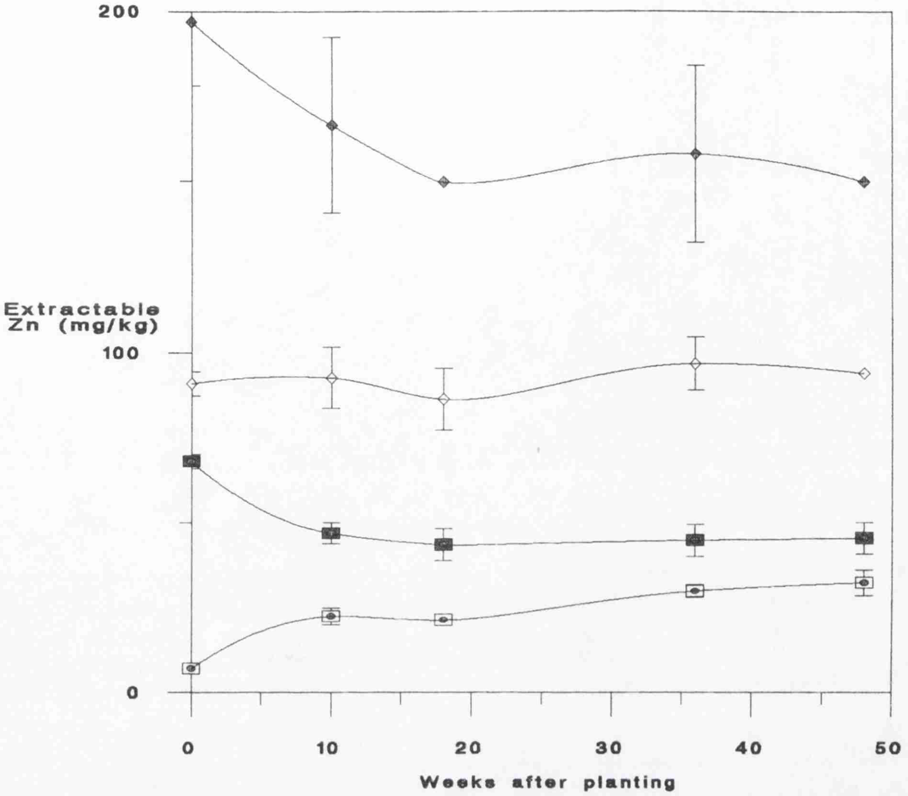


Fig 5.20a Grass pot samples of Zinc contaminated soil

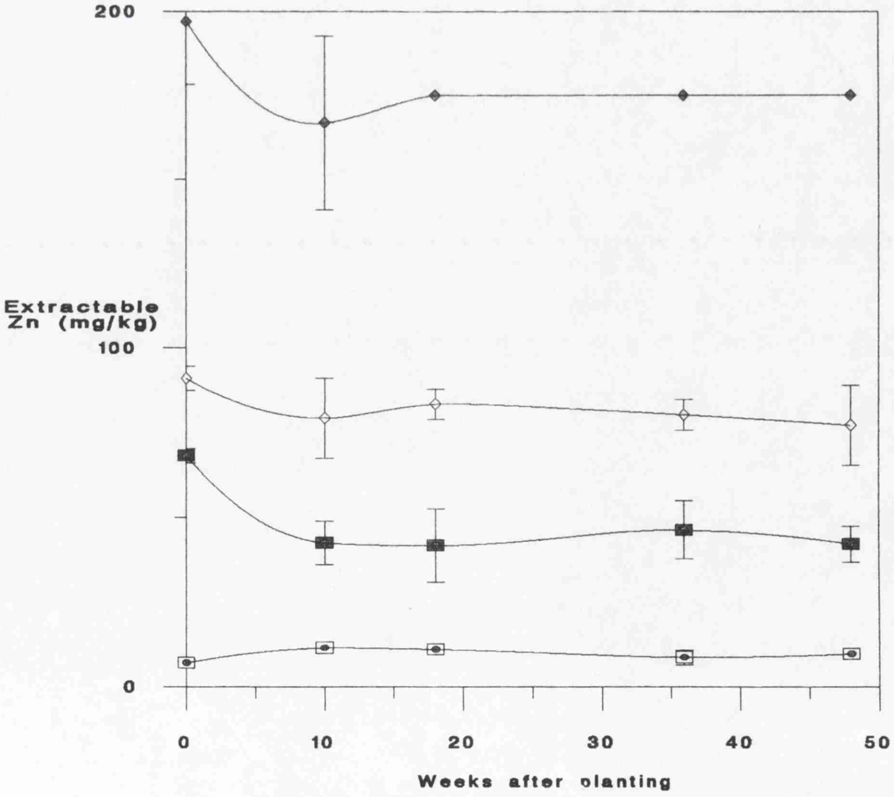


Fig 5.20b Control pot samples of Zinc contaminated soil

▣ Calcium chloride
■ Acetic acid
◇ EDTA
◆ Acid oxalate

It is clear from the Figures 5.1ab to 5.20ab that $CaCl_2$ and acetic acid extractable Cu and Fe were not measurable and large quantities of these elements were extracted with EDTA and acid oxalate in both sets of pots for all the soils. This indicates that exchangeable and specifically adsorbed Cu and Fe were very low and a large quantity of these elements was associated with organic and oxide bound fractions. Results reported here were with agreement with others. McLaren and Crawford (1973) reported very small amounts of Cu in $CaCl_2$ and acetic acid fractions and found a large proportion of Cu in organic and oxide bound fractions. Liang et al. (1991) also reported large amounts of copper in organic and oxide bound fractions. They concluded that organic and oxide-bound Cu are the important sources of available Cu for plants. Although, EDTA and acid oxalate extracted more Mn and Zn than $CaCl_2$ and acetic acid, the amount of Mn and Zn extracted with $CaCl_2$ and acetic acid were appreciable. This indicates that Mn and Zn are easily available to plants, as exchangeable and specific adsorbed pools are considered to be most available pools of plant.

It is also observed from the Figures that Cu extracted with EDTA from the Zinc contaminated soil, Zn extracted with $CaCl_2$ from Dreghorn (Arkleston), Dreghorn (Aucincruive) and Zinc contaminated soil, Fe extracted with all the extractants from all the soils and Mn extracted with all the extractants for four soils, increased with time over the first 10 weeks and there was no difference between the other dates of sampling. There are many possible reasons for an increase of extractable amounts of these metals. Use of certain fertilizers can create acidic soil reactions and cause reduced pH. Marked increases in the availability of Fe and Mn should be expected when soil pH decreases. Continuous supply of fertilizer and water during the growing period for the production of ryegrass reduced the soil pH and may cause an increase in soluble Fe. The development of anaerobic conditions will cause the solution of Fe through reduction. Occluded trace metals will be released on dissolution of Fe and ferromanganese minerals. Bloomfield (1969) observed that anaerobic incubation of a soil followed by aeration had the effect of generally increasing the quantity of extractable Fe and trace elements. In this experiment continuous supply of nitrogeneous fertilizers were applied for the growth of plants, which may cause a decrease of soil pH. A decrease in soil pH will mobilize Mn from various fractions and increase its concentration in the solution, often in other more soluble and bioavailable fractions such as the exchangeable and organic (Sims and Patrick, 1978). Sims (1986) reported that the exchangeable Mn, a plant-available form, was high at low soil pH levels (< 5.2). While, Shuman (1991) reported that in poorly drained soils, the organic fraction Mn and the Mn-oxide fraction Mn are high compared with well-drained soils. Activities of the micro-organisms present in the soil also increased the mobility of the micronutrients in soil.

Generally it is noticed that amounts of Cu, Fe, Mn and Zn extracted with $CaCl_2$ show a little increase with time. The possible reason for the increase in this pool might be due to the adsorption of microelements during the growing time, may be released from the surface of roots by the exchange process because of initimate contact that exists between roots and soil particles. Tisdale et al. (1985) suggested that ions (such as H^+ ions) may exchange with those held on the surface of clay and organic matter in soils. Calcium chloride extractable microelements may also be released from the dead root tissues. Washing of some roots present in soil can release substances which may dissolves some oxides and cause the release of nutrients into the solution. Shuman (1982) reported the release of oxide bound Zn into solution. Bakhsh (1988) also observed that $CaCl_2$ extractable Zn increased with time.

The decrease in the extractable amount of Cu, Fe, Mn and Zn in first 10 week of sampling may be either due to direct uptake by the plants or due to movement to maintain the levels in the other fractions. Another possible cause of decrease is the adsorption of microelement by the clay minerals or associated with the suspended organic materials.

5.3.2 Plant uptake of soil Cu, Fe, Mn and Zn

Cumulative total amounts of Cu, Fe, Mn and Zn taken up by the grass tops are represented in Figures 5.21 to 5.24. In general all five soils showed two linear portions of the graphs indicating different rates of uptake of Cu, Fe, Mn and Zn by the grass. In the the initial growth stages the metal uptake went up to a certain level and then became in equilibrium with soil Cu, Fe, Mn and Zn with time.

The growth period between the different harvests varied considerably and the uptake data presented in Table 5.1 are therefore expressed in terms of uptake/week. Variations in ryegrass uptake at different harvests were generally similar for Cu, Fe, Mn and Zn, with the highest uptake rate occuring at the 4th (22 week) harvest and the lowest at the last harvesting.

(Bakhsh, 1988)

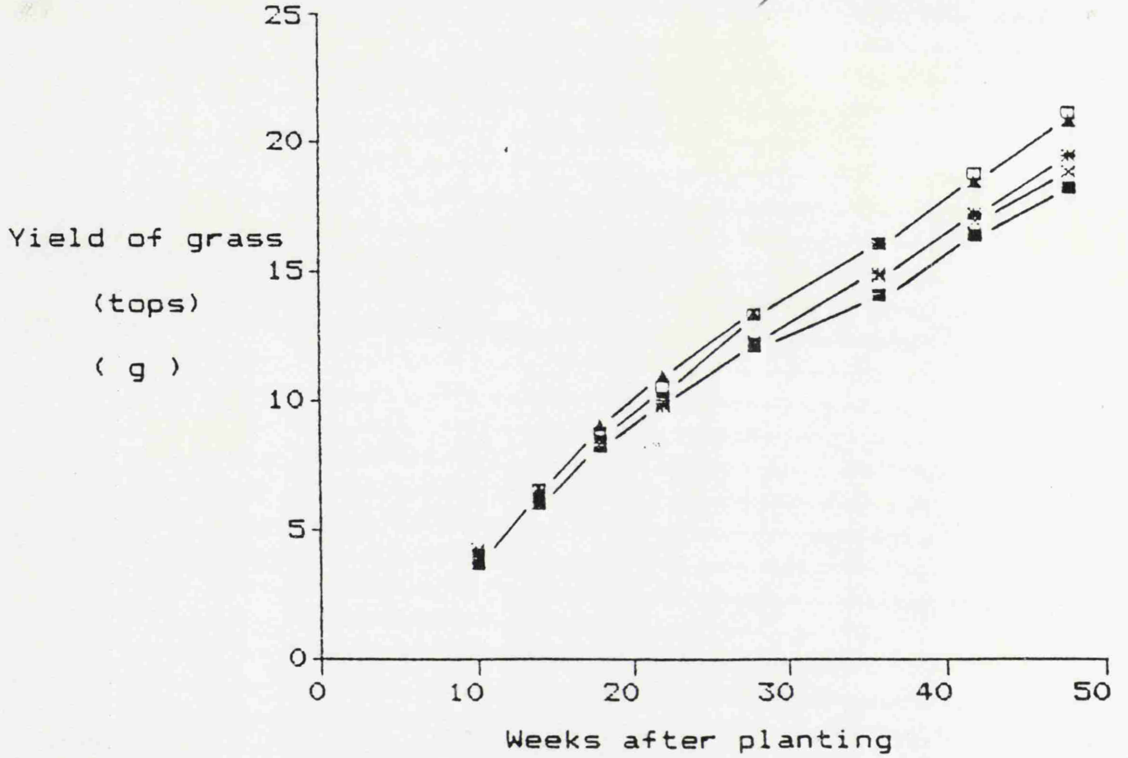


Fig. 3.2 Cumulative yield of grass (tops).

Dreghorn (Arkleston) (*); Dreghorn (Auchincruive) (x);
Midelney topsoil (□); Midelney subsoil (■);
Zinc contaminated soil (▲).

Table 5.2 Grass yield (g pot^{-1}) and concentration of metals (μg g^{-1}) at different harvesting times (weeks)

Soil	10	14	18	22	28	36	42	48
	Weeks							
Dreghorn (Arkleston)								
Grass yield	3.9	2.3	2.2	1.4	2.4	2.7	2.3	2.3
Cu concentration	4.2	6.7	7.1	8.5	0.0	0.0	3.6	4.9
Fe concentration	57.0	70.0	60.3	86.0	36.7	33.3	31.0	39.7
Mn concentration	108.3	214.7	190.3	496.7	176.7	206.7	174.0	343.0
Zn concentration	22.5	42.5	43.8	55.2	54.9	47.9	31.4	37.8
Dreghorn (Auchincruive)								
Grass yield	4.2	2.2	2.1	1.4	2.4	2.5	2.0	2.0
Cu concentration	2.5	4.4	7.9	6.2	0.0	0.0	2.6	2.9
Fe concentration	44.3	74.0	57.0	95.7	42.7	35.0	14.7	14.3
Mn concentration	164.0	272.0	243.3	920.0	303.3	353.3	120.0	130.0
Zn concentration	17.6	24.1	31.2	39.4	32.6	28.6	32.1	36.8
Midelney (Topsoil)								
Grass yield	4.0	2.5	2.2	1.8	2.8	2.7	2.7	2.4
Cu concentration	4.7	6.0	4.6	7.9	0.0	0.0	2.7	4.2
Fe concentration	84.7	67.3	49.7	91.0	54.7	54.7	44.3	35.0
Mn concentration	36.0	73.0	101.0	148.0	135.7	166.0	126.7	146.7
Zn concentration	28.6	31.3	36.2	42.8	37.2	30.6	28.0	34.4
Midelney (Subsoil)								
Grass yield	3.6	2.4	2.2	1.8	2.1	1.9	2.3	1.9
Cu concentration	3.8	4.3	3.5	6.7	0.0	0.0	5.4	4.3
Fe concentration	113.7	76.7	55.7	84.7	102.0	102.7	110.7	102.7
Mn concentration	54.7	68.3	72.7	115.8	99.7	99.7	104.7	105.7
Zn concentration	7.7	8.5	6.5	11.3	4.5	2.0	4.0	3.5
Zinc contaminated soil								
Grass yield	4.0	2.5	2.5	1.9	2.4	2.8	2.3	2.4
Cu concentration	8.7	12.0	13.0	12.1	3.7	0.0	9.8	10.2
Fe concentration	47.7	56.0	67.3	71.0	47.3	20.7	28.0	33.0
Mn concentration	35.0	95.0	104.0	139.3	104.7	57.3	107.7	130.3
Zn concentration	65.6	88.5	126.5	138.7	214.0	94.9	164.7	150.7

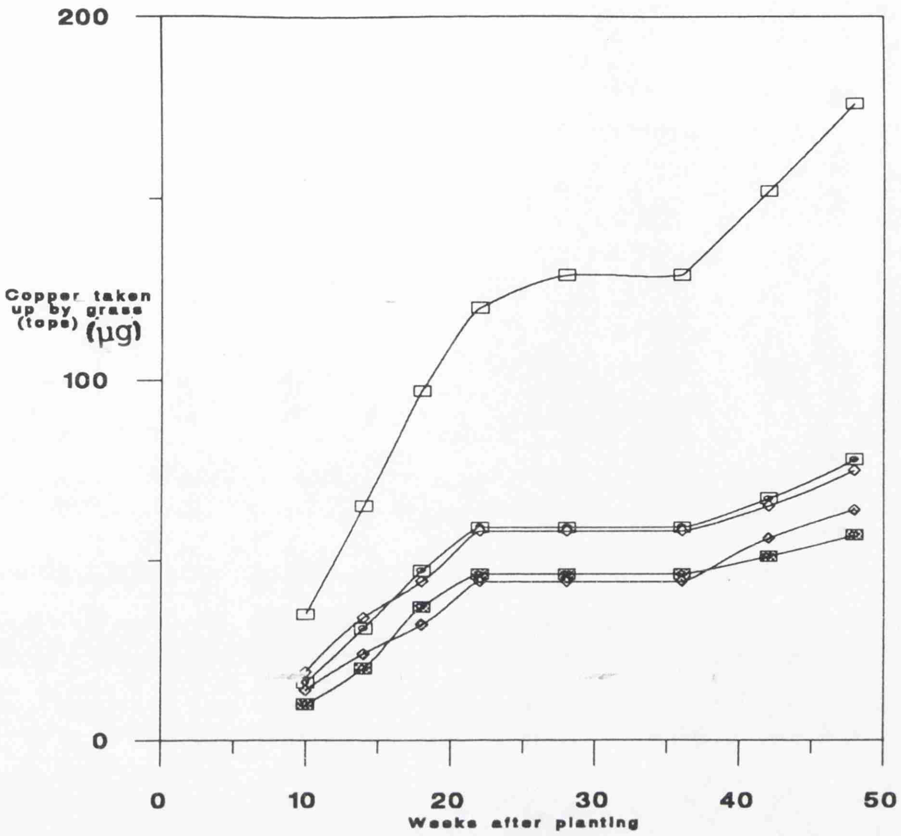


Fig. 5.21 Cumulative amount of Cu taken up by grass (tops)

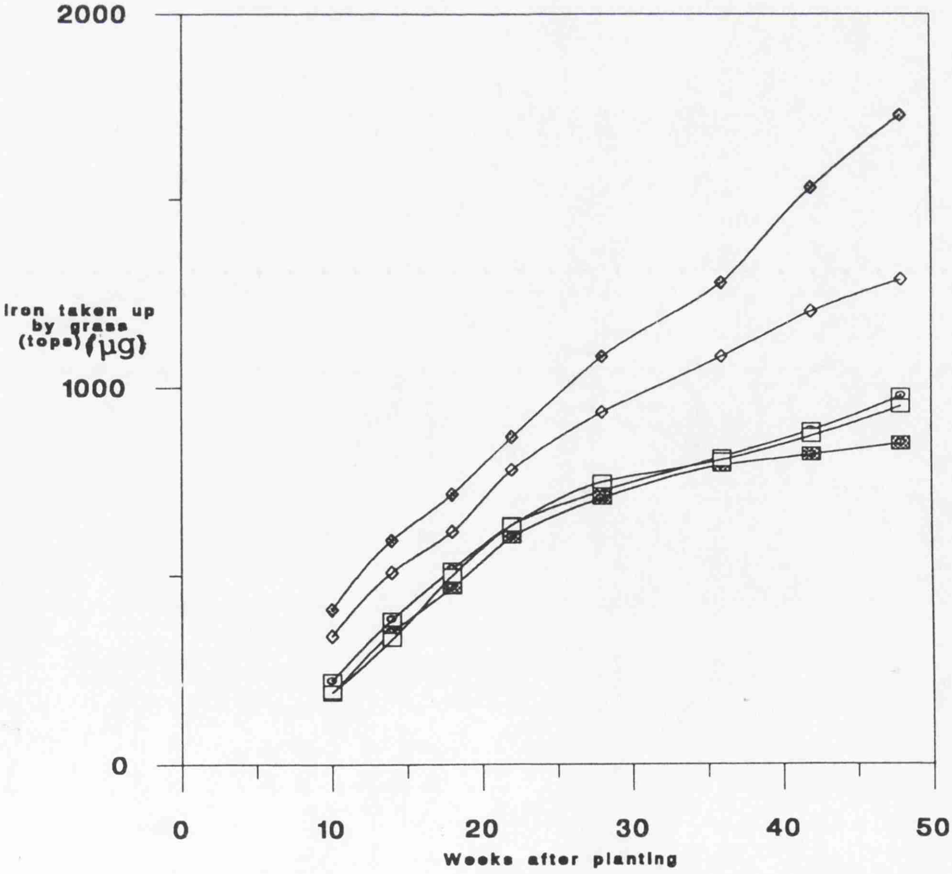


Fig. 5.22 Cumulative amount of Fe taken up by grass (tops)

⊡ Dreghorn (Arkleston)
▩ Dreghorn (Auchincruive)
◇ Midelney (Top soil)
◈ Midelney (Sub soil)
□ Zinc contaminated soil

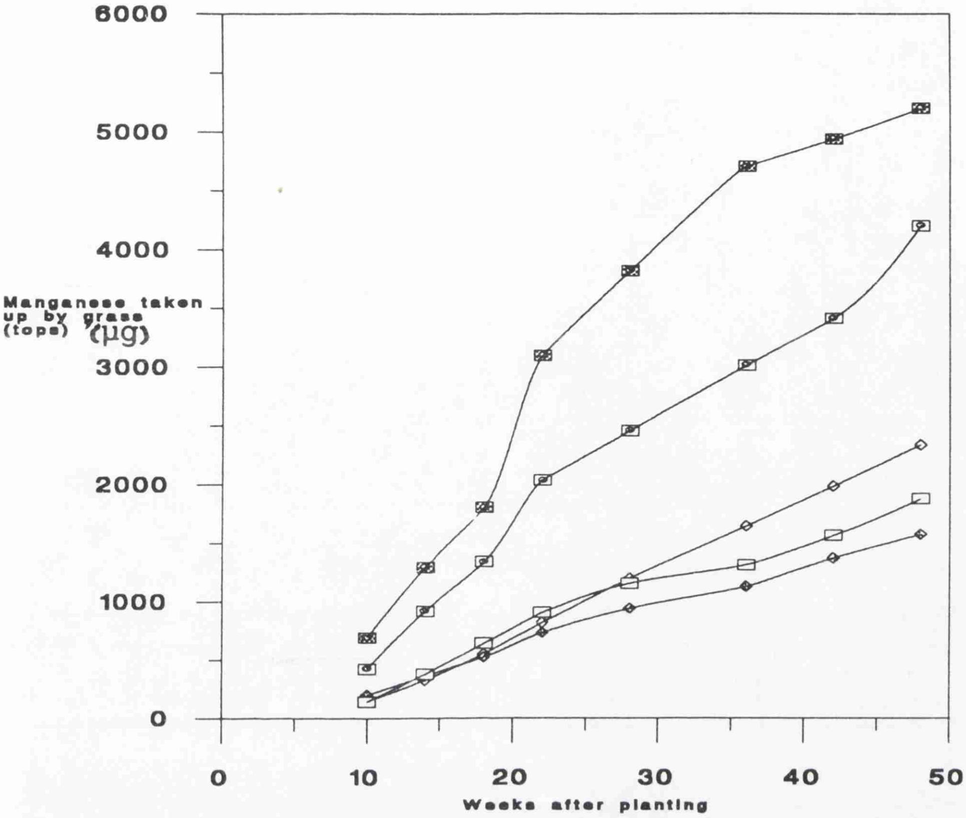


Fig. 5.23 Cumulative amount of Mn taken up by grass (tops)

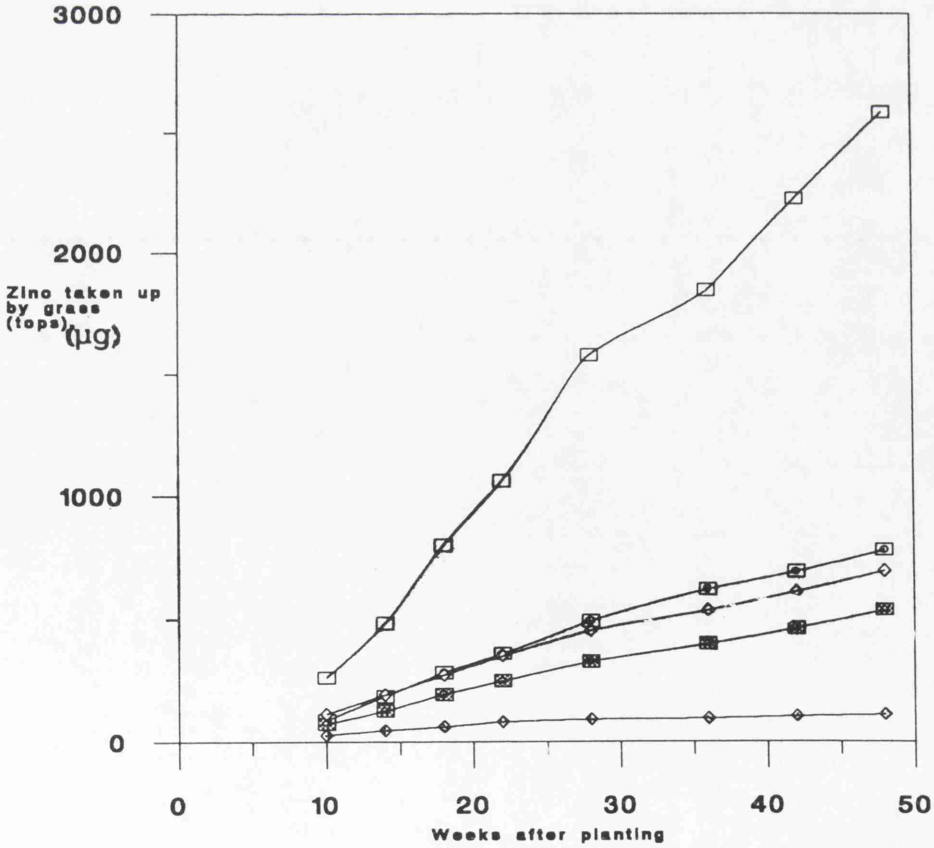


Fig. 5.24 Cumulative amount of Zn taken up by grass (tops)

- ⊡ Dreghorn (Arkleston)
- ▨ Dreghorn (Auchincruive)
- ◇ Mideiney (Top soil)
- ◇ Mideiney (Sub soil)
- □ Zinc contaminated soil

Table 5.1 Rates of metal uptake by different ryegrass harvests in five soils (μg/week).

	Harvesting time (Weeks)							
Soils	10	14	18	22	28	36	42	48
Cu uptake								
1	1.6	2.2	2.6	2.7	2.1	1.6	1.6	1.6
2	1.0	1.4	2.1	2.1	1.6	1.3	1.2	1.2
3	1.9	2.4	2.4	2.6	2.1	1.6	1.6	1.6
4	1.4	1.7	1.8	2.0	1.6	1.2	1.3	1.3
5	3.5	4.6	5.4	5.5	4.6	3.6	3.6	3.7
Fe uptake								
1	22.2	27.4	28.7	28.6	25.9	22.6	21.1	20.3
2	18.6	24.9	26.1	27.4	25.2	22.0	19.6	17.7
3	33.9	36.2	34.2	35.5	33.3	30.0	28.6	26.8
4	40.9	42.4	39.7	39.4	38.6	35.4	36.4	35.9
5	19.1	23.6	27.7	28.8	26.7	22.4	20.7	19.8
Mn uptake								
1	42.2	65.4	74.2	92.3	87.6	83.7	81.2	87.5
2	68.9	92.1	100.0	140.4	136.3	130.5	117.6	108.3
3	14.4	23.3	30.4	37.0	42.6	45.6	47.2	48.7
4	19.7	25.8	28.9	33.1	33.5	31.3	32.6	32.7
5	14.0	26.9	35.4	41.0	41.2	36.5	37.2	39.0
Zn uptake								
1	8.8	13.3	15.7	16.3	17.5	17.2	16.5	16.2
2	7.4	9.1	10.7	11.3	11.6	11.1	11.0	11.2
3	11.4	13.7	15.1	15.9	16.2	14.9	14.6	14.5
4	2.8	3.4	3.4	3.7	3.3	2.6	2.5	2.3
5	26.2	34.5	44.4	48.3	56.3	51.2	52.9	53.8

1 = Dreghorn (Arkleston) 2 = Dreghorn (Aucincruive)
3 = Midelney (Top soil) 4 = Midelney (Sub soil)
5 = Zinc contaminated soil

It is observed from Table 5.1 that uptake rates of Cu and Zn were higher in Zinc contaminated soil, whereas, Midelney (Sub soil) and Dreghorn (Aucincruive) soil showed a higher uptake rate of Fe and Mn. Higher uptake rates of Cu and Zn by the grass in Zinc contaminated soil might be due to the higher amounts of Cu and Zn present in Zinc contaminated soils or may be due to the higher yield of grass in this soil. The uptake of Fe and Mn was different from that of Cu and Zn. Midelney (Sub soil) showed lower Fe values, but the uptake rate of Fe was higher in this soil, indicating that quantity of metal present in the soil is not directly related to the uptake of plants. Most authors agree that availability of soil lead to plants is low and relatively large differences in soil contents result in only small increases in plant contents. Marten and Hammond (1966) grew bromegrass on lead-contaminated soil in a glasshouse and found no statistical difference between plants growing on soils containing 12, 59 and 95 ppm lead. Higher uptake rate of Mn in Dreghorn (Aucincruive) soil might be due to the higher values of $CaCl_2$ extractable Mn. The amount of Mn in Dreghorn (Aucincruive) soil extracted with $CaCl_2$ was higher than all other soils, which is considered to be the most available pool of plants.

There are many possible explanations for the variations in metal uptake between the different harvests. These may result from changes in the availability of the metals in the soil or from changes in the plant behaviour. Alternatively, environmental factors may be indirectly responsible, affecting uptake by influencing the behaviour of the soil and or plants. Increased plant growth frequently causes reduced nutrient contents because of the dilution effect that occurs where nutrient uptake does not increase to the same extent as growth. Fleming and Murphy (1968) have reported trace element contents of grass harvested on a number of occasions throughout the growing season. Copper contents of ryegrass decreased with advancing maturity of the plant, though Zn contents were little affected. Lane and Fleming (1967) suggested that high contents of Cu in the first cuts of ryegrass were a reflection of Cu reserves in the seed. However, in the present study the amounts of Cu, Fe, Mn and Zn were not determined in the ryegrass seed. Therefore it is difficult to say that whether the increase in the initial harvesting may be due to the seed or any other reason.

A number of environmental factors such as lighting, temperature and soil moisture content have been found to affect the uptake of trace elements. Ozanne (1955b) reported that the absorption and translocation of Zn by clover was strongly affected by light conditions.

A number of workers have demonstrated that plant uptake of heavy metals increases as the soil moisture content is increased. In this experiment water was added to the pots as required but the amounts used were not measured and differing moisture conditions may have developed in the individual pots, so any affects on uptake would be expected to be of a random nature. None of these factors satisfactorily explains the observed variations in metal uptakes with time, but nevertheless, the generally similar behaviour of all treatments does suggest that a common cause affects the variation in metal uptakes between the different harvests, which may be due to changing availability of the metals in the soil.

It is also observed that reduced uptake of metals with time may be the result of depletion of the readily-available metals pools in the soils. Although no measurements were made of the quantities of metals in the plant roots, which are likely to contain higher concentrations than the tops.

Chapter 6

Conclusion

The main objectives of this thesis were to understand the chemistry and behaviour of trace elements in terms of their distribution and solubility in the soil and availability to plants. A large number of chemical extractants were used for the removal of micronutrients from the soil. However, it is very difficult to study all the extractants hence just a few common successful ones have been chosen and discussed in this project.

Metal associated with exchangeable and specifically adsorbed pools were removed by using simple metal salts. Two different concentrations of the same reagents were used to see the effect of reagent concentration on the removal of exchangeable metals from the soil. Metals associated with organic matter and oxide bound fraction were removed by using different pairs of organic and oxide extractants. Soil extracts of the different reagents were passed through 0.45 μm filter paper to see the effect of filtration on the availability of micronutrients. A pot experiment was conducted to see the uptake of plant and seasonal changes on the distribution of microelements in various soil pools by using different extractants.

Although conclusions have been drawn at the end of each chapter, it was necessary to draw a general conclusion covering the whole project.

The following general conclusion were drawn.

1) Extraction of exchangeable metals with different salts of acetate, chloride and nitrate showed a non detectable level of Co, Cu and Ni, indicating a large proportion of these metals were associated with organic matter and oxide bound fractions. Different salts of nitrates and chlorides extract a large quantity of Mn and Zn and very small amount of Fe. Whereas, NH_4OAC extracted more Fe than Mn and Zn, which indicates that kind of cation and the type of soil also play an important role in the replacement of micronutrients. Extractable amounts of Fe, Mn and Zn were increased by increasing the concentration of the reagent, suggesting that higher concentrations of various salts extract a fraction of micronutrient which was retained by the soil when extracted with lower concentrations of the reagent.

2) Different pairs of extractants did not show any marked differences for the removal of micronutrients associated with organic and oxide bound fractions. It was concluded from the present study that NH_4EDTA and acid oxalate are the best extractants for the removal of organic matter and oxide bound fraction, respectively. It was also concluded that extractable amounts of Cu, Fe, Mn and Zn were decreased after filtration through membrane filter paper.

3) It was concluded from the pot experiment that exchangeable and specifically adsorbed pools of Cu and Fe were very low, whereas large quantities of Mn and Zn were present in these pools, which are considered to be the

most available pools for plant uptake. It was also found that extractable amounts of Cu and Zn decreased, while Fe and Mn increased in the first 10 weeks of sampling and thereafter remained unchanged. The soil with high contents of Cu and Zn showed higher uptake rate of Cu and Zn. Soil with high Mn values in the exchangeable fraction showed a higher uptake of Mn, while the uptake of Fe was higher in the soil of lower Fe values, suggesting that total content of the element in the soil is not always a good indicator of the availabilty to plants.

The results reported in this study are preliminary in that the methods are still being improved and only a few soils were included. The method will be applied to study micronutrients in fractions over many more soil types to allow correlations with soil properties. For extraction of exchangeable metals with simple metal salts it may be nessary to increase the weight of soil, as small weights of soil could not produce reliable analysis for Co, Cu and Ni. It will be helpful to use the smaller pore size of membrane filters for clarifying the soil extract in future work. The effects of management practices and addition of lime and fertilizer on the distribution of microelements among fractions needs to be studied in detail to expand the understanding of micronutrient soil chemistry and to assist in the search for better methods of soil extraction.

Before relating these results to the environmental conditions of the Dera Ismail Khan region of Pakistan, it seems worth explaining the prevailing climatic conditions.

Dera Ismail Khan District is in the southern part of North West Frontier Province of Pakistan. It is a semi-arid region, with annual rainfall of about 10 inches per annum. Soil texture ranges from sandy to clayey with high pH. Organic matter in the soil is very low. Some areas have silt deposits brought about by the summer torrent floods from the western Sulaiman mountain. There is canal, tubewell and rainfed agriculture. Application of organic manures is very low. Some rich farmars apply synthetic fertilizers to their crops in canal and tubewell irrigated areas. Water conservation practices are not good, for example, the water channals are not cemented and there is much loss of water.

Keeping in view, the above facts it would be very difficult to implement these results without local investigation in that area. But very importantly, with the scientific knowledge gained during this stay in the U.K. and the practical skill developed from this training, it is possible to continue this type of work in Pakistan. Furthermore, levelling the soil and constructing cemented water courses by On Farm Water Management Project can influence the distribution of micronutrients in the soil. Newly constructed Chasma Right Bank Canal will be irrigating 2.5 million acres of land in Dera Ismail Khan. The existing cropping pattern will be changed. The intensive type of cropping would lead to the differences of the micronutrient availability. On the other hand, high evapo-transpiration rate due to warm climate could result in the build up of excess micronutrients in the root zone of the plants. It will be, therefore, imperative to carry out the same type of research project to evaluate the soils of D.I.Khan regarding their micronutrient levels, distribution and their availability to plants. This is an excellent case for purposeful continuation of research work on the extraction mechanism of trace elements deficiencies and toxicities.

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