SOIL MANGANESE AND SOME FACTORS AFFECTING ITS AVAILABILITY

by

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ABSTRACT OF THESIS

Studies were carried out to investigate the effects of seedbed cultivation practices on the availability of soil manganese to barley. Generally, overall seedbed consolidation had little effect on manganese uptake, but the availability of the element was found, in many cases, to be considerably enhanced where soil had been compacted by repeated passes of tractor wheels during cultivation operations. The effect of this wheeling phenomenon on manganese uptake was governed by the method of fertiliser application. Often, the soil beneath the wheel tracks was found to be more acidic and to have higher concentrations of $CaCl_2$-extractable manganese. At a number of sites the soil pH was highly correlated with the logarithm of the extractable soil manganese. Possible causes of enhanced manganese availability in the more compacted soil were (1) soil acidification brought about by nitrification of ammoniacal fertiliser and/or by H^+ ion exudation as a result of enhanced availability of fertiliser (NH_4^+,K^+); (2) a greater exudation of compounds able to dissolve insoluble manganese; (3) contact reduction mechanisms.

The principles of radioisotopic exchange and isotopic dilution analysis were applied to the study of soil manganese using radioactive ^{54}Mn. The addition of the tracer to a wide range of soil types showed that the rate of disappearance of ^{54}Mn from soil solution, and its distribution in various fractions, differed greatly between the soils. Generally, the radioisotope labelled all the soil fractions determined, with the majority of ^{54}Mn associated with the water-soluble + exchangeable, organically bound and easily reducible oxide fractions. When the soils were subjected to air and oven-drying both the native and radioactive manganese behaved in similar fashion. However, the changes in native manganese were proportionately greater than ^{54}Mn in the former two fractions while in the easily reducible fraction the reverse was true. Net gains of ^{54}Mn were observed in the resistant and residual manganese fractions as the moist soils were dried, probably because of occlusion or oxidation of the radioisotope in these fractions.

An assessment of the manganese labile pool using ^{54}Mn was also carried out. The two techniques exployed - chemical extraction and plant uptake - were found to be of limited value, which was solely or partly due to sorption of ^{54}Mn. Also, measurements of the labile pool by plant uptake were found to be markedly affected by different levels of soil consolidation.

The chemical and microbiological release of manganese under waterlogged conditions was investigated using sterilised and unsterilised soils. During the 28 day submergence period, a direct microbial contribution to the release of manganese appeared to be small in three of the four soils investigated. Chemical reduction was thought to be attributable to the reaction of manganese oxides to microbially synthesised organic compounds and/or to enzymatic systems that remain operative following sterilisation by gamma irradiation.

DECLARATION

I declare that I have composed this thesis myself. The work embodied in it is the result of my own investigations except where reference has been made to published literature.

S.P. Goldberg

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SECTION 1

GENERAL INTRODUCTION

Since the early 1960's, there has been a general shift in cereal production in Scotland from the traditional oat crop to the more economically valuable barley. Unlike oats, the tolerance of barley to the inherently acid soils of Scotland is limited, and it has therefore been necessary to lime soils to more suitable pH levels (6.0-6.5). This practice as well as a possible combination of other factors - e.g. modern seedbed cultivation practices, more intensive farming resulting in greater crop removal of manganese from the soil, different macronutrient fertilisers, newer barley varieties, changing climatic conditions - has increased the incidence of manganese deficiency to alarming proportions. Indeed, in the county of Angus, manganese deficiency has occurred with such frequency and severity in recent years that farmers have been advised to spray their crops as a precautionary measure prior to any visible symptoms appearing. Spray applications however, although beneficial, often do not eliminate the problem altogether and the practice requires a more labour-intensive management programme.

Severe manganese deficiency can result in complete crop failure, and decreases in grain yield of 200-2000 kg ha^{-1} are common where the deficiency is not so acute. Apart from economic yield of the crop manganese deficiency results in adverse effects on grain quality. This was emphasised in 1978 where livestock (pigs) fed on manganese-deficient grain suffered from manganese deficiency (Speirs, 1980).

These factors highlight the need for a better understanding of soil manganese, and the work described in this thesis is an attempt to investigate various physical, chemical and microbiological factors that affect its availability to plants.

SECTION 2

LITERATURE REVIEW

LITERATURE REVIEW

The importance of manganese as an essential trace element in biological systems has been known for many years. Raulin (1863) presented the first clear demonstration of its essential nature in the fungus *Rhizopus (Ascophora) nigricans*. The role of the metal as a micronutrient to higher plants was first established by McHargue (1923) in his work with oats, soyabean and tomato, and later confirmed by Samuel and Piper (1928).

2.1 Functions of Manganese in Plants

The role of manganese in plant cell structure and metabolism is of major significance. Its importance in the maintenance of chloroplast (Possingham *et al*, 1964; Teichler-Zallen, 1969; Constantopoulos, 1970) and ribosome (Lyttleton, 1960) structure has been demonstrated. Manganese activates non-specifically many enzyme reactions involved in carbohydrate breakdown, as well as organic acid, nitrogen and phosphorus metabolism (Nason and McElroy, 1963). It appears to be an essential constituent of the enzyme pyruvate carboxylase, and of the NAD malic enzyme system found in leaves of aspartate-type C_4 plants (Scrutton *et al*, 1966; Hatch and Kagawa, 1974). The principal locus of manganese deficiency in plants is identified in photosystem II of photosynthesis where the metal appears to be a specific constituent of the oxygen-evolving system (Cheniae, 1970). The work of Anderson and Pyliotis (1969) suggests that deficiency results in the inactivation of the Hill Reaction, i.e. the photosynthetic release of molecular oxygen by light-illuminated chloroplasts. Dieckert and Rozacky (1969) isolated a protein containing manganese from peanut seeds which they named manganin. However, no functional role has yet been determined for this protein (Rains, 1976). Manganese is also known to influence the level of auxin in plant tissue. Morgan *et al* (1966) found that toxicity symptoms in cotton were due to low levels of indoleacetic acid (IAA). It is postulated that excessive concentrations of manganese inactivate auxin firstly by the oxidation of auxin protectors, followed by accelerated oxidation of IAA by endogenous peroxidase (Morgan *et al*, 1966; Stonier *et al*, 1968; Rains, 1976).

2.2 Symptoms of Manganese Deficiency and Toxicity

Manganese differs from other trace elements in that both deficiency and toxicity are widespread in agriculture. Deficiencies are most commonly found in small grains and soyabean. However, maize, peanut, cotton, sweet potato, sugarbeet, potato, sorghum and mint are also affected (Sparr, 1970). Descriptive terms denoting deficiency in crops, such as 'grey speck' of oats, 'marsh spot' of peas and 'speckled yellows' of sugarbeet are not as widely used now as they once were when the cause of the symptoms was unknown (Tisdale and Nelson, 1966). Gill and Vear (1958) described a considerable variety of visual symptoms found in different crops including a yellowing of leaves, especially in the young ones, the spotting of white, brown or black dead tissue between the green veins of young leaves, and subnormal leaf and stem turgidity.

Crinkle leaf of cotton, stem streak necrosis of potato and internal bark necrosis of apple trees are physiological disorders associated with manganese toxicity. Excessive levels in the plant are characterised by marginal chlorosis and the cupping of young leaves. Speckling in older leaves is associated with localised manganese accumulations (Berger and Gerloff, 1947; Adams and Wear, 1957; Shelton and Zeiger, 1970).

2.3 Varietal Susceptibility to Manganese Deficiency

According to Nyborg (1970) different plant species vary to a large extent in their susceptibility to manganese deficiency. For example, in work with cereal species he found that susceptibility ratings (based on plant symptoms and yields) were of the order oats > wheat > barley. He also demonstrated varietal differences in sensitivity in oats and to a lesser extent in wheat. The two barley cultivars, on the other hand, manifested no significant differences.

Work on varietal differences in barley relating to uptake efficiency or tolerance to soils low in manganese is scant; this was evident after a review of literature in Field Crops Abstracts (1960-1980). However, recent work (unpublished) at the East of Scotland College of Agriculture suggests that modern varieties

(e.g. Midas) are more susceptible to manganese deficiency than older ones (eg. Ymer). Reasons for this are partially or entirely physiological (Morris, 1980; Speirs, 1980).

2.4 Forms of Manganese in Soil

Manganese exhibits a variety of oxidation states, from Mn(II) to Mn(VII) identified in various compounds and ions, for example $MnOH^+$, $Mn(OH)_2$, $Mn(OH)_3$, MnO_2, MnO_3^-, MnO_4^{2-} and MnO_4^- (Gregg, 1963; Remy, 1966). Studies have shown that in soils only the II, III and IV oxidation states are known to exist (Naftel, 1934; Leeper, 1939; Mann and Quastel, 1946; Heintze and Mann, 1947). The ease of transformation of the metal between oxidation states and its occurrence in multiple oxidation states in a number of non-stoichiometric oxides are factors that make the chemistry of soil manganese and its compounds very complex. Furthermore, manganese oxides can exist in several different crystalline and amorphous states, and form co-precipitates with iron and other oxides (Ponnamperuma *et al*, 1969; McKenzie, 1972).

2.4.1 Manganese (II)

Manganese (II) can exist in soil solution or associated with soil surfaces, either organic or inorganic (Leeper, 1947; Hodgson, 1963). Various workers have studied the adsorption of Mn(II) on oxide material. Morgan and Stumm (1964) described the adsorption of Mn(II) onto MnO_2 as a Mn^{2+}-H^+ exchange reaction while Hem (1964) showed it to be slightly adsorbed on iron-oxide precipitates. Jenne (1968) advanced the theory that hydrous oxides control the fixation of heavy metals. According to McKenzie (1972), Mn(II) is associated with a large number of oxides and oxyhydroxides where it substitutes for Mn(IV). Soil clays can fix divalent Mn as a result of precipitation, oxidation to higher oxides, physical entrapment in the clay lattice wedge zones, and strong adsorption at exchange sites (Reddy and Perkins, 1976). Page (1964), in his work on extractable soil manganese, found that it behaves as a typical divalent cation obeying laws that govern cation exchange phenomena. He observed that in varying the soil-solution ratio and the strength of extractant, the amount of manganese in solution was proportional to the amount of soil in suspension (Figure 2.1).

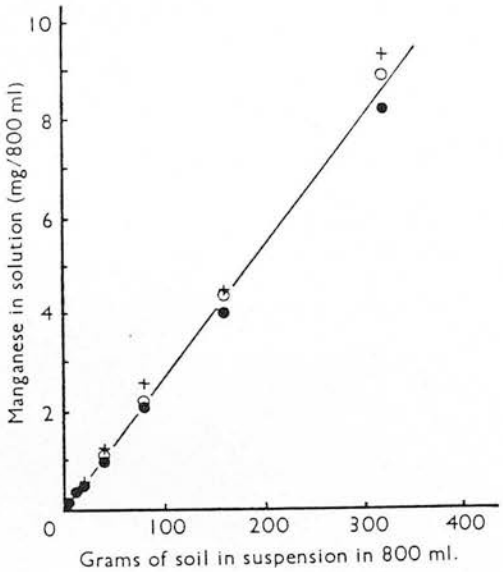
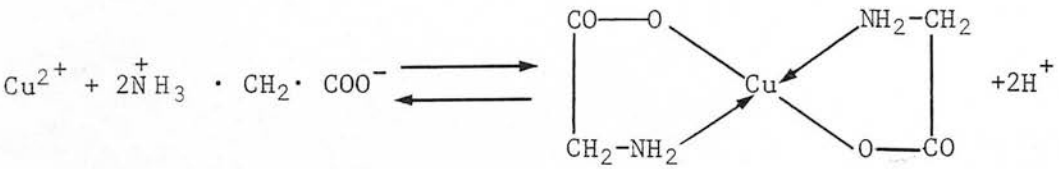


Figure 2.1. Effect of volume ratio on manganese extracted from soil

● Extraction with N $Ca(NO_3)_2$ solution.
\+ " " 2N " "
○ " " 4N " "

Since Main and Schmidt (1935) first demonstrated the association of Mn(II) with proteins, amino acids and related organic compounds, conflicting evidence has arisen over the years as to whether Mn(II) and other transition elements can exist in soils as insoluble metallo-organic complexes or chelates. Bremner *et al* (1946) first hypothesised the existence of Mn(II) complexes in soils when they noted that solutions efficient in extracting polyvalent metals from soils (i.e. pyrophosphate) were also good solvents for organic matter. However, Heintze and Mann (1946) observed that during pyrophosphate extractions, both commercial and laboratory-made hydrated manganese dioxides were reduced by soil organic matter. In later work (1947, 1949) they showed that Mn(II) could exist in soils in a form not readily exchangeable with NH_4^+ or Ca^{2+} ions and its recovery was greatly enhanced if Cu, Cd, Co, Ni or Zn were added to the extracting solution. Hemstock and Low (1953) argued that this effect was due to the displacement of manganese from chelate complexes by the added heavy metals, because they found, in experiments with synthetic manganese oxides (i.e. in the absence of organic matter) that the presence or absence of copper had no effect.

However, an alternative mechanism emerged when Beckwith (1955b) demonstrated a reducing effect arising from liberated H^+ ions when copper salts were added to a system containing manganese oxides and an organic soil. He showed how a chelation reaction with a weak acid such as glycine would cause it to titrate as a stronger one in the presence of copper:



However, he did provide evidence that organic matter complexes were also formed with Mn(II) and other metals, from titration curves of acid washed organic soils determined in the presence or absence of the metal ion in question.

More recent work by Geering *et al* (1969), using resin exchange, spectrophotometric and polarographic techniques, has shown that 84 to 99% of the manganese in a soil solution can exist in the form of organically bound Mn(II). The binding of divalent metals with organic matter has generally been considered to be due partly to the formation of chelation complexes with aromatic carboxylate and hydroxyl groups (Stevenson and Ardakani, 1972). However, according to Bloom and McBride (1979), no direct evidence for chelation has been found. Indeed, nuclear magnetic resonance and electron spin resonance studies have shown that Mn(II), unlike Cu(II), does not form an inner sphere complex with fulvic acid but instead is bound electrostatically (Gamble *et al*, 1976; Alberts *et al*, 1976).

2.4.2 Manganese(III)

The possibility of oxides of manganese(III) existing in soils was first considered by Naftel (1934) and later by Leeper (1939). Manganese(III) in soil extracts of buffered pyrophosphate solutions was identified by Dion and Mann (1946) as a soluble complex salt of manganic pyrophosphoric acid $H(MnP_2O_7)$. They assumed that Mn(III) occurred in soil as a highly hydrated manganic oxide $Mn_2O_3 \cdot xH_2O$ and proposed that the first product of biological oxidation of Mn(II) was Mn_2O_3. New evidence has recently arisen confirming their findings

(Alexander, 1977). The trivalent state is known to be stable in oxides of hausmannite (Mn_3O_4) and bixbyite ($(Fe,Mn)_2O_3$), and because of the ionic size it is likely that the lower valency manganese in manganese dioxides is Mn(III) (McKenzie, 1970).

2.4.3 Manganese (IV)

Apart from suggestions by Mulder and Gerretsen (1952) that organically bound Mn(IV) may be of importance in soils, the metal in this state is thought to exist exclusively as oxide material. Mixed-valency oxides of Mn(II), (III) and (IV) are known to exist in soils (Ponnamperuma *et al*, 1969). The most common minerals of manganese occurring in Australian soils have been found by Taylor *et al* (1964) to be lithiophorite, hollandite and birnessite, having general formulas Li_2Al_8(Mn(II), Co, Ni)$_2$ Mn(IV)$_{10}O_{35}.14H_2O$, Ba(Mn(IV), Fe(III))$_8O_{16}$ and (Ca, Mg, Na_2, K_2)$_x$Mn(IV)Mn(II)(O, OH)$_2$.
Well crystallised birnessite is probably the earliest of the Mn(IV) oxides to be formed in soil and results from chemical or biological oxidation of lower valency manganese (McKenzie, 1972).

2.5 Forms Available for Plant Uptake

Plants absorb manganese primarily in the Mn(II) form (Leeper, 1947; Fujimoto and Sherman, 1948; Wier and Miller, 1962). Recent evidence of Garcia and de la Puente (1977) suggests that the trivalent form is also available, but no indication of Mn(IV) absorption has been found (Russell, 1973). Hodgson (1963) states that "the degree of availability of micronutrients in soils is a function of their partition among different forms ..." A dynamic equilibrium is thought to exist between the various forms of soil manganese (i.e. water soluble, exchangeable, reducible, non-reducible, inert) and several cycles (see Appendix A) have been proposed in which the metal alters between available and non-available states (Mann and Quastel, 1946; Dion and Mann, 1946; Fujimoto and Sherman, 1948; Ghanem *et al*, 1971). Levels in soil, whether deficient, adequate or toxic are greatly influenced by a host of interdependent parameters - chemical, physical and environmental. Small changes in these factors can greatly influence the manganese status of the soil.

2.6 Factors Affecting Manganese Availability

The following review attempts to cover those factors which influence the availability of natural and applied manganese in soils. However, no factor is mutually exclusive from any other and a full comprehension of the influence of one factor will be incomplete unless others are considered simultaneously.

2.6.1 Soil pH

The availability of manganese is more dependent on soil pH than that of the other micronutrients (Lucas and Davis, 1961). McHargue (1923) was amongst the first investigators to note its importance. He described toxicity symptoms in plants when manganese was added in the absence of lime, and yield increases if liming preceded manganese addition. Many workers over the years have reported similar relationships. Mann (1930) and Piper (1931) noted that raising soil pH with amendments of magnesium or calcium carbonate decreased manganese content in the crop. Acidifying the soil with HCl addition increased plant manganese concentration (Piper, 1931). Leeper (1947) stated that deficiencies were most commonly found in soils of pH $\geqslant$ 7, while in acid soils, plants could manifest toxicity symptoms. Chambers and Gardner (1951) reported a decrease of 0.24 kg ha^{-1} in the manganese content of winter wheat when a loamy soil was limed from pH 4.3 to 6.5, and Rich (1956) found pH and other soil factors correlated with manganese content in peanut leaves. Singh and Pathak (1969) and White *et al* (1970) were able to alleviate manganese toxicity symptoms in oats and potatoes by liming acid soils. Uptake by oats of radioactively labelled manganese as manganese sulphate was found to be greatest on acid (pH 4.5) and least on alkaline (pH 8) soils (Deb and Scheffer, 1970).

Solution and exchangeable levels of soil manganese are similarly influenced by the pH. The Mn(II) form will generally predominate in acid soils, while Mn(III) occurs more readily at pH 7. Higher manganese oxides such as manganese dioxide (MnO_2) in which the Mn(IV) ion exists will prevail under alkaline conditions (Tisdale and Nelson, 1966). Mann (1930) and Fujimoto and Sherman (1948) noted decreases in the solubility of soil manganese as pH

increased due to the addition of magnesium and calcium carbonates. A five-fold decrease in exchangeable manganese was reported by Heintze (1946) when a clay loam was limed from pH 4.5 to 7.9, while the liming of a silt loam from pH 4.6 to 6.5 decreased exchangeable manganese levels 20 to 50 times (Christensen *et al*, 1950). Bãjescu (1968), when adding manganese sulphate to soil, found the ion most effectively immobilised at pH 7.8 to 8.5. He also found that the immobilisation was linearly related to pH over a range of 6.0 to 8.5.

The mechanism involved for the observed relationship between pH and manganese availability and extractability has not been definitely established. Several theories have been put forward over the years; these are summarised below.

The oxide theory of non-availability was postulated by Piper (1931). As pH increases, the inorganic formation of insoluble higher oxides of manganese is favoured (Leeper, 1935; Fujimoto and Sherman, 1948). If the concentration of Mn(II) ions in solution is converted to its negative logarthmic value (pMn), thus expressing concentration in the same form as pH (negative logarithm of the hydrogen ion concentration), straight line relationships between pMn and pH are obtained for various manganese compounds - MnO, MnO_2, $Mn(OH)_3$, $MnCO_3$, Mn_2O_3, Mn_3O_4, MnOOH (Eriksson, 1952; Hemstock and Low, 1953; Lindsay, 1972). This would suggest a direct relationship between the two ions, with Mn(II) ions becoming available or unavailable as the result of cation exchange reactions with hydrogen ions. Morgan and Stumm (1964) stated that the cation exchange capacity of the hydrous oxides is strongly pH dependent and increases with increasing pH.

A second aspect of the oxide theory of non-availability has been suggested in which the Mn(II) ion is oxidised to higher valency states through biological activity (Gerretsen, 1937; Mann and Quastel, 1946). These higher oxides (both of inorganic and organic origin) are autocatalytic in nature due to large surface area, and will serve to accelerate the oxidation of Mn(II) ions (Leeper, 1947; Fujimoto and Sherman, 1948). In this case soil reaction would indirectly

affect oxide formation via its control on microbial metabolism. Page (1962), however, in studying pH-manganese relationships in Scottish soils presented evidence contrary to the oxide theory. From a number of field sites he determined regression equations of pH versus pMn (water-soluble) and compared these to the theoretical relationships predicted from the equations of Eriksson (1952) and Hemstock and Low (1953). He was unable to explain the behaviour of manganese in the field in terms of the formation or solution of oxide material by changes of acidity. In his laboratory studies he found the relationship between pH and pMn to be curved, whereas higher oxide formation would have implied a linear relationship. Furthermore, in extracting a soil with lime water solutions he showed that soil manganese adopted its changed pH relationship in a time interval short enough to exclude the microbial influence. He therefore attributed the pH-manganese interaction to the formation of manganese complexes with organic matter. However, Bromfield and David (1978) referred to Page's laboratory studies and made the observation that the relationship between soluble manganese and pH for their biologically prepared manganese oxide was identical to his.

Further evidence against the role of organic matter was provided by Walker and Barber (1960), who showed that chelated manganese decreases with rising pH. Passioura and Leeper (1963) indicated that in some Australian soils, the complexing of manganese with organic matter in a non-exchangeable form was of little significance. More recent work on strongly acid brown soils from Western Germany suggests that the Mn(II) levels in soil solution are not directly affected by inorganic manganese compounds or organic manganese complexes but rather by the amount of exchangeable manganese present which responds rapidly to pH changes (Khanna and Mishra, 1978).

The importance of the pH-manganese relationship is strongly emphasised by the fact that, in soil tests, better predictions of manganese availability to plants are obtained if soil pH is considered together with the particular extractant. Cox (1968), in developing yield response predictions for soyabeans, included pH with the amount of manganese extracted with dilute acid. A comparison of a number of extracting methods using 63 different soils

revealed that a simple ammonium acetate extraction, with a correction for pH, was the most efficient method of predicting manganese availability in maize (Browman *et al*, 1969). Also the correlation of DTPA-extractable manganese with manganese concentrations in wheat and soyabean was shown to depend on soil pH (Shuman and Anderson, 1974).

2.6.2 Microorganisms

Microorganisms may affect micronutrient availability in soils in several ways:-

1. The liberation or immobilisation of the ion during organic matter decomposition; this will be dependent on the concentration of the element which is in excess of the microbiological demand.
2. The oxidation or reduction of the element resulting from electron transfer from substances which serve as energy sources to those substances which may become products of respiration.
3. Indirect influences on soil chemistry (concomitant with microbial activity) such as changes in soil pH and oxidation-reduction potential (Alexander, 1977).

2.6.2.1 Liberation or immobilisation

Microbial cells rarely contain more than 0.05 percent of manganese. Therefore, according to Alexander (1977), immobilisation of the metal into microbial tissue is considered to be of no significance.

2.6.2.2 Oxidation and reduction

The most widely studied and probably the most important effects of microorganisms on micronutrient availability involve the oxidation and reduction of manganese and iron (Hodgson, 1963). Indeed, Mann and Quastel (1946) postulated a manganese cycle in soil based almost entirely on biological influences (see Appendix A).

Oxidation

The microbial oxidation of manganese has been observed by many workers since the early part of the century. Schorler (1904) and

Beijerinck (1914) were the first to record the phenomenon. Both bacterial and fungal organisms are known to be responsible (Beijerinck, 1914; Bromfield and Skerman, 1950; Timonin *et al*, 1972). Active organisms isolated include the bacterial genera *Arthrobacter, Bacillus, Corynebacterium, Klebsiella, Mettallogenium, Pedomicrobium and Pseudomonas*. The fungal genera include *Cladosporium, Curvularia, Fusarium* and *Cephalosporium* (Alexander, 1977). Actinomycetes have also been found responsible (Timonin *et al*, 1972; Bromfield, 1978a). Two organisms are occasionally required for the oxidation of manganese to insoluble MnO_2 (Skerman and Bromfield, 1949; Bromfield and Skerman, 1950).

Alexander (1977) stated that the biological oxidation of the metal is not too sensitive to acidity and is greatest at pH 6.0-7.5. Bromfield (1978a) was able to isolate an acidophilous actinomycete from an acid soil (pH 5.0) that was capable of oxidising Mn(II) ions over a pH range of 5.0 to 6.5 while Ivarson and Heringa (1972) provided evidence of oxidation by the soil fungus *Cephalosporium* at a pH as low as 4.5. Bromfield and Skerman (1950) stated that manganese oxidising fungi are probably important in acid soils. Although autooxidation of Mn(II) occurs above pH 8.0, microbial oxidation has been reported at levels as alkaline as pH 8.9 (Alexander, 1977). Bacterial oxidation has not been reported below pH 5.5. This may be related directly to inhibition of growth at lower pH. Bacteria and actinomycetes are known to function better at the intermediate and higher pH values, while fungi can flourish at all soil pHs (Brady, 1974).

In early work on bacterial oxidation of manganese, the presence of the oxides was established by their brown colouration or by a blue colour induced with benzidine. Despite the usual designation as MnO_2, the precise chemical structure of the biologically formed oxide is not understood.

Bromfield (1958a), using X-ray diffraction, could not distinguish any crystalline forms and concluded that the products were amorphous in nature. Hariya and Kikuchi (1964), in their study on manganese precipitation by bacteria in mineral springs, found the initial

amorphous precipitates later recrystallised to birnessite and pyrolusite. Manganese dioxide formed by microorganisms from natural black manganese sludges was shown to contain both birnessite and nsutite (Schweisfurth and Gattow, 1966). Alexander (1977) states that the microbiological oxides seem to be intermediary between the trivalent, Mn_2O_3 and tetravalent, MnO_2 forms.

Several mechanisms for the microbial contribution of Mn(II) oxidation have been proposed. One is based on the hypothesis that oxidation is nonspecifically accomplished by the microbial production of hydroxy-acids and by the pH increase that occurs in their immediate vicinity. Manganese oxidation is known to occur in alkaline solutions that contain citrate, tartrate, malate, lactate or gluconate, all of which are products of carbohydrate decomposition (Alexander, 1977). Bromfield and Skerman (1950) demonstrated an increase in pH from 5.8 to 7.6 resulting from bacterial oxidation of manganese in a citrate medium; they termed the oxidation nonbiological. Therefore in the presence of a hydroxy-acid, any effect that creates an alkaline environment will bring about nonbiological oxidation. Correspondingly, hydroxy-acid production in an already alkaline system will result in the same change (Alexander, 1977).

Bromfield (1956) proposed another mechanism for manganese oxidation by microorganisms, i.e. the direct role of enzymes. He found that oxidation by *Corynebacterium* grown on yeast extract media was inhibited by copper and mercury, suggesting the involvement of a heavy metal-containing enzyme. The chemical 2,4-dichlorophenol also inhibited oxidation, indicating the involvement of catalase. He thought it probable that manganese oxidation occurred inside the cell. Trimble and Ehrlich (1968) successfully isolated a manganese-oxidising enzyme from *Arthrobacter* growing on Atlantic ferromanganese nodules. The negative charge of the oxide caused adsorption of Mn(II) which was then oxidised by the bacterium. Likewise, manganous oxides formed from soil *Arthrobacter* were found to rapidly absorb Mn(II) ions from aqueous solution. However, in the absence of *Arthrobacter*, absorbed Mn(II) was not oxidised (Bromfield and David, 1976).

The biological influence on manganese deficiency has been readily demonstrated. Gerretsen (1937) noted that deficiency symptoms in oats were not manifested unless certain bacteria were present. Healthy plants could be grown if the soil was sterilised with formaldehyde. Timonin (1946) produced data to show that varieties of oats susceptible to manganese deficiency had higher contents of manganese-oxidising bacteria in their rhizosphere. Furthermore, the population of oxidisers was greatly reduced, deficiency symptoms did not occur, and grain yields increased if plants were grown on sterilised soil. Similarly, increased manganese availability was observed by Smith (1963) and Sonneveld and Voogt (1975) on fumigated and steam-sterilised soils, respectively, and was attributed to the suppression of manganese-oxidising microorganisms. Improvement of bacterial activity by drainage, liming or organic matter addition may result in manganese deficiency (Timonin and Giles, 1952; Bauerenfeind, 1960). Field observations reviewed elsewhere (Cheng and Ouellette, 1971) support this view.

Conflicting evidence has often been reported in the literature. Leeper and Swaby (1940) noted that many near-neutral soils, although exhibiting active biological oxidation of Mn(II) ions, nevertheless supplied adequate amounts of manganese for plant growth. Barber and Lee (1974) found that manganese uptake by barley grown in solution cultures was stimulated by the presence of microorganisms. Contrary findings have been noted for oats but not for rape (Bromfield, 1978b). Bacterially formed manganese oxides have been shown to be readily available to oats grown in sand culture (Bromfield, 1958a) and to be extractable with solutions that are commonly employed to estimate soil manganese availability to plants (Bromfield and David, 1978).

Bromfield (1978b) explained manganese uptake on the basis of the rates of two opposing processes:- the rate of biological oxidation of Mn(II) ions and the rate of manganese release from oxides.

Reduction

The transformation of higher-valency manganese oxides to the available Mn(II) forms can be accomplished by several microbially mediated mechanisms. Firstly, as carbon dioxide is evolved, as a

result of organic matter decomposition its dissolution in soil water will form carbonic acid:-

$$H_2O + CO_2 \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-.$$

The ensuing decrease in soil pH can result in increased manganese availability (Tisdale and Nelson, 1966). Neutral or calcareous soils are especially sensitive to small changes in the carbon dioxide concentration - the reduction in pH being approximately proportional to the logarithm of the partial pressure of carbon dioxide, (Bradfield, 1941). Secondly, reduction can occur under anaerobic conditions, resulting from soil compaction or flooding, as oxide material is used as a source of oxygen by microorganisms (Leeper, 1947; Fujimoto and Sherman, 1948). However, Trimble and Ehrlich (1968) were able to isolate two strains of bacteria which reduce manganese dioxide under both aerobic and anaerobic conditions. The exact mechanism for this reduction is not well understood but it is thought that a higher manganese oxide may serve as an electron acceptor for respiratory enzymes. Alexander (1977) represented the reaction as $RH_2+MnO_2 \rightarrow Mn(OH)_2+R$ where RH_2 denotes the enzyme. Thirdly, organic matter decomposition will result in the formation of microbiologically synthesised acids which can effectively regenerate the divalent ion from higher oxides. These acids may be organic, e.g. fatty acids, hydroxy-carboxylic and polycarboxylic acids (Stevenson and Ardakani, 1972), or inorganic. An example of the latter was provided by the work of Garey and Barber (1952) and Vavra and Frederick (1952), who noted an increase in manganese solubility and availability after sulphur addition to soil and solution cultures. Biogenesis of sulphuric acid resulting from the oxidation of sulphur by *Thiobacillus thiooxidans* and the subsequent drop in pH was found to be partially responsible. Metabolic products of other species of bacteria such as *Clostridium*, *Micrococcus* and *Pseudomonas*, as well as a high percentage of the fungi and the actinomycetes, can also effect the nonenzymatic transformation of Mn(IV) to Mn(II) (Alexander 1977).

Since reducing conditions favour the production of the divalent ion, deficiency of manganese is unlikely to be a problem unless amounts of native soil manganese or organic matter are inadequate. Manganese toxicity, on the other hand, is frequently found in

conditions where oxygen supply is limited or absent, especially in flooded soils. Indeed Graven *et al* (1965) noted an increase in the manganese content in alfalfa from 426 μg g^{-1} to a toxic level of more than 6,000 μg g^{-1} after 72 hours of flooding. They suggested that the susceptibility of alfalfa to manganese toxicity may account for its sensitivity to poorly aerated soils. Jha and Siddiqui (1965) found that the destruction of mango trees in flood-affected areas of Bihar State, India, was related to unusually high levels of manganese. Toxicity symptoms have also been reported on apple and pear trees growing in irrigated or waterlogged soils even at near-neutral pH conditions (Grasmanis and Leeper, 1966). Manganese toxicity in paddy rice has been reported by Mukhopadhyay *et al* (1967).

2.6.2.3 Indirect influences

The overall effect of microbial activity in altering the pH or oxidation - reduction potential of a soil will depend on the buffering capacity and poise* of the system and also on the organic substrate. The increase in pH of nearly two units by manganese-oxidising organisms observed by Bromfield and Skerman (1950) occurred on a citrate medium. Hirte (1970) after inoculating soil with high nitrogen amendments, recorded a marked increase in pH associated with microbial proliferation. However, due to subsequent accumulation of organic acids and other substances, the pH slowly decreased to ultimate values lower than in untreated soils.

2.6.3 Organic Matter

2.6.3.1 Effects on manganese uptake

The influence of organic matter on the manganese status of soil and plants has been recognised for many years. Arrhenius (1924) observed that heavy applications of manure were beneficial to manganese-deficient plants, and exchangeable manganese was found to be significantly increased in field plots amended with manure over a 32-year period (Schollenberger and Dreibelbis, 1930). Piper (1931) suggested the practice of green manuring as a means of controlling

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*Poise is defined as the resistance to changes in potential of a redox system upon the addition of small amounts of oxidant or reductant (Ponnamperuma, 1972).

manganese deficiency. An application of sugarcane leaves to a manganiferous soil from Hawaii increased exchangeable manganese after 24 hours. Likewise, the manganese concentration in cowpeas grown on the amended soil was found to be substantially increased (Fujimoto and Sherman, 1948). Miller and Ohlrogge (1958) added manure and manure extracts to a clay loam and found subsequent increases in both soil extractable and plant manganese.

Contradictory results have also been widely reported. Rich (1956) found that, among other soil factors, the level of organic matter was not significantly related to the manganese content of peanut leaves, and Atkinson *et al* (1958) found the manganese uptake by plants to be less after application of farmyard manure. Cotter and Mishra (1968) incubated soils with different amounts of organic matter and noted a loss of exchangeable manganese (at the lower percentages of organic matter) after incubation periods of 5 or more days. Addition of peat moss was found to reduce available manganese in gravelly loam and clay loam soils from Quebec, while carbohydrate amendments slightly depressed the manganese content in potato leaves (Cheng and Ouellette, 1971). Russell (1973) claimed that certain organic compounds can form insoluble complexes with manganese.

2.6.3.2 Mechanisms

The effect of organic matter on inorganic soil constituents is very complex. According to Hodgson (1963), its effect on manganese is particularly pronounced relative to other micronutrients. Early workers (Arrhenius, 1924; Schollenberger and Dreibelbis, 1930; Piper, 1931) attributed the beneficial effects of organic matter on manganese availability to increased microbiological activity in which the oxidation-reduction equilibrium status of the soil shifted in the direction of greater reduction. Leeper (1947) further refined the theory of organic matter - microbiological interaction. He stated that the biological oxidation of organic matter in soil could proceed at such a rapid rate that atmospheric oxygen would soon be depleted. As a result, anaerobic organisms would proliferate and utilise the higher oxides as a source of oxygen - the oxide reduction resulting in the liberation of Mn(II) ions. However, in organic soils, the higher manganese oxides do not commonly occur

(Heintze, 1957).

Christensen *et al* (1950) and Cotter and Mishra (1968) suggested that the reductive effect of organic matter on manganese may be chemical as well as biological. The latter authors found manganese levels in soil increased in less than 3 hours after organic matter amendments and concluded that microbial depletion of atmospheric oxygen could not occur in such a short time. Indeed, recent work has shown that various leachates of organic substances have been effective in dissolving manganese oxides (Zajicek and Pojasek, 1976).

The effect of organic matter on soil manganese status and availability is greatly influenced by soil pH, and the nature of the organic material. These factors are considered separately below.

Soil pH

The ability of plants to utilise higher manganese oxides results from the continuous process of organic matter decomposition. The reducing effect of organic matter is optimum when its biological oxidation is rapid (Ghanem *et al*, 1971). Leeper (1947) observed that the reduction of oxides by organic matter is more likely to occur at low pH. Mattson (1948), however, stated that an alkaline soil reaction enables organic matter to become highly reducing. This is in accord with the evidence that microbiological activity, which is strongly influenced by pH, is favoured at intermediate and higher pH levels (Brady, 1974). Effects of organic amendments on manganese release in limed relative to unlimed soils are short-lived, due to a more rapid decomposition of the organic matter followed by a rapid fixation of released Mn(II) ions (Christensen *et al*, 1950). Sanchez and Kamprath (1959) observed that addition of peat decreased acid-extractable manganese in limed soils but an increase occurred if no lime was added. They also stressed the importance of organic matter content in determining manganese availability in soils of pH $\geqslant$ 6. Manganese deficiencies are seldom found in soils of pH $\geqslant$ 7 unless a certain amount of organic matter is present (Mulder and Gerretsen, 1952; Russell, 1973). However, Cotter and Mishra (1968) noted very high levels of exchangeable

manganese (300 μg g^{-1}) on an alkaline alluvial soil (pH 8.0) which had been amended with cotton seed hulls.

Changes in soil pH brought about by additions of organic matter of different acidity have also been considered as a mechanism influencing manganese availability (Christensen *et al*, 1950).

Effect of organic matter composition

Amendments of organic matter having high C:N ratio will contain large amounts of easily oxidisable substances such as starch and sugars. Leeper (1947), and Fujimoto and Sherman, (1948) postulated that rapid biological oxidation would soon create anaerobic and hence reducing conditions but this seems likely to occur mainly in those soils in which a combination of fine texture and high water content inhibits the supply of oxygen to the microorganisns.

The inherent content of manganese in organic matter is also an important consideration. Atkinson *et al*, (1954) studied trace element contents of fresh and composted farmyard manure from a variety of livestock. Manganese contents ranged from 75 to 249 μg g^{-1} on a dry matter basis with an average of 201 μg g^{-1}. They found that, with the exception of zinc, average quantities were considerably less than the minimum amounts commonly used when treatment was applied as a chemical fertiliser. However, they stated that the regular application of manure should in many cases suffice in alleviating trace element deficiency symptoms. Fresh plant residues are known to vary greatly in manganese content and their subsequent decomposition may affect availability to future plant generations. Concentrations from leaves of forest trees in Kenya ranged from 90 to 5,000 μg g^{-1} in the dry matter. Different species of lupins contained from 500 to over 50,000 μg g^{-1} manganese, while barley content was approximately 100 μg g^{-1} (Chamberlain and Searle, 1963).

2.6.4 Climatic Effects

Manganese in any given soil can exhibit a most pronounced variation in availability to plants; both short term and seasonal fluctuations have been noted. DeLong *et al* (1940) and Kosegarten (1956) found that exchangeable manganese decreased following rainy periods. Higher amounts of water-soluble and exchangeable manganese

have been extracted from soils during summer months (McCool, 1934; Nozdrunova *et al*, 1958). Sherman and Harmer (1942) on the other hand, noted increased levels of exchangeable manganese in spring. The study of Metson *et al* (1979) on seasonal variation in the chemical composition of pasture plants showed manganese content to be the most variable of the six elements studied (Si, Al, Fe, Zn, Cu, Mn); enhanced uptake was noted during the summer months. This is in agreement with the observation of Mederski and Wilson (1955), who found that manganese deficiency symptoms in young soyabean plants disappeared during the summer. Likewise, Fujimoto and Sherman (1945) found manganese toxicity to be more prevalent during summer but markedly diminished during the winter and spring months.

Various workers have attempted to isolate the climatic or environmental parameters that influence manganese uptake and availability in the field. Temperature and/or light intensity appear to play a very significant role. Munns *et al* (1963) showed that seasonal variations in manganese uptake were correlated with temperature, and Epstein (1971) suggested that reduced manganese uptake at low soil temperature prevented severe manganese toxicity in potatoes. He found the manganese ratio of tops to roots to be much lower at the lower soil temperature and thought this could be due to restricted root development. On the other hand, Löhnis (1951) observed that bean plants were able to tolerate a higher concentration of manganese in their tops without injury if grown at higher temperatures, but speculated that other external conditions may also exert an influence. Sutton and Hallsworth (1958) grew lucerne under two temperature and light intensity regimes. Growth in the lower light intensity environment resulted in lower manganese concentrations in the plant material, while temperature had no effect on uptake. This is in agreement with the work of McCool (1935) who found that the shading of bean plants reduced manganese content by approximately half.

Relative humidity may be another important factor on manganese absorption when applied as a foliar spray. The results of Mederski and Hoff (1958) indicated that high rates of manganese absorption could be expected at low vapour pressure gradients between leaf surface and atmosphere. Similar results were obtained by Rossi and

Beauchamp (1971) where quantities of manganese absorbed by soyabean plants were much greater at 70% than at 25% relative humidity.

2.6.5 Effects of Soil Physical Conditions on Availability of Manganese

Various physical conditions, when imposed on soil, profoundly affect levels of extractable manganese. The influence that such factors as drying, heating and wetting have on soils in laboratory conditions may well provide an explanation of what occurs in soil in its natural state. Steam treatment or autoclaving, although unrealistic in relation to field conditions, is nevertheless a widespread practice in the maintenance of glasshouse soils and is therefore considered here. Those fractions of manganese most affected by physical treatments, i.e. water-soluble, exchangeable and easily reducible, are generally considered to be the forms of soil manganese available for plant uptake during a growing season.

1. Air-Drying

Kelley and McGeorge (1913) found the content of water-soluble manganese increased considerably as moist soil was air-dried. Increases in the exchangeable manganese fractions from the moist to the air-dried state were demonstrated by Fujimoto and Sherman (1945) and Zende (1954) for Hawaiian and Australian soils respectively. Later work on Indian soils showed all forms of active manganese, i.e. water-soluble, exchangeable and easily reducible, to be increased upon air-drying (Singh and Pathak, 1970). Storage of air-dried samples will also result in increases in exchangeable manganese. Indeed, Boken (1958) reported increases of almost 600% in exchangeable manganese of some air-dried soils stored at room temperature for one year. Substantial increases upon storage have also been reported by other workers (e.g. Fujimoto and Sherman, 1945; Zende, 1954; Bartlett and James, 1980).

2. Wetting

As the moisture content of an air-dried soil is increased to field capacity, exchangeable manganese levels decrease (Fujimoto and Sherman, 1945; Christensen *et al*, 1950). However, decreases in manganese levels with wetting take place only in well-drained

soils. If moisture content is increased beyond field capacity, reduction processes occur transforming higher valency manganese to the Mn(II) form. McCool (1934) found more manganese in extracts of soil held at high water content than in soils of lower moisture. Once a soil is waterlogged large quantities of Mn(II) become liberated (Ponnamperuma, 1972).

3. Heating

According to Fujimoto and Sherman (1945), heating soils affects exchangeable manganese levels in the same way as air-drying, but to a greater degree. They incubated a soil at constant temperatures of 40°, 50° and 60°C, and sampled it periodically over a number of days. Exchangeable manganese increased with time, and reached greater concentrations at the higher temperatures. After subjecting a number of their soils to oven temperatures of 105°C, as much as 100-fold increases in exchangeable manganese concentrations were observed in some cases.

The increase in exchangeable manganese concentrations is a function of both the storage period and storage temperature (Boken, 1952). There does, however, appear to be a temperature above which extractable manganese levels decrease. Nishita and Haug (1974) found the greatest quantities of water-soluble and exchangeable manganese were, in three out of four soils, extracted from samples heated at 200°C. Above that temperature both forms of manganese decreased markedly. The authors attributed this to the conversion of Mn(II) to insoluble oxide forms.

4. Autoclaving

The steam-heating of soil further enhances the release of extractable forms of soil manganese. Autoclaving soils was found to increase levels of exchangeable manganese to many times those brought about by oven drying (Fujimoto and Sherman, 1945). Water-soluble, exchangeable and reducible forms of manganese were increased in steam-sterilised Indian soils (Singh and Pathak, 1970). The quantity of manganese released can be so great as to cause detrimental effects to crops. For example, Sonneveld and Voogt (1975) reported manganese toxicity in lettuce grown on previously steam-

sterilised glasshouse soils.

2.6.5.1 Mechanisms controlling release and fixation of manganese

The literature on this subject contains a number of contradictory experimental observations and conflicting theories. This is not surprising in view of the complexities of the interactions between pH, type and amount of organic matter and reserves of active manganese oxides, as well as the wide variations in the quantities of manganese present in soils.

Fujimoto and Sherman (1945) postulated that increases or decreases in extractable manganese levels in dry and wet soils could be governed for the most part by a dehydration-hydration action on manganese oxide material. When they heated a soil at increasing temperatures and measured exchangeable manganese after one and two hours, the results resembled the dehydration curves of various soil minerals (Figure 2.2).

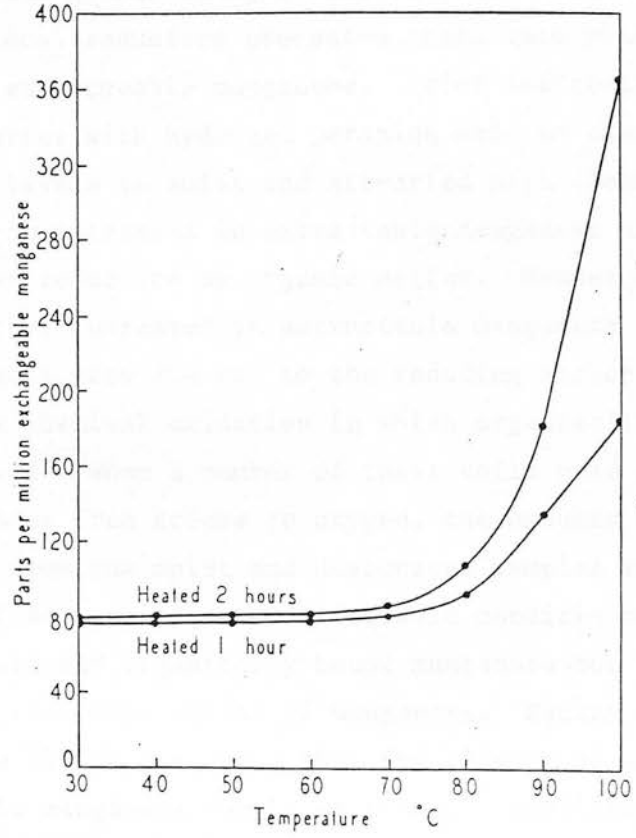


Figure 2.2. Effect of temperature on the level of exchangeable manganese.

Furthermore, the release of manganese by heating seemed to have a critical temperature (70°C) which indicated that the soil manganese, in whatever form it existed, was undergoing some sudden chemical or physical change at that temperature. Fujimoto and Sherman (1945) thought that a certain portion of manganese in the soil existed as a complex hydrated oxide of the type $(MnO)_n(MnO_2)_n(H_2O)_n$ which, under a drying atmosphere, would destabilise as its water of hydration was eliminated; soluble manganese (Mn(II)) and manganese dioxide would result. This reaction, which they thought to be reversible, was later represented in the following manner (see Appendix A).

$$(MnO)_x(MnO_2)_y(H_2O)_2 \rightleftharpoons xMnO + yMnO_2 + zH_2O$$

(Fujimoto and Sherman, 1948).

The organic matter fraction is thought to play a dominant role in increasing extractable manganese levels as a moist soil is air-dried. Boken (1952) thought that in soils relatively high in humus content, local reduction processes could take place, increasing levels of exchangeable manganese. Prior destruction of the soil organic matter with hydrogen peroxide made no change in extractable manganese levels in moist and air-dried soil (Zende, 1954). It was assumed that increases in extractable manganese after drying was due to slow reduction by organic matter. Hammes and Berger (1960) proposed that increases in extractable manganese levels upon air drying a soil were due not to the reducing action of organic matter but to its chemical oxidation in which organically bound manganese was released. When a number of their soils were dried in a desiccator without free access to oxygen, the amounts of manganese extracted from the moist and desiccated samples were equal. However, removal of soil moisture under aerobic conditions influenced markedly acid-soluble and organically bound manganese but had little effect on easily reducible oxides of manganese. Recent work by Khanna and Mishra (1978) has shown that the effects of air-drying on extractable manganese levels of two soil profiles were more pronounced for subsurface samples than for corresponding humus-rich surface samples. Furthermore, air-drying forest soils rich in organic matter did not result in significant changes in extracted

manganese concentrations. They therefore discounted the rôle of organic matter but provided no evidence that oxide material was the source of increased manganese concentrations with drying.

Boken (1958) found that for a number of sandy and loamy soils of low carbon content ($\leqslant 4\%$), an inverse relationship existed between percentage organic matter and the relative increase in the exchangeable manganese content in soils after drying and storage for one year. A similar relationship was observed for two profiles of acid brown forest soils (Khanna and Mishra, 1978). Zende (1954), on the other hand, found no clear relationship between manganese release and organic matter content. He ruled out any connection, since the reducing compounds needed to act on manganese oxides would make up less than 1% of the total organic matter content. However, the observations of Boken (1958) were based on data from a far greater number of soils.

The inherent pH of the soil may be a significant factor in determining the extent of manganese released with drying. Zende (1954), using four Australian soils each adjusted to two different pH values by the addition of lime, found that the absolute increases in exchangeable manganese after air-drying declined with increasing pH. Furthermore, two calcareous peats (pH 8.0) showed no increase in extractable manganese upon air-drying. The results are in keeping with the observation of Leeper (1947) that oxide reduction by organic matter is more likely to occur at lower pH (see above, p.18). Later work by Boken (1958) on a number of air-dried mineral soils revealed no relationship between soil pH and increases in extractable manganese with storage.

2.7 Manganese in Waterlogged Soils

One of the objects of this work was to study the chemical and microbiological release of manganese in submerged soils. Therefore, some of the aspects concerning the behaviour of manganese under waterlogged soil conditions are reviewed.

2.7.1 Background

The flooding of an aerobic soil results in marked chemical changes in soil constituents, brought about by microbial activity.

Organisms obtain needed energy through a series of chemical reactions that involve electron transfer from energy sources to those substances that may become products of respiration. Substances that donate electrons are oxidised and those substances serving as electron recipients are reduced. Oxygen is the soil component which most easily accepts electrons and its concentration usually declines to undetectable levels within a day after soil flooding (Ponnamperuma, 1972). In the absence of free oxygen, other oxidised constituents, eg. NO_3^-, Mn(IV), Fe(III), SO_4^{2-}, can accept electrons, and are thus transformed to their reduced counterparts NH_4^+, Mn(II), Fe(II) and S^{2-}, respectively. Under anoxic conditions organic compounds are no longer fully oxidised to CO_2 and H_2O but instead are transformed into organic acids and methane (Russell, 1973).

2.7.2 Redox Potential (Eh)

Redox potential is a measure of the intensity of oxidation or reduction of the soil and is the single electrochemical property that distinguishes a flooded from a well-drained soil (Ponnamperuma, 1972). In the same way that the pH of a system can be measured as the difference in electrical potential (voltage) between two suitable electrodes, so can the state of oxidation or reduction. According to Patrick and Mahapatra (1968), the ranges of oxidation-reduction potentials usually encountered in well-drained and waterlogged soils are: oxidised, +400 to +700 mV; moderately reduced, +200 to +400 mV; reduced, -100 to +200 mV; and highly reduced, -300 to -100 mV. Nitrate and manganese dioxide are reduced at fairly high redox potentials whereas the reduction of sulphate occurs at much lower potentials in strictly anaerobic conditions. The potentials at which various soil constituents are reduced is shown in Table 2.1.

The submergence of an aerobic soil usually results in a sharp decrease in redox potential within the first few days; Eh then increases to a maximum before decreasing again asymptotically with time. The actual cause of Eh changes is dependent on the initial aerobic potential of the soil, temperature, type and content of organic matter, and nature and content of electron acceptors, as well as the duration of flooding (Ponnamperuma, 1965, 1972).

Table 2.1 Sequence of reduction of oxidation-reduction systems in waterlogged soils*

Reaction	Redox potential in mV at pH_7, 25°C	Sequence
$O_2 + 4H^+ + 4e^- \rightleftharpoons 2H_2O$	830	0
$NO_3^- + H_2O + 2e^- \rightleftharpoons NO_2^- + 2OH^-$	430	1
$MnO_2 + 4H^+ + 2e^- \rightleftharpoons Mn^{2+} + 2H_2O$	410	2
$Fe(OH)_3 + e^- \rightleftharpoons Fe(OH)_2 + OH^-$	-130	3
$Fe(OH)_3 + 3H^+ + e^- \rightleftharpoons Fe(OH)_2 + OH^-$	-140	
$CH_3COCOOH + 2H^+ + 2e^- \rightleftharpoons CH_3CHOHCOOH$	-180	4
$CH_3CHO + 2H^+ + 2e^- \rightleftharpoons CH_3CH_2OH$	-190	
$SO_4^{2-} + H_2O + 2e^- \rightleftharpoons SO_3^{2-} + 2OH^-$	-490	5
$SO_3^{2-} + 3H_2O + 6e^- \rightleftharpoons S^{2-} + 6OH^-$	-200	
$2H^+ + 2e^- \rightleftharpoons H_2$	-420	6
$CO_2 + 2H^+ + 2e^- \rightleftharpoons HCOOH$	-620	7
$H_3PO_4 + 2H^+ + 2e^- \rightleftharpoons H_3PO_3 + H_2O$	-700	
$H_3PO_3 + 2H^+ + 2e^- \rightleftharpoons H_3PO_2 + H_2O$	-920	8
$H_2PO_2^- + H^+ + 2e^- \rightleftharpoons P + 2H_2O$	-930	
$P + 3H^+ + 3e^- \rightleftharpoons PH_3$	-360	

* Source: Ponnamperuma (1965).

The presence of manganese contributes significantly to changes in redox potential. Positive potentials may be maintained even 6 months after submergence in soils low in organic matter (< 1.5%) or with high manganese content (> 2000 μg g^{-1}). On the other hand soils low in active manganese (and iron) with more than 3% organic matter can become highly reduced (-200 to -300 mV) within two weeks of submergence (Ponnamperuma and Castro, 1964; Ponnamperuma, 1965). Yuan and Ponnamperuma (1966) found synthetic MnO_2 retarded decreases in redox potential in a flooded soil and thus prevented adverse effects of excess Fe(II) and other reduction products to rice.

2.7.3 pH

Within several weeks of submergence the pH of acid soils usually increases while a decrease in pH is observed in alkaline soils. Flooding therefore results in the convergence of soil pH to relatively stable values between 6.0 and 7.0 (Ponnamperuma, 1972). However, the pattern of changes is influenced by a number of factors. Besides low temperature (Cho and Ponnamperuma, 1971) or the presence of nitrates (Yamane, 1958) which both retard pH changes, the nature and content of the organic matter and oxidised soil components are very important. Soils high in organic matter and active iron can attain pH values of approximately 6.5 within several weeks of submergence. Much slower pH changes may be observed in acid soils low in organic matter or active iron, and values may not reach more than 5.0 even months after submergence (Ponnamperuma, 1972, 1976). Manganese hydroxide ($Mn(OH)_2$) may be the dominant soil constituent controlling pH changes in soils low in active iron but high in manganese content, whereas CO_2 tension may be important in soils low in both active iron and manganese (Ponnamperuma, 1965).

2.7.4 Transformations of Manganese

Upon submergence of a soil, Mn(III) and Mn(IV) oxides are reduced to the soluble Mn(II) form. This increase in the concentration of Mn(II) has been reported by many workers (eg. Robinson, 1930; Piper, 1931; Ponnamperuma, 1972) and together with the disappearance of oxygen and nitrate, is one of the first measurable effects of reducing conditions in flooded soils (Patrick and Turner, 1968). By labelling their soil with radioactive ^{54}Mn, Patrick and Turner (1968) studied

changes in four forms of soil manganese, viz., water-soluble, exchangeable, easily reducible and residual. The most striking effect observed was the conversion of easily reducible manganese to the exchangeable form. Water-soluble manganese increased only after several weeks and residual manganese was unaffected by water-logging. Sims and Patrick (1978) while noting similar findings concerning water-soluble and exchangeable manganese, also observed that only trace amounts of liberated manganese became associated with the organic fractions. Other manganese transformations known to occur in submerged soils include precipitation of water-soluble manganese as $MnCO_3$, and the reoxidation of the divalent ion when diffusing or moving by mass flow to oxygenated soil interfaces (Ponnamperuma, 1972). The kinetics of these transformations vary markedly between different soils and are determined mainly by the organic matter and active manganese content of the soil (Figure 2.3).

Amounts of water-soluble and exchangeable manganese released during flooding were found by Gotoh and Patrick (1972) and Sims and Patrick (1978) to be inversely related to soil pH and redox potential. Gotoh and Patrick (1972) noted that decreases in redox potential at all pH levels markedly enhanced the transformation of reducible manganese to the water soluble and exchangeable form. At pH 5 only a small decrease in a high redox potential (+700 mV) increased Mn(II) concentrations in the system; at a potential of +500 mV, i.e. when still far from being in a 'reduced' state, almost all the reducible manganese was already converted to the Mn(II) form. They concluded that in acid soils, the effects of acidity were dominant over those due to redox potential. At higher pHs (6-8), manganese reduction to the water-soluble and exchangeable form was accomplished only at much lower redox potentials (+200 - +300 mV).

In an attempt to understand the complex chemistry of manganese transformations in flooded soils and the manganese systems involved, stability field diagrams have been developed that represent those conditic of Eh and pH that are compatible with Mn(II) ions and pure manganese oxides in chemical equilibrium in H_2O (Figure 2.4). Ponnamperuma *et al*, (1969), and Gotoh and Patrick (1972) are amongst a number of workers that have attempted to relate the activity of Mn(II) ions in solution,

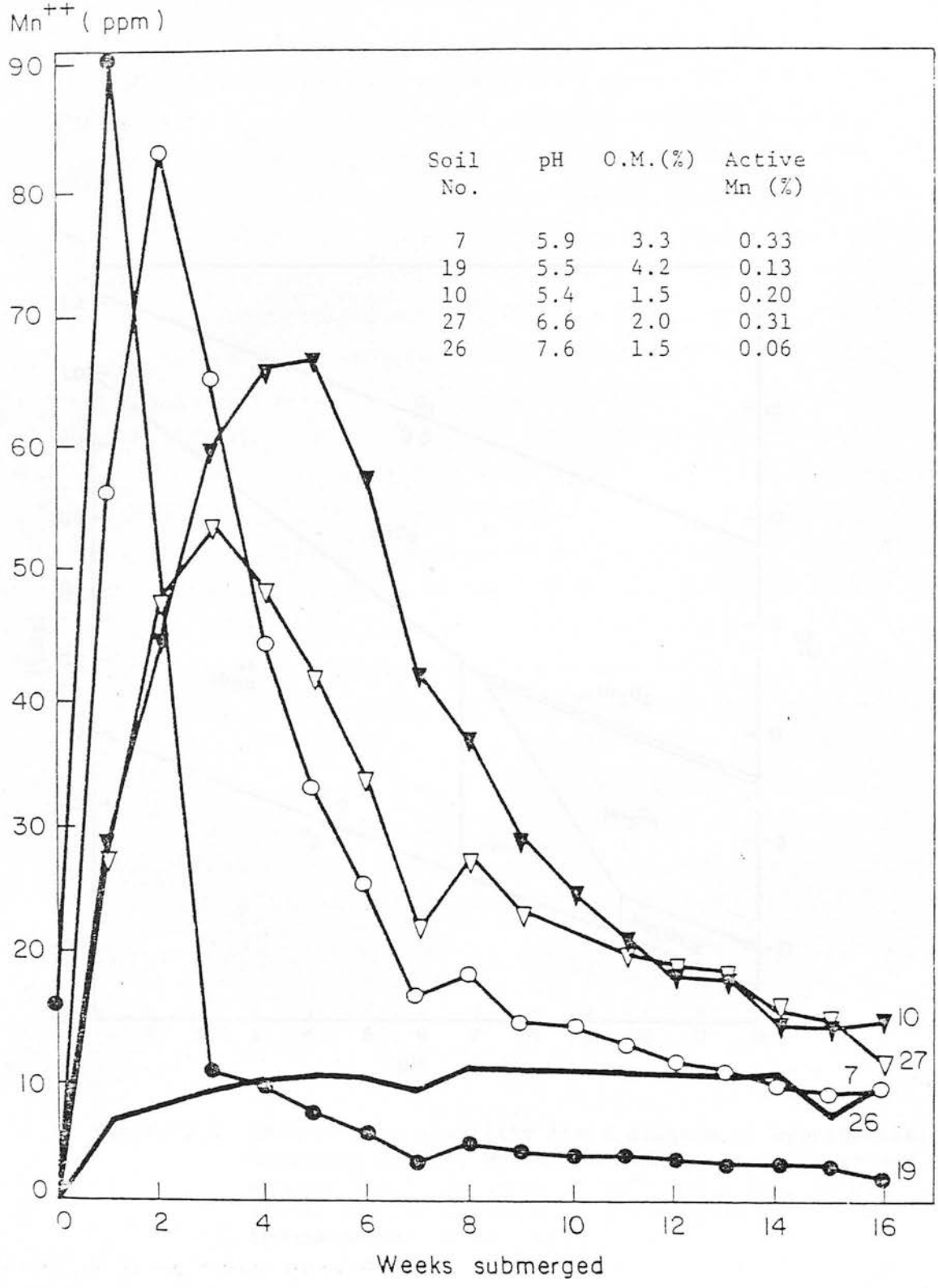


Figure 2.3 Concentrations of water-soluble manganese following soil submergence (Ponnamperuma, 1977)

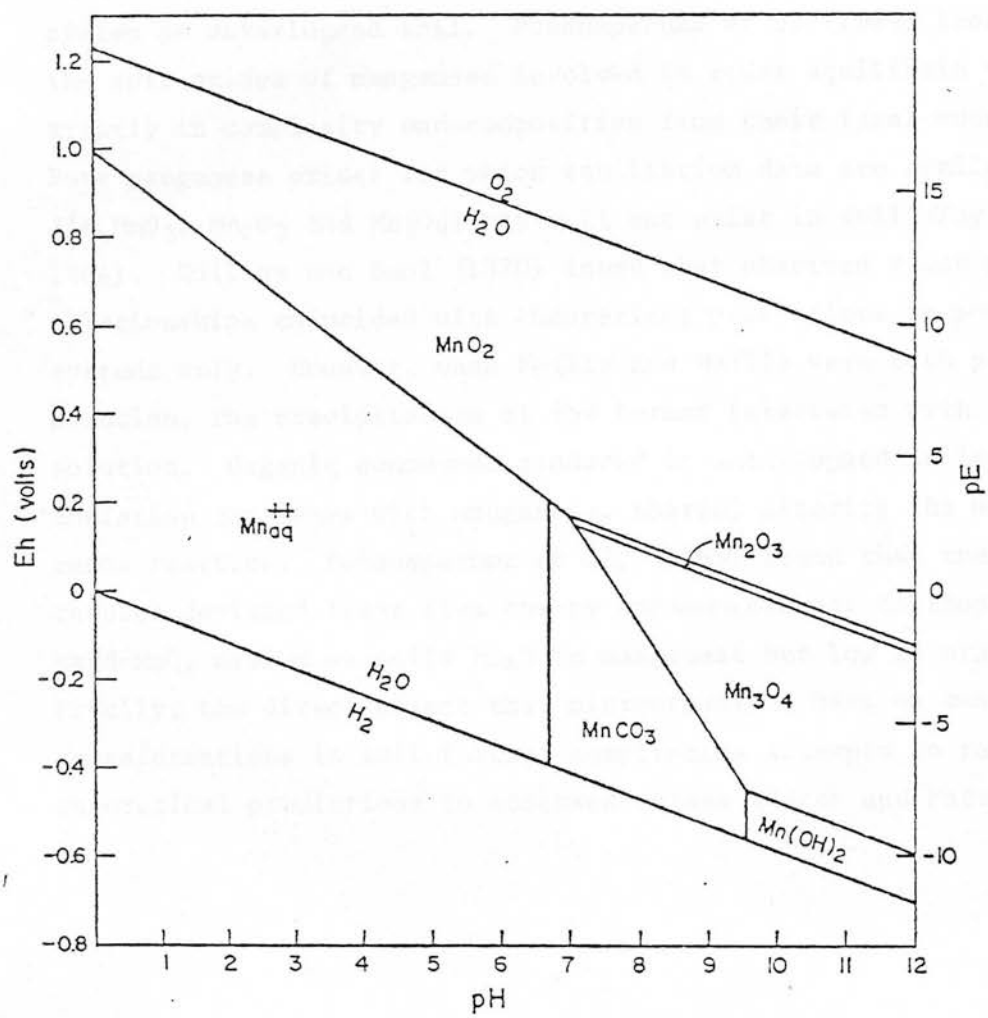


Figure 2.4 Example of a stability field diagram of hypothetical manganese oxides, $Mn(OH)_2$ and $MnCO_3$ relative to an aqueous Mn(II) activity of 10^{-4} and a P_{CO_2} of 0.1 atm at a total pressure of 1 atm at 25^0C (Ponnamperuma *et al*, 1969).

calculated from pure systems of known composition, to those observed experimentally after soil submergence. The former authors, in studying waterlogging effects on sixteen Philipines rice soils, found the redox potential to be lower, for a given concentration of Mn(II) in solution, than expected from theoretical equations. Gotoh and Patrick (1972) reported similar discrepancies between the theoretical and observed relationships on a Louisiana rice soil. These discrepancies are not surprising, given the complex chemical and biological processes that occur in such a heterogeneous system as waterlogged soil. Ponnamperuma *et al* (1969) thought that the soil oxides of manganese involved in redox equilibria varied greatly in complexity and composition from their ideal counterparts. Pure manganese oxides for which equilibrium data are available (i.e. MnO_2, Mn_2O_3 and Mn_3O_4) may well not exist in soil (Taylor *et al*, 1964). Collins and Buol (1970) found that observed redox potential relationships coincided with theoretical predictions in mono-elemental systems only. However, when Fe(II) and Mn(II) were both present in solution, the precipitation of the former interfered with Mn(II) in solution. Organic compounds produced in waterlogged soils may form chelation complexes with manganese, thereby altering the metal's redox reaction. Ponnamperuma *et al*, (1969) found that their observed results deviated least from theory and were closest to those of a sand-MnO_2 medium on soils high in manganese but low in organic matter. Finally, the direct effect that microorganisms have on manganese transformations in soil further complicates attempts to relate theoretical predictions to observed values (Gotoh and Patrick, 1972).

SECTION 3

SOIL CONSOLIDATION AND MANGANESE AVAILABILITY

3.1 INTRODUCTION/EXPERIMENTAL

For some years, poor growth and development of barley crops sown on light-textured soils has been reported often by farmers in many areas of Scotland. The occurrence of 'puffy' or loosely consolidated seedbeds is frequently a factor, and the poor growth is often accompanied by symptoms of manganese deficiency.

Advisory reports have drawn attention to the fact that deficiency symptoms are often not uniform throughout fields and that 'patchy' areas of better tissue growth are frequently encountered. The degree of soil consolidation in these fields appeared to influence the occurrence or severity of deficiency symptoms, with better growth of barley on the more compacted areas.

Work on the effect of soil consolidation on manganese availability has been minimal, but a few authors (e.g. Passioura and Leeper, 1963; Batey, 1971) have noted that compacting certain soils enhances uptake.

In order to investigate this relationship, field trials were set up during 1978-1980. As a direct consequence of findings in the field a number of laboratory and glasshouse experiments were designed in the hope of clarifying the cause and effect relationship of enhanced availability of manganese on compacted soil.

A pot experiment investigating the translocation of manganese within the plant was also carried out.

The following chapter consists of three main subdivisions:- field investigations, laboratory experiments and glasshouse (pot) experiments. General analytical methods concerning soil and plant analysis common to all the sections are outlined below.

3.2 GENERAL ANALYTICAL METHODS

3.2.1 Soils

Due to the large number of soil samples required for analysis from the field sites, it was thought that air-drying would be the most convenient practice. Air-drying a soil facilitates sieving, mixing and handling, and minimises the difficulties inherent in the analysis of non-homogeneous samples.

The main physical and chemical changes that occur when moist soils are air-dried have been considered by various workers over the years. In a recent paper by Bartlett and James (1980) the authors discussed some of these changes and stressed that their importance is too often ignored in soil investigations.

One of the major effects of air-drying a soil concerns the increase in solubility and extractability of manganese (see Section 2.6.5), and such changes must be kept in mind when interpreting experimental data.

Several experiments were carried out to investigate the effects of air-drying on concentrations of extractable soil manganese. These studies, which are outlined in Appendix B.1 emphasised the importance of a standardised drying technique which was suited to rapid routine analysis. It was decided that all soil samples (from the field investigations as well as from the laboratory and pot experiments) were to be air-dried for a period of 7 days prior to analysis. Soils were spread evenly over plastic-coated paper (approximately 1 cm deep) and left for this period in a drying room at a temperature of 20 ± 3°C. The soils were then sieved (<2 mm) and the appropriate determinations carried out.

pH (H_2O)

Soil pH was measured in a 1:2.5 suspension of soil and distilled water on a Pye Unicam meter (Model 290 Mk) using a combined glass/calomel electrode. The electrode was calibrated using standard buffer solutions at pH 4.0 and 7.0. Soil and water were intermittently shaken for 20 minutes prior to measurement.

pH ($CaCl_2$)

Following the pH (H_2O) determination a 0.5 ml aliquot of 1M $CaCl_2$ was added (giving a solution equivalent of 0.01M $CaCl_2$) and the suspension shaken intermittently (over 20 minutes) before measurement.

Organic matter content

Organic carbon was determined using the modified Tinsley method (Bremner and Jenkinson, 1960) using 0.5 g air-dried soil (<0.2 mm). Organic matter was determined by multiplying the per cent carbon by 1.72 (Allison, 1965).

Cation exchange capacity (C.E.C.) and total exchangeable bases (T.E.B.)

The C.E.C. and T.E.B. were determined according to the methods described by Metson (1956).

Particle size distribution

Particle size was carried out by the standard pipette method according to Black (1965). The soil particles were separated according to U.S.D.A. size limits (sand 2.00 to 0.05 mm, silt 0.05 to 0.002 mm and clay less than 0.002 mm).

Elemental determinations

Manganese ($CaCl_2$ - extractable)

Immediately following the air-drying process the soils were extracted with 0.05M $CaCl_2$ (1:10 soil-solution ratio) on a rotary shaker for one hour. Following centrifugation (7000 rpm for 5 minutes), the supernantant solutions were analysed by aspirating into an atomic absorption spectrophotometer (Instrumentation Laboratory Model 251). Manganese determined in this manner is hereafter referred to as extractable manganese.

Manganese (soil solution)

Soil solution was obtained by centrifuging moist soil at 7000 rpm for 20 minutes in perforated polythene centrifuge tubes (McLaren *et al*, 1978). The solution was analysed for manganese by atomic absorption.

Phosphorus, potassium, magnesium and iron (ammonium acetate extractable)

Soils were extracted with a 1M ammonium acetate solution buffered to pH 4.5 (1:5 soil-solution ratio). Samples were shaken for 30 minutes, centrifuged and filtered.

Phosphorus in the supernatant was determined colorimetrically according to a modification of the method of Salt (1968).

Potassium was determined by aspirating the solution into a flame photometer (Corning, Model 400) while magnesium was determined by atomic absorption.

Ammonium and nitrate

Soil samples (20 g) were shaken for 30 min. in 50 ml 1M KCl. After filtration through Whatman No.42 filter paper appropriate dilutions were made. Ammonium and nitrate were determined colorimetrically according to the methods of Crooke and Simpson (1971) and Henriksen and Selmer-Olsen (1970), respectively.

3.2.2 Plant Material

All samples were oven-dried at 105°C for 24 hours and finely ground (<0.2 mm) prior to analysis. Two digestion methods were employed depending on the elemental analysis desired.

Digestion Method 1 - for manganese

Tissue samples (0.5 g) were weighed into 50 ml pyrex beakers. An acid mixture consisting of 10 ml conc. nitric acid and 2 ml perchloric acid (72% w/w) was added and the tissue digested on a hot sand bath. In some cases an additional amount of the acid mixture was necessary to ensure complete digestion. After the acid mixture had completely volatilised, the beakers were removed and cooled. About 10 ml of 2M HCl were added and the contents of the beaker slowly boiled for several minutes. After cooling the solutions were diluted to 25 ml and mixed before filtering through Whatman No.541 paper (MAFF, 1973). Determinations were made by atomic absorption.

Digestion Method 2 - for nitrogen

Tissue samples (0.25 g) were weighed into a 200 mm x 25 mm boiling tube graduated at 50 ml. An aliquot (4 ml) of a selenium-sulphuric acid digestion mixture prepared according to Crooks and Scott (unpublished) was added followed by 4 x 0.5 ml aliquots of H_2O_2 (30%). The contents of the tubes were boiled at 300°C until the solution cleared followed by a further hour of boiling. After cooling the contents were diluted to 50 ml (modified method of O'Neill and Webb, 1970). Nitrogen was determined colorimetrically by the method of Crooke and Simpson (1971).

3.2.3 Preparation of Standards

All elemental determinations in the solutions were made by comparison with standards. Manganese standard solutions were prepared in 1N HCl. The remainder were prepared in solutions similar to those used for the extraction or digestion procedure.

3.3 FIELD INVESTIGATIONS - MATERIALS AND METHODS

Field investigations consisted of soil and plant analysis from seedbed consolidation trials and similar analysis of samples from the fields of commercial farms in South-Eastern Scotland where manganese deficiency symptoms were in evidence (Figure 3.1).

3.3.1 Seedbed Consolidation Trials

Trials were carried out in conjunction with the Crop Production, Advisory and Development Department at the East of Scotland College of Agriculture. These trials had other objectives in addition to the investigation of the manganese deficiency problem, e.g. the effects of seedbed consolidation and depth of sowing on emergence and subsequent growth. These aspects are beyond the scope of this thesis, and only the results concerning soil and plant manganese are presented here.

Some of the characteristics of the soils used in the seedbed consolidation trials are given in Table 3.1.

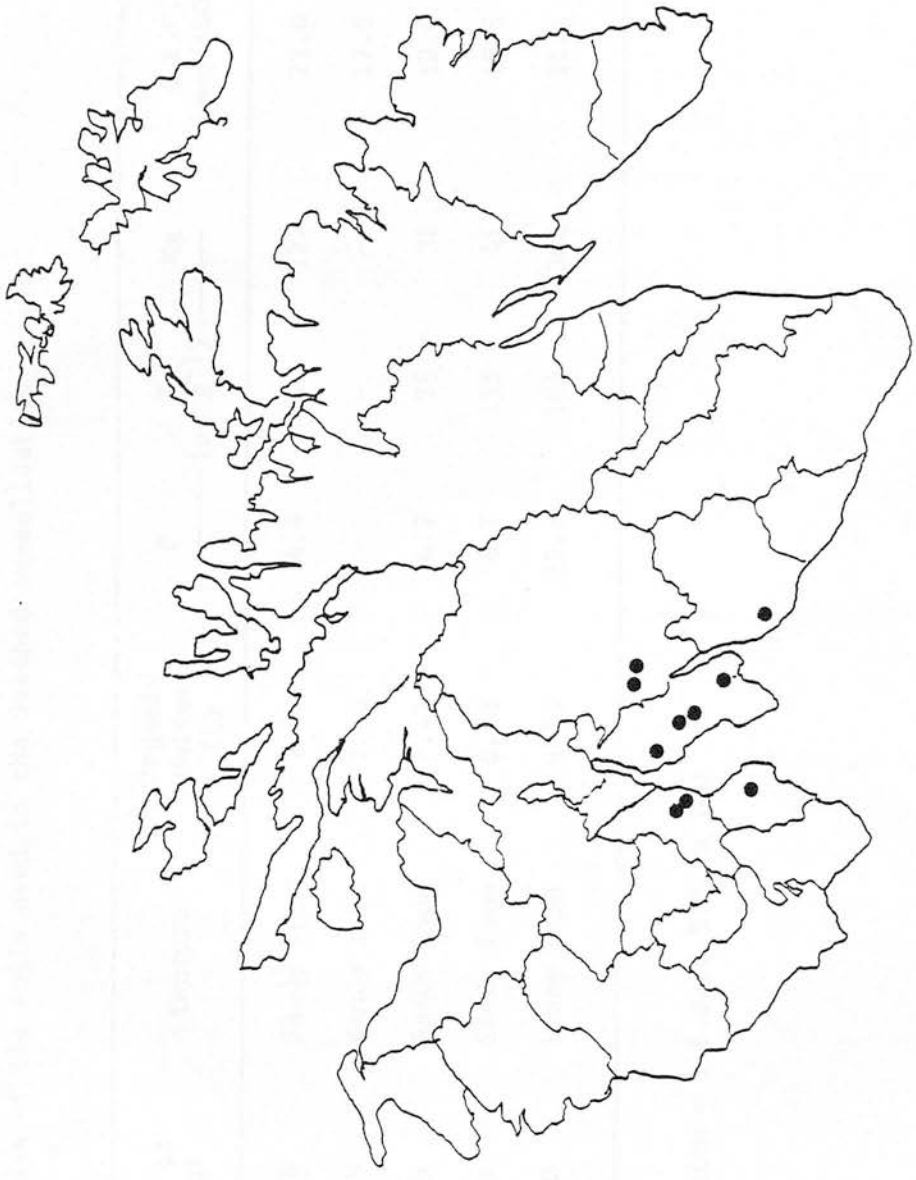


Figure 3.1 Location of sites of field investigations for manganese deficiency.

Table 3.1 Some properties of the soils used in the seedbed consolidation trials

Soil Series	Year of Trial	Texture	Organic Matter (%)	P	K	Mg	C.E.C. (meq/100g)	% B.S.*
				(μg g^{-1})				
Giffordtown	1978	Sandy loam	7.17	4.4	140	127	22.0	84.1
Macmerry	1978	Sandy loam	7.56	-	-	-	17.5	85.7
Hexpath	1979	Sandy loam	7.41	6.7	79	32	12.0	87.5
Darvel	1979	Sandy loam	6.05	6.7	155	41	18.5	48.6
Carpow	1980	Loamy sand	9.88	35.8	164	308	11.5	95.7

* % B.S. - % base saturation = T.E.B./C.E.C. x 100

3.3.1.1 1978 Trials

Experiments were undertaken on soils of the Giffordtown and Macmerry series. On the former soil the barley variety Sundance was used, while plots on the latter were sown with the variety Athos.

Description

Trials were of a split plot design consisting of 3 replicate blocks. The treatments consisted of 3 fertiliser rates applied to main plots and 6 cultivation treatments applied to subplots. The experimental layout is presented in Appendix B.2.

Treatments

1. Seedbed consolidation

For the study of manganese uptake, 3 seedbed conditions were chosen out of six:-

1. Loose (L): triple K*, deep/normal drilling/rolled.
2. Normal (N): dutch harrow/heavy rolled/normal drilling/rolled.
3. Compacted (CP): power harrow/rolled with tractor wheels/normal drilling/rolled.

2. Fertiliser application rates

Three rates of fertiliser were broadcast on the plots prior to cultivation and drilling. At the trial on the Giffordtown soil varying quantities of N-P-K fertiliser (25-10-10) were added. On the Macmerry soil only the nitrogen applications (Nitrochalk - 25% N) differed while a P-K fertiliser (0-20-20) was applied at a constant rate (Table 3.2).

---

* Triple K - a harrow with 'C'-shaped forward raked springed tines.

Table 3.2 Fertiliser application rates on the Giffordtown and Macmerry soils, 1978

Site/Rate		Quantity applied (kg ha^{-1})		
		N	P_2O_5	K_2O
Giffordtown	N1	62	25	25
	N2	94	38	38
	N3	125	50	50
Macmerry	N1	40	50	50
	N2	70	50	50
	N3	100	50	50

3.3.1.2 1979 Trials

Experiments were conducted on soils of the Hexpath and Darvel series. Both trials were sown with the variety Porthos.

Description

The design consisted of 3 replicate blocks of 18 treatments. The treatments were:-

1. A basic set of 4 cultivations x 2 methods of fertiliser application (broadcast and combine-drilled) x 2 spray treatments with $MnSO_4$ (with and without), the cultivations being as follows:-

   1. Loose (L): triple K, deep/normal sowing depth/harrow.

   2. Normal(N): rotera*/harrow drill/harrow.

   3. Compacted 1 (CP1): wheeled by tractor wheels once/ drilled/harrowed.

   4. Compacted 3 (CP3): wheeled by tractor wheels thrice/ light harrowed/drilled/harrowed.

2. A treatment involving cultivation No.2 above (N), together with fertiliser being broadcast down the length of the plot

* Rotera - a power harrow with vertical reciprocating tine.

on the plough furrow prior to cultivation and drilling (with and without $MnSO_4$ spraying). This treatment is hereafter referred to as NBB.

The experimental design is presented in Appendix B.3.

Fertiliser application

Commercial fertiliser (25-9-9) was applied on the Hexpath soil to give equivalents of 109 kg ha^{-1} N, 39 kg ha^{-1} P_2O_5 and 39 kg ha^{-1} K_2O. Another fertiliser (20-10-10) supplied 100 kg ha^{-1} N, 50 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} K_2O to the Darvel soil. The amendments (with the exception of treatment NBB) were either broadcast on the surface at drilling time (BA) or combine-drilled (C).

Foliar spray of $MnSO_4$

Barley growing in plots receiving this treatment were sprayed at three different growth stages (GS) during the season at the following rates:-

GS 21 at 2 kg $MnSO_4$ ha^{-1}

GS 25 at 3 kg $MnSO_4$ ha^{-1}

GS 31 at 2 kg $MnSO_4$ ha^{-1}

3.3.1.3 1980 Trial

This experiment was carried out on a soil of the Carpow series, and like the previous year, plots were sown with the barley variety Porthos.

Description

This trial consisted of a single replicate design of 4 cultivations x 3 fertiliser applications x 2 fertiliser rates x 2 manganese spray treatments (with and without) set out in 4 blocks of 12. The experimental design is shown in Appendix B.4.

Treatments

1. Seedbed consolidation

Consolidation was similar to the L, N, CP1 and CP3 cultivations in the 1979 trials.

2. Fertiliser

Commercial fertiliser (25-9-9) was applied either by:-

1. Broadcasting over the plough furrow prior to cultivation and drilling-BB. This fertiliser application method was the same as that used in treatment NBB in the 1979 trial.

2. Broadcasting at drilling time-BA (similar to 1979 trial on N, L, CP1 and CP3 cultivations).

3. Combine-drilled-C (similar to 1979 trial on N, L, CP1 and CP3 cultivations).

The fertiliser rates are given in Table 3.3.

Table 3.3 Fertiliser application on the Carpow soil, 1980

Rate	Quantity applied (kg ha^{-1})		
	N	P_2O_5	K_2O
N1	48	17	17
N2	78	28	28

3. Foliar spray of $MnSO_4$

$MnSO_4$ spray was applied at the same rate and growth stages as described for the 1979 trials. With the exception of grain yield determinations, no other analyses were carried out on plots treated with manganese sulphate.

3.3.2 Survey of Fields of Commercial Farms

In 1978 and 1979 visits were made to commercial farms in different parts of S.E. Scotland where reports from agricultural advisers indicated that manganese deficiency symptoms were evident. These fields had been cultivated and sown according to current commercial practice, with no unusual experimental treatments being involved, and were thus representative of normal conditions in the area.

3.3.3 Field Measurements of Soil Consolidation

Bulk Density

Determinations were made by driving a 331 cm^3 stainless steel cylinder into the soil. The contents were weighed (for moisture analysis, oven-dried at 105°C overnight and reweighed. A correction for stone volume was made by sieving stones from the soil, weighing, and assuming a particle density of 2.65 g cm^{-3}.

Penetrometer Measurements

Measurements were carried out by a Farnell Soil Assessment Cone Penetrometer Model 244 using a cone index scale calibrated 0-300. A 30° cone (1.29 cm^2 cross section) was pushed vertically into the soil to a depth of 15 cm. The maximum reading during this insertion was noted. Cone index readings were multiplied by 0.175 to convert cone resistance to kg cm^{-2} (Soane *et al*, 1971).

3.4 FIELD INVESTIGATIONS - RESULTS AND DISCUSSION

3.4.1 Field Work, 1978

Soil and plant samples were taken on a number of occasions during the growing season. The first soil and plant samples were taken at 6 and 8 weeks, respectively, after sowing the Giffordtown soil. These dates were 2 and 4 weeks after emergence, thus the soil was sampled in the middle of the period between emergence and plant sampling, in the hope that this would be more representative of the uptake period than sampling at the beginning or end. The first soil and plant samples from the experiment on the Macmerry soil were taken 8 and 10 weeks after sowing, respectively.

Effects of consolidation

Results for both fields are shown in Table 3.4. Values represent the means over all the fertiliser treatments. The data show that the different consolidation treatments had a minimal effect on bulk density; the differences observed were not significant. Likewise, soil pH and soil and plant manganese concentrations were not detectably affected by the different consolidation treatments.

Table 3.4 Effect of cultivation treatment on bulk density, soil pH and soil and plant manganese on the Giffordtown and Macmerry soils, 1978 (mean of 3 replications and 3 fertiliser rates)

Soil	Consolidation	Bulk density ($g\ cm^{-3}$)	pH (H_2O)	Mn, soil ($\mu g\ g^{-1}$)	Mn, plant ($\mu g\ g^{-1}$)
Giffordtown	L	0.88	6.0	3.5	22.3
	N	0.90	6.0	3.5	22.6
	CP	0.95	6.1	3.4	19.4
Macmerry	L	1.05	6.6	8.5	12.1
	N	1.05	6.7	7.2	11.3
	CP	1.13	6.7	7.9	10.8

Effects of Fertiliser Rates

The effect of different fertiliser application rates on the soil pH and extractable manganese as well as manganese content in the plant is shown in Table 3.5 for both the soils (also included are data showing manganese content in the ears at harvest). Increasing the amount of fertiliser significantly increased ($P<0.001$) the uptake of manganese on the Giffordtown soil while the observed increases on the Macmerry soil were not significant. Fertiliser rate had no significant effect on pH or extractable manganese.

The effect of increased manganese uptake with fertiliser rate may be attributable to several factors. These will be discussed later in the thesis.

A comparison of soil and plant manganese concentrations on the Giffordtown and Macmerry soils

A comparison of concentrations of soil and plant manganese in both fields (Table 3.4) shows that despite the much higher concentrations of extractable soil manganese in the Macmerry soil, tissue concentrations were about half those observed in the crop growing on the Giffordtown soil. The water-soluble manganese concentrations in

Table 3.5 Effect of fertiliser application rate on soil pH and on soil and plant manganese on the Giffordtown and Macmerry soils, 1978 (mean of 3 replications and the 3 tillage treatments)

Soil	Fertiliser Rate	pH (H_2O)	Mn, Soil (µg g^{-1})	Mn, Plant (µg g^{-1})	Mn, Ears (µg g^{-1})*
Giffordtown	N1	6.0	4.0	17.9	6
	N2	6.1	3.0	19.8	8
	N3	6.0	3.5	25.9	15
Macmerry	N1	6.6	8.1	10.6	
	N2	6.8	6.6	11.1	
	N3	6.7	8.8	12.5	

* At harvest - single replication only.

the soils were determined to see whether they were more related to uptake by the plant. A water extraction of both soils (1:10 soil-solution ratio) yielded 0.19 µg g^{-1} and 0.41 µg g^{-1} water-soluble manganese for the Giffordtown and Macmerry soils, respectively. These concentrations were only about 5% of those in the $CaCl_2$ extract. However, the ratio of water-soluble manganese concentrations in the two soils was virtually identical to that in $CaCl_2$ ($\sim$2). Thus, water extractions appear to be no better at predicting manganese uptake than $CaCl_2$; although, since the two soils had been kept in the air-dried state for different lengths of time, it should be kept in mind that the concentrations measured cannot be taken as absolute comparisons. Page *et al* (1962) also doubted the value of water-soluble manganese in predicting plant uptake.

The use of a different barley variety at each trial (Sundance vs Athos) could provide a clue to account for the discrepancy between extractable manganese concentrations and uptake (see Section 2.3). Further work in this respect would be worthwhile.

Site effects on the Giffordtown soil

Contrary to the field trial on the Macmerry soil, symptoms of manganese deficiency in the crop were quite evident on the Giffordtown

field. The severity of deficiency symptoms was not uniform, however (Figure 3.2). Furthermore, within each individual plot better plant growth was evident within two narrow strips where tractor wheels had passed over the ground several times during cultivation and sowing operations. Plant growth between the tractor wheel tracks was very poor and manganese deficiency symptoms much more evident (Plates 3.1 and 3.2). In Area C visible differences in wheel tracks and the non-wheeled area were not as marked as those observed in Areas A and B.

The sampling of the soil and plant material for analysis (and subsequent interpretation of results) was designed, therefore, to distinguish between the various deficient areas of the field as well as between the wheel tracks and on the wheeled areas in individual plots.

Effects of wheel tracks

Results of the first tissue sampling showed (Figure 3.3) a very marked increase in manganese concentration in the plants growing in the wheel tracks, for both Areas A and B (which encompassed the plots of the first replicate, within which visible differences in the plants were most evident). If barley is considered manganese deficient at concentrations ≤ 20 µg g^{-1} (Batey, 1971), all of Area B came within this category. Area A, however, although showing symptoms of manganese deficiency, was well above this minimum level in most instances. Similar effects of wheeling were observed in the manganese content in the barley ears at harvest time (Figure 3.4).

Generally, extractable manganese concentrations from corresponding soil samples in Areas A and B were correlated with the concentrations observed in the plants (Figures 3.5 and 3.6). Area C also showed marked differences in manganese content on wheel-tracked and non-wheeled sections of the plot (Figure 3.6). It is also worthwhile to note that the effect of higher levels of extractable manganese in the wheel tracks was maintained throughout the season.

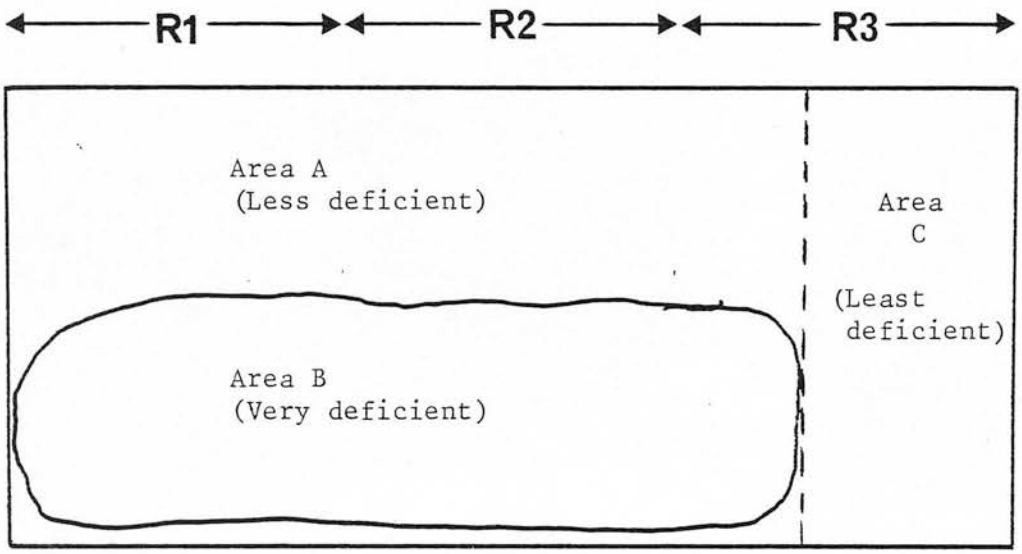


Figure 3.2 Approximate location of areas visibly affected by manganese deficiency at the Giffordtown experimental site, 1978.

R - replicate

3.1

3.2

Plates 3.1 and 3.2 Effect of tractor wheel compaction on barley growth and colour on individual plots at the field trial on the Giffordtown soil, 1978

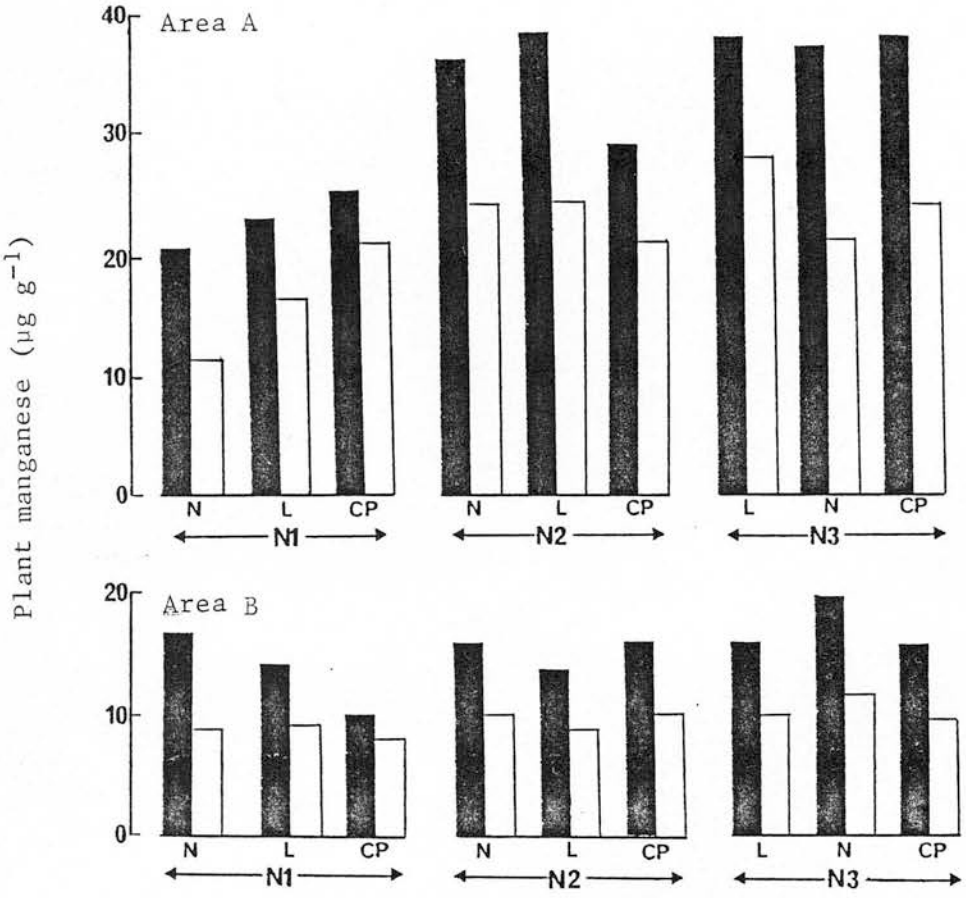


Figure 3.3 The concentration of manganese in barley growing from the wheel-tracked and non-wheeled sections of some plots (Replicate 1) on the Giffordtown soil, 1978 (refer to Figure 3.2).

■- wheel-tracked; □- non-wheeled

N, L, CP - seedbed consolidation treatments

N1, N2, N3 - fertiliser application rates

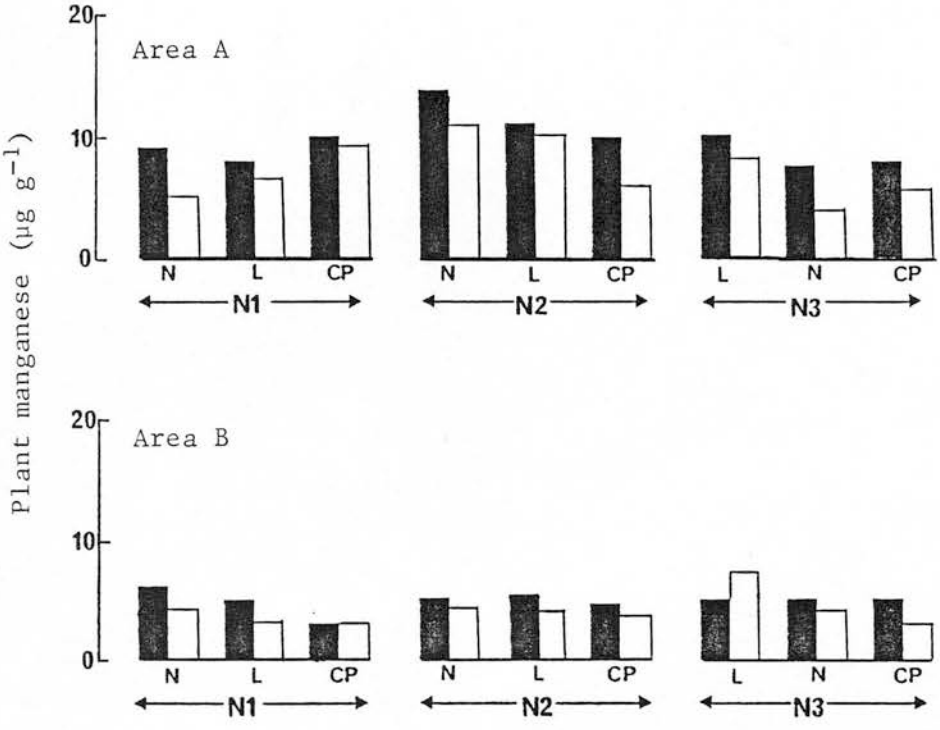


Figure 3.4 The concentration of manganese in barley ears at harvest from the wheel-tracked and non-wheeled sections of some plots (Replicate 1) on the Giffordtown soil, 1978 (refer to Figure 3.2).

■- wheel-tracked; □- non-wheeled

N, L, CP - seedbed consolidation treatments

N1, N2, N3 - fertiliser application rates

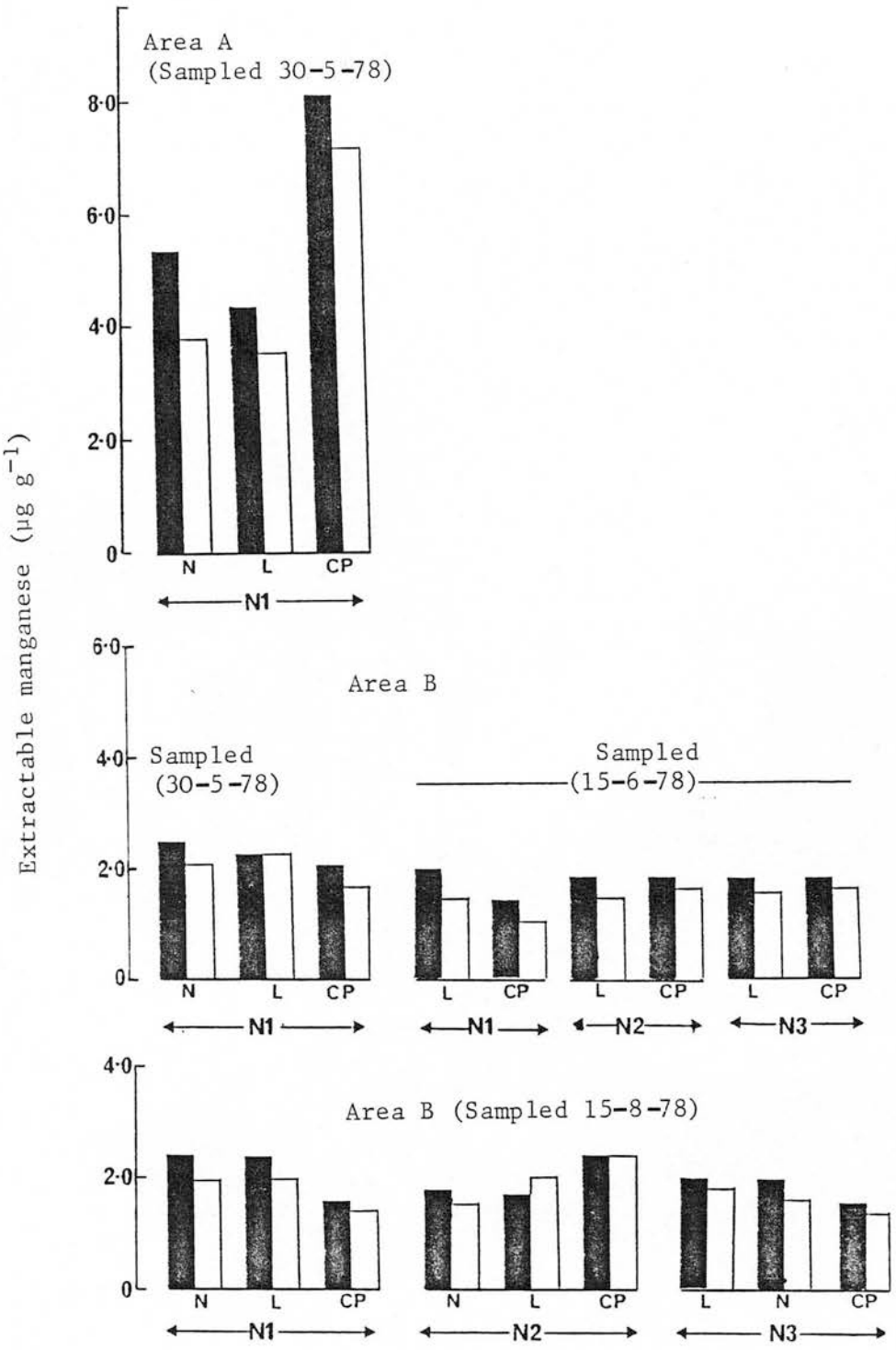


Figure 3.5 The concentration of extractable manganese from the wheel-tracked and non-wheeled sections of some plots (Replicate 1) on the Giffordtown soil, 1978 (refer to Figure 3.2).

■- wheel-tracked; □- non-wheeled

N, L, CP - seedbed consolidation treatments

N1, N2, N3 - fertiliser application rates

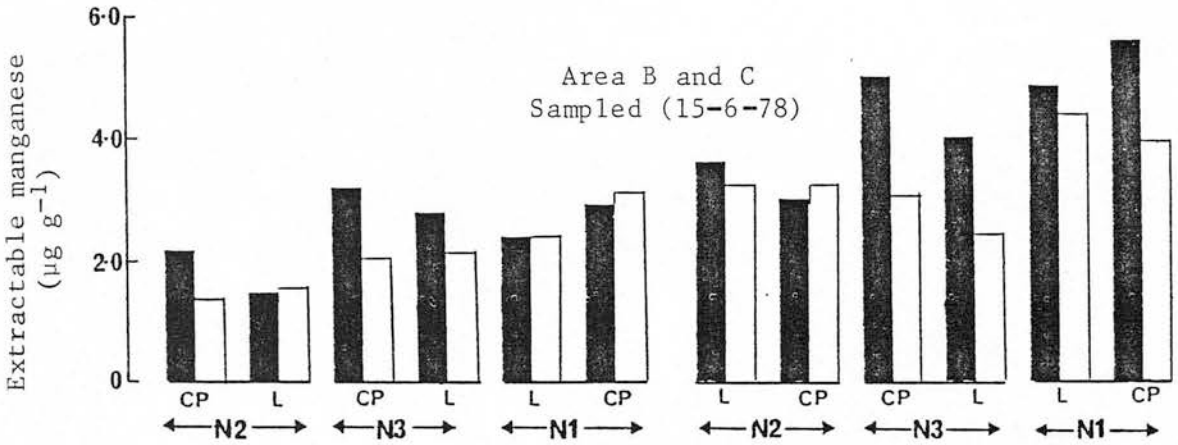


Figure 3.6 The concentration of extractable manganese from the wheel-tracked and non-wheeled sections of some plots (Replicates 2 and 3) on the Giffordtown soil, 1978 (refer to Figure 3.2).

■- wheel-tracked; □- non-wheeled

N, L, CP - seedbed consolidation treatments

N1, N2, N3 - fertiliser application rates

Penetrometer measurements in the wheel tracks and non-wheeled areas of the individual plots showed higher readings in the former, regardless of the overall seedbed consolidation imposed on the whole plot (Table 3.6). Although these measurements indicated a greater soil resistance (compaction) in the wheel tracks this was not detected in bulk density determinations. This could be explained by considering the larger depth of soil extracted (7.5 cm) for bulk density determinations which would mask any surface compaction resulting from tractor wheelings.

Results summarised in Table 3.7 show that in both Areas A and B, the pH was reduced within the wheel tracks and these differences were reflected in manganese uptake by the plant. The greater penetrometer readings in the wheeled areas suggested that soil compaction might have an indirect effect on manganese availability via its influence on the pH. However, compaction was not the only factor involved, because although within Areas A and B comparable relative changes occurred in pH and extractable manganese, the actual values for manganese in Area B were less than half those in Area A although the corresponding penetrometer readings were slightly greater. The data also showed that changes in extractable manganese and in manganese content of the grain at final harvest were not, by themselves, good indicators of yield. Dry grain yield (Table 3.7) was found to be greater on the wheeled sections of the plots in both Areas A and B. However, the yield on the wheel tracks of Area B was over 1.5 times greater than the yield on non-wheeled - Area A, yet manganese concentration in the ears from the former was much less (see data for samples taken on August 23). Furthermore, plants in Area B were growing on soil with less extractable manganese. 'Edge' effects may partially be responsible in accounting for increases in the yields from the wheel tracks compared with immediately adjacent non-wheeled areas. Competition for nutrients, space and moisture would be lessened because of the poor growth on either side of the track. However, such 'edge' effects cannot account for the observed variations between comparable situations in Areas A and B of the trial.

Results of soil and plant analysis at the various sampling times during the growing season were found for the most part to be

Table 3.6 Soil compaction as measured by penetrometer readings on the wheel tracks and non-wheelings of Areas A and B at the site on the Giffordtown soil, 1978

Seedbed Consolidation	Penetrometer readings (kg cm^{-2})*							
	Area A - Wheeled		Area A-Non-wheeled		Area B - Wheeled		Area B-Non-wheeled	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
L	14.5	1.8	10.2	2.1	16.8	2.4	9.6	1.8
N	22.4	2.6	12.6	2.4	23.3	2.6	10.7	3.0
CP	18.7	1.8	13.5	1.4	23.3	1.9	18.2	1.9

* mean of 10 measurements.

S.D. = standard deviation.

Table 3.7 Summary of data for the first replication in Areas A and B and on wheel tracks and non-wheeled sections of the plots at the site on the Giffordtown soil, 1978

Date of Sampling	Analysis	Area A		Area B	
		Wheel tracks	Non-wheeled	Wheel tracks	Non-wheeled
	Penetrometer†	18.6	12.1	21.1	12.8
30-5*	pH (H_2O)	5.7	5.9	6.3	6.4
	pH ($CaCl_2$)	5.2	5.3	5.8	5.9
	Mn, soil ($\mu g\ g^{-1}$)	6.0	4.8	2.2	2.0
8-6	Mn, plant ($\mu g\ g^{-1}$)	31.0	21.4	15.3	9.7
23-8	Mn in ears at harvest	9.4	7.1	4.7	4.0
	Dry grain yield ($g\ m^{-2}$)	460	320	520	290

† mean readings from Table 3.6.

* at N1 rate only.

significantly different between the wheel-tracked and non-wheeled areas of individual plots. Paired t-tests on the various data were carried out, and the results are summarised in Table 3.8.

Soil pH and extractable manganese

Extractable manganese in all soil samples, whether from wheel tracks, from non-wheeled areas or for bulk samples from entire plots, showed a very high correlation with pH (Figure 3.7). The manganese concentration is plotted as $\log[Mn^{2+}]$ because pH is itself a log scale and an increase in the H^{+} ion concentration increases manganese solubility (Lindsay, 1972). These results are in agreement with those of other workers (e.g. Page, 1962) concerning the marked influence of soil pH on the solubility of manganese.

Wheel-track effects on a field of the Corby soil series

During the course of the 1978 season manganese deficiency symptoms developed on a field on the Corby soil series (gravelly loam; O.M. 10.02%) which was the site of a barley variety trial. Wheel-tracking effects were clearly seen within several variety plots and in much of the surrounding area outwith the experimental site. Analysis of soil and plant samples showed the same effects as those observed on the trial on the Giffordtown soil, i.e. a reduced pH, increased availability of soil manganese and greater manganese uptake by plants growing in the wheel tracks (Figure 3.8).

Other nutrients (in wheel-tracked and non-wheeled areas)

Since the determination of other trace elements was not routinely carried out, it cannot be said conclusively that the greater levels of soil manganese found in wheeled areas were peculiar to that element only. However, determinations of EDTA-extractable soil copper (1:5 soil-solution ratio shaken for 1 hour in 0.04M EDTA) on some of the soil samples from the Giffordtown and Corby fields showed that the solubility of the element was unaffected by the presence of wheel tracks. The routine determination of iron in $CaCl_2$ extracts did not prove satisfactory. Levels extracted from air-dried samples were very low and poor agreement between duplicate analyses prevented any detection of wheel-tracking effects similar to those of manganese.

Table 3.8 Summary of the results of paired t-tests for the significance of difference between wheel tracks and non-wheelings of soil pH and soil and plant manganese on the Giffordtown soil, 1978

Analysis	Sampling date	Section of field sampled*	Significance of difference between wheel tracks and non-wheelings
Mn, plant	8-6-78	Replicate 1, Area A	$p < 0.001$
		" , " B	$p < 0.001$
Mn, plant (ears only)	24-8-78 (harvest)	" , " A	$p < 0.001$
		" , " B	$p < 0.10$
Mn, soil pH ($CaCl_2$)	15-6-78	Replicates 1, 2 and 3) of Area B)	$p < 0.01$ $p < 0.05$
Mn, soil pH ($CaCl_2$)	15-8-78	Replicate 1, Area B	$p < 0.01$ $P < 0.10$

* Refer to Figures 3.2-3.6.

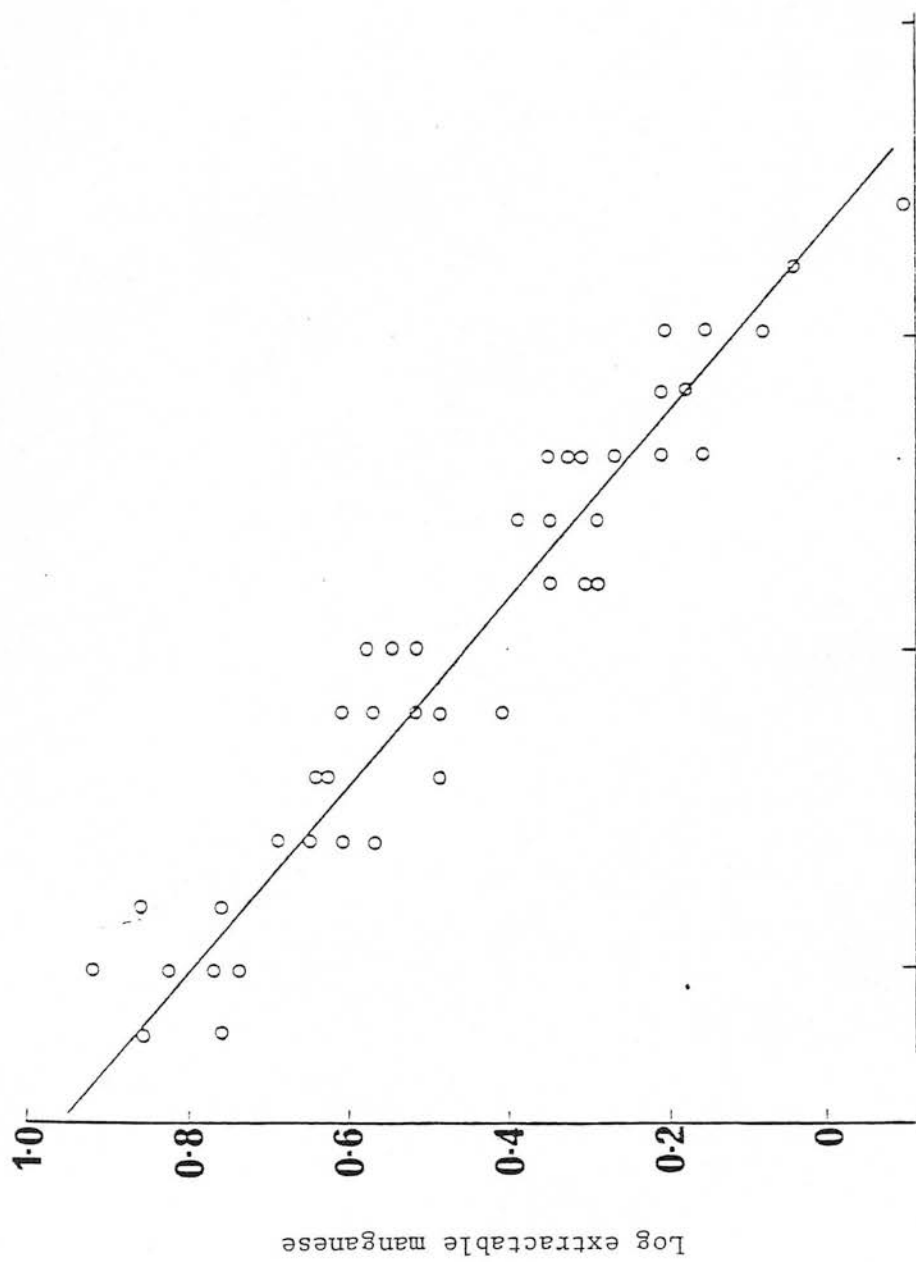


Figure 3.7 The relationship between pH (H_2O) and extractable manganese on the Giffordtown soil.

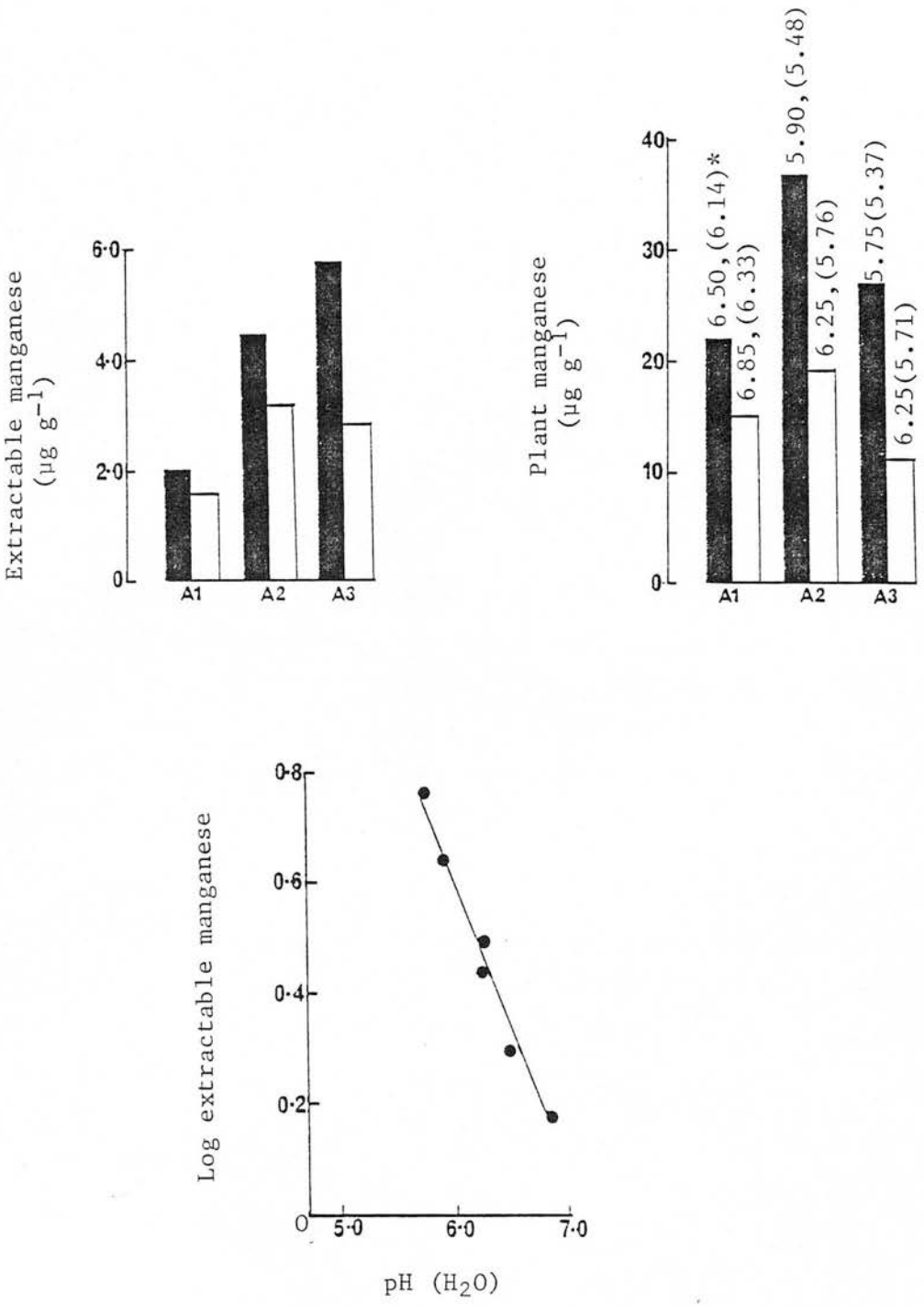


Figure 3.8 Results of soil and plant analysis from wheel-tracked and non-wheeled areas on a field of the Corby soil, 1978.

A1, A2, A3 - sampling sites on the field

*values represent pH readings - 1st value pH (H_2O); 2nd value, in brackets, pH ($CaCl_2$)

■ wheel-tracked; □ -non-wheeled.

According to Ponnamperuma (1977), the concentration of water-soluble iron is extremely low in aerobic soils since it is governed by the solubility of Fe(III) oxide hydrates. Thus, it is understandable that the $CaCl_2$ solution used in this study, being a relatively weak extractant, would not be expected to solubilise adequate amounts of iron for analysis. The use of a stronger and more acidic extractant (ammonium acetate buffered at pH 4.5) solubilised enough iron for accurate determinations, but the analysis of six pairs of wheel-tracked and corresponding non-wheeled soils did not show a relationship similar to that of manganese.

Summary of 1978 field data

Results of field experiments on soils of the Giffordtown and Macmerry series indicated that different cultivation treatments had no significant effect on levels of manganese in the soil and plant. Increasing rates of fertilisers increased manganese uptake (significantly on the Giffordtown soil) but had no observable effect on pH or the extractable manganese status of the soil.

Penetrometer measurements on the band of soil running on either side of each treatment on the Giffordtown trial indicated a compacting effect resulting from the repeated passes of tractor wheels during cultivation. Soil pH was more acid in these wheeled areas and available manganese higher than in the less compact soil between the wheel tracks. Similar results were noted at another site of a barley variety trial on a soil of the Corby series.

3.4.2 Field Work, 1979

Field trials were designed to investigate the effects of cultivation and fertiliser placement (either broadcast or drilled with the seed) on the manganese status of the soil and plant, as well as on plant yield. It was thought that the wheel-tracking effect could be influenced by methods of fertiliser application, and sampling was carried out on the wheel tracks and non-wheeled areas of each plot to see if trends occurred which were comparable to those observed in 1978. Manganese sulphate spray was applied to half the plots to determine any beneficial effects on plant manganese content and yield and to test interactions with tillage. Analysis of the

various soil parameters, i.e. bulk density, pH and extractable manganese were found not to differ significantly between the plots receiving manganese spray and those not sprayed. Soil data are therefore presented as the mean of both treatments.

Effects of consolidation

The effects of the different cultivation techniques on soil pH and on soil and plant manganese are summarised in Table 3.9. Cultivation treatments CP1 and CP3 significantly increased ($p<0.01$) soil bulk density relative to the other treatments for the Hexpath soil, but little effect was observed on the Darvel soil. As in the two trials the previous year, cultivation treatments were found not to affect significantly pH or manganese concentrations in the soil and plant (irrespective of spray treatment) at either trial. No discrepancy was noted between the two sites in the relationship between extractable soil manganese and plant concentrations (Table 3.9) unlike the situation found in the Giffordtown and Macmerry trials of 1978 (Table 3.4).

Visible symptoms of manganese deficiency were not noticeable on the Hexpath field despite levels below the supposed minimum required found in the tissue at harvest. This absence of deficiency symptoms was similar to what was observed on the Macmerry soil in 1978. Occurrence of manganese deficiency would seem unlikely on the Darvel field, given the low soil pH and relatively high levels of extractable manganese. The concentration of manganese in the plants reflected this.

Effects of wheel tracks due to fertiliser application methods

As previously described (see Section 3.3.1.2) the plots that had received the four standard cultivation procedures (L, N, CP1 and CP3) had fertiliser either broadcast at drilling (following seedbed cultivation) or combine-drilled. The influence of these fertiliser application methods on soil pH and extractable manganese in the wheel-tracked and non-wheeled areas of the plot is shown in Table 3.10. Paired t-tests on the soil data showed no significant differences in pH and extractable manganese in the wheeled and non-wheeled areas. Plant manganese concentrations at the first sampling were

Table 3.9 Effects of different cultivation treatments on bulk density, soil pH and soil and plant manganese (averaged over entire plot) on the Hexpath and Darvel soils, 1979

Soil	Consolidation	Bulk density ($g\ cm^{-3}$)	pH (H_2O)	pH ($CaCl_2$)	Mn, soil* ($\mu g\ g^{-1}$)	Mn, Plant ($\mu g\ g^{-1}$)			
						First sampling†		Harvest (whole plant)	
						With Mn spray	Without Mn spray	With Mn spray	Without Mn spray
Hexpath	L	1.25	6.06	5.62	2.5	64.0	17.5	15.0	12.6
	N	1.22	6.10	5.72	2.5	65.8	17.0	14.6	13.2
	CP1	1.33	6.10	5.73	2.4	56.0	17.2	14.6	16.4
	CP3	1.36	6.24	5.85	2.2	65.2	18.0	15.8	15.9
Darvel	L	0.94	5.69	5.31	5.1	54.3	25.6	28.0	26.0
	N	0.91	5.65	5.22	6.2	51.1	27.5	29.1	26.2
	CP1	0.97	5.78	5.40	5.1	59.3	24.1	28.5	24.3
	CP3	0.97	5.57	5.19	5.9	50.0	28.8	27.4	25.9

* Sampled 3 weeks after sowing.

† First sampling was 6 weeks and 10 weeks after sowing for the Hexpath and Darvel soils respectively.

Tissue samples for sprayed plots were taken 6 days and 10 days following spray applications on the Hexpath and Darvel soils respectively.

Table 3.10 Soil pH and extractable manganese on wheel-tracked and non-wheeled areas of plots as affected by two methods of fertiliser application on the Hexpath and Darvel soils, 1979

Soil	Soil sampling date	Area of individual plots	Fertiliser application method					
			Broadcast at drilling			Combine-drilled		
			pH (H_2O)	pH ($CaCl_2$)	Mn, soil ($\mu g\ g^{-1}$)	pH (H_2O)	pH ($CaCl_2$)	Mn, soil ($\mu g\ g^{-1}$)
Hexpath	25-4-79	Wheel-tracked	6.13	5.75	2.4	6.19	5.80	2.3
		Non-wheeled	6.05	5.67	2.5	6.13	5.73	2.4
Hexpath	24-5.79	Wheel-tracked	6.37	6.05	1.8	6.44	6.14	1.8
		Non-wheeled	6.46	6.10	1.9	6.45	6.10	1.7
Darvel	7-5-79	Wheel-tracked	5.64	5.22	5.7	5.71	5.32	4.9
		Non-wheeled	5.66	5.28	5.9	5.68	5.29	5.0

higher on non-wheeled than in wheeled areas, on the sprayed plots only. This was especially evident at the site on the Darvel soil (Table 3.11). The effect was probably attributable to an overlapping of the spray in the plot centre, since spraying was carried out along both sides of the plot, and not a consequence of plant uptake. At harvest however, no significant differences were observed between adjacent wheelings and non-wheelings regardless of the method of fertiliser application.

The fifth cultivation treatment-NBB (see Section 3.3.1.2) - differed from the broadcast treatment described above in that fertiliser was broadcast prior to drilling rather than applied simultaneously. Such a treatment was thought likely to result in additional fertiliser accumulating in the region of the wheel tracks as a result of, first, the soil surface being depressed before receiving fertiliser and then more fertilised soil being displaced laterally into the depressions.

A slight visible effect of wheel tracks on growth was indeed noted on the plots receiving fertiliser in this manner at both sites early in the season (Plate 3.3), but the differences in tissue colour and growth disappeared after 12 weeks. Unlike the plants growing on the Giffordtown and Corby soils in the previous year, no deficiency symptoms were apparent in the non-wheeled areas at either site.

The results of soil and plant analysis for cultivation treatment NBB are shown in Table 3.12. On the plots receiving the manganese sulphate spray, the plant manganese concentrations were increased to such an extent that the effects of any soil treatment were masked. On the unsprayed plots the data of the first tissue sampling on the Hexpath soil showed manganese concentrations to be consistently higher in the wheel tracks relative to the non-wheeled areas; this effect however disappeared at harvest. A similar situation was found on the Darvel soil at the first sampling and the effect was maintained in 2 out of 3 plots at harvest. The differences in extractable soil manganese and pH on wheel-tracked and non-wheeled areas at both sites were found not to be significant at the 5% level. The results however do suggest that fertiliser placement may play a

Table 3.11 Plant manganese on wheel-tracked and non-wheeled areas of plots as affected by two methods of fertiliser application on the Hexpath and Darvel soils, 1979

Soil	Area	Fertiliser broadcast at drilling				Fertiliser combine-drilled			
		First sampling*		Harvest (whole plant)		First sampling*		Harvest (whole plant)	
		Mn, plant with Mn spray	Mn, plant without Mn spray	Mn, plant with Mn spray	Mn, plant without Mn spray	Mn, plant with Mn spray	Mn, plant without Mn spray	Mn, plant with Mn spray	Mn, plant without Mn spray
		($\mu g\ g^{-1}$)							
Hexpath	Wheel-tracked	55.2	19.8	10.4	10.6	57.5	16.2	12.6	9.3
	Non-wheeled	69.0	19.2	11.4	12.4	69.2	14.5	12.0	9.6
Darvel	Wheel-tracked	40.7	23.0	22.8	19.2	43.6	28.0	20.9	22.4
	Non-wheeled	66.8	26.9	22.2	22.0	63.7	28.1	21.0	24.8

* First sampling was 6 weeks and 10 weeks after sowing for the Hexpath and Darvel soils respectively.

Tissue samples from sprayed plots were taken 6 days and 10 days following spray application on the Hexpath and Darvel soils respectively.

Plate 3.3 Cultivation Method NBB - The effects of tractor wheel compaction on barley growth and colour at the field trial on the Darvel soil, 1979

Table 3.12 Soil pH and soil and plant manganese on the wheel tracks (W) and non-wheeled areas (NW) on plots receiving cultivation treatment NBB on the Hexpath and Darvel soils, 1979

Soil	Plot No.	pH (H_2O)		pH ($CaCl_2$)		Mn, soil ($\mu g\ g^{-1}$) Sampled 14-6-79		Mn, plant ($\mu g\ g^{-1}$) Sampled 14-6-79		Mn, plant ($\mu g\ g^{-1}$) Harvest	
		W	NW	W	NW	W	NW	W	NW	W	NW
Hexpath	9*	6.25	6.20	5.64	5.69	2.2	2.1	63.0	66.0	11.8	8.2
	14	6.16	6.22	5.43	5.69	2.7	2.6	26.0	19.0	9.0	7.8
	25	6.03	6.03	5.43	5.41	2.6	2.6	32.0	20.0	8.8	9.0
	31*	5.86	5.85	5.17	5.25	4.1	3.8	74.0	57.0	19.8	12.5
	43*	5.99	6.05	5.49	5.47	2.8	2.7	76.0	75.0	12.5	10.0
	47	5.79	5.90	5.24	5.28	4.4	3.3	61.0	28.0	11.5	13.0
						Sampled 19-6-79		Sampled 28-6-79			
Darvel	7*	6.26	6.24	5.76	5.75	3.6	4.0	47.2	62.8	19.2	20.5
	17	5.65	5.79	5.04	5.14	7.5	6.4	42.5	34.5	36.5	23.0
	22*	5.47	5.57	4.87	4.90	8.6	6.6	55.2	73.8	23.2	17.5
	34	5.16	5.24	4.67	4.62	13.4	13.0	66.2	46.2	36.8	27.0
	41	5.73	5.43	5.14	4.74	11.0	10.7	37.0	35.5	17.5	23.0
	49*	5.40	5.39	4.79	4.83	10.6	10.2	54.0	53.5	29.2	27.5

* Plots receiving manganese spray applications.

role in bringing about the wheel-tracking phenomenon and accounting for differences in manganese availability. This could be due to better nutrient availability to the growing plant.

Effects of cultivation treatment and fertiliser application method on yield

Yield data as influenced by consolidation and fertiliser application is given in Tables 3.13 and 3.14 respectively. There was a significant decrease ($p<0.05$) in yield with the heavier consolidation treatments (CP1 and CP3) relative to the light consolidation (L and N) on the Hexpath soil. This was thought to be attributable to a combination of excessive moisture at early growth stages and bird damage (Gill, 1980). The differences in yield on the Darvel soil were not significant at the 5% level.

Combine-drilling significantly increased ($p<0.05$) yields on the Hexpath soil but not significantly on the Darvel (Table 3.14).

The effect of manganese spray on yield

The use of manganese sulphate spray (averaged over the consolidation treatments or fertiliser application methods) did not significantly affect yield at either site (Tables 3.13 and 3.14). (There were also no significant interactions between spray treatment and tillage on yield.)

The translocation of manganese within the plant

The results of the plant analysis in Tables 3.9 and 3.11 show a much higher concentration of manganese in the sprayed plots relative to the unsprayed plots at the first sampling. However, it can be seen that the differences between sprayed and non-sprayed plots disappeared at harvest. Since grain yields between the two treatments did not differ significantly (Tables 3.13 and 3.14) it is unlikely that the decreased manganese concentrations in the sprayed plots between the first sampling and harvest was caused by a dilution effect. This suggests that manganese applied early in the season may be lost due to the shedding of older leaves, or by being washed off the plant with rainfall, rather than being translocated to the younger parts of the plant as they develop.

A survey of the literature showed that the findings of various investigators concerning the occurrence or non-occurrence of translocation of manganese were contradictory. It was therefore decided to

Table 3.13 The effects of cultivation treatment on yield on the Hexpath and Darvel soils, 1979 (at 15% moisture content)*

Soil	Consolidation	Yield (t ha^{-1})		Mean
		With Mn spray	Without Mn spray	
Hexpath	L	4.99	5.04	5.02
	N	5.04	5.00	5.02
	CP1	4.53	4.83	4.68
	CP3	4.87	4.83	4.85
	Mean	4.85	4.92	
Darvel	L	5.51	5.33	5.42
	N	5.68	5.80	5.74
	CP1	5.75	5.74	5.74
	CP3	5.63	5.80	5.72
	Mean	5.64	5.67	

* Data supplied by Dr J C Holmes - Crop Production, Advisory and Development Department.

Table 3.14 The effects of fertiliser application on yield on the Hexpath and Darvel soils, 1979 (at 15% moisture content)*

Soil	Fertiliser application method	Yield (t ha^{-1})		Mean
		With Mn spray	Without Mn spray	
Hexpath	BA	4.74	4.87	4.80
	C	4.98	4.99	4.98
	Mean	4.86	4.93	
Darvel	BA	5.56	5.63	5.60
	C	5.71	5.72	5.72
	Mean	5.64	5.68	

* Data supplied by Dr J C Holmes - Crop Production, Advisory and Development Department.

investigate if indeed applied manganese was mobile within the plant; this could be assessed with the use of radioactive ^{54}Mn. The experiment is described in Appendix B.5. Initially a qualitative assessment of translocation was made. Plants were sprayed either at the 2-, 4- or 5-leaf stage; the 4th, 5th and 8th leaves respectively were gamma-counted to determine ^{54}Mn activity. The later leaves all showed count rates above background, indicating that some translocation did take place.

Attempts were later made to make a quantitative estimate of the ^{54}Mn translocated. Several of the shed sprayed leaves from different plants (sprayed at the 5- and 8-leaf stages) were combined into one vial and counted, followed by oven-dry weight determinations. Flag leaves and ears from the same plants were treated in similar fashion. Results showed that only 0.64% to 1.3% and 0.20% to 0.27% of the combined activity of the sprayed leaves were found in the flag leaves and ears, respectively. Although these figures can only be viewed as general estimates it does indicate that very little manganese is translocated to other parts of the plant from the point of application. In a 1980 field trial, manganese deficiency symptoms were noted in the flag leaves of barley that had been sprayed earlier with $MnSO_4$, thereby substantiating these findings. Joham and Amin (1967) noted similar findings in cotton.

Commercial farm survey

A survey of a number of fields showing the wheeling effect was conducted during the growing season. Relevant information concerning each site is shown in Table 3.15. It was hoped that such a survey would shed some light on the various conditions under which this effect occurred and help resolve the question of why different levels of soil extractable and plant manganese were found in the wheeled and non-wheeled areas.

Soil samples were taken from beneath several (usually 3) strips of darker green plants (presumed to be wheel tracks) and adjacent light green (manganese deficient) plants. Each sample was made up from 5 sub-samples taken with an auger from 0-15 cm depth. Corresponding plant samples were taken from random areas of each strip.

Table 3.15 Some physical and chemical properties of the soils investigated in the commercial farm survey, 1979

Farm	Soil Series	Texture	Drainage Class*	Penetrometer readings (kg cm^{-2})†				Organic Matter (%)	P	K	Mg
				Wheel tracks		Non-wheeled areas					
				Mean	S.D.	Mean	S.D.		—— ($\mu g\ g^{-1}$)‡ ——		
1	Aldbar	Sandy loam	Free	-	-	-	-	10.50	10.6	137	287
2	Humbie	Sandy loam	Imperfect	29.0	5.4	18.6	3.8	4.97	12.7	64	51
3	Macmerry	Sandy loam	Imperfect	34.3	4.4	23.4	5.0	7.34	1.4	144	445
4	Darleith	Sandy clay loam	Free	44.8	5.1	21.0	4.2	10.83	6.4	437	445
5	Carpow	Loamy sand	Free	43.8	5.1	30.1	6.0	9.88	25.9	164	326
6	Caprington	Sandy loam	Imperfect	35.9	8.7	21.7	7.4	5.99	6.5	68	226
7	Winton	Sandy loam	Imperfect	32.6	4.1	15.8	3.5	12.44	89.0	179	194

* Source: Soil Survey Map of Scotland

† Mean of 10 readings

S.D. - Standard Deviation

‡ Mean of all samples taken

Plant analysis confirmed the presence of high concentrations of manganese as a result of spraying with $MnSO_4$, in all samples except those from Farm No.7. Therefore only the data from this one farm are presented here (Table 3.16). Results of soil analyses are also given in Table 3.16, and regressions of pH vs log extractable manganese are shown in Figure 3.9. The data show a significantly depressed pH ($p<0.01$) and higher extractable manganese ($p<0.001$) in the wheel tracks thereby confirming the effect seen the previous year.

It is of interest to note that in all the fields surveyed, with the exception of the field at Farm No.1, the fertiliser had been broadcast applied.

Summary of 1979 field data

As in the previous year, different cultivation techniques did not have any great effect on levels of available manganese at either trial. Bulk density was significantly increased on the Hexpath soil by cultivation practices designed to produce more compacted seedbeds. No increases in bulk density were noted on the Darvel soil, however. Yield responses at the trial on the Hexpath soil only, were found to be significantly increased on combine drilled plots relative to the plots receiving broadcast applications of fertiliser. The decrease in yield with heavier consolidation treatments was thought to result in unfavourable moisture relationships as well as bird damage. No significant yield responses with manganese spray application were detected at either site.

Plant and soil samples from fields of commercial farms showing a pattern of alternating strips of light and dark green tissue generally gave similar results to those observed the previous year on the Giffordtown and Corby soils - i.e. a depressed (more acid) pH accompanied by greater quantities of available manganese in the wheel tracks, and, at the one site definitely known not to have been sprayed, a correspondingly greater concentration of manganese in the plant.

These phenomena were not observed on the field trial plots in which fertiliser was applied after seedbed consolidation. A slight effect was noted however, where fertiliser was applied to the plough furrow prior to seedbed cultivation, indicating that fertiliser

Table 3.16 Soil pH and soil and plant manganese concentrations on fields showing symptoms of manganese deficiency and wheel-tracking effects on 7 commercial farms, 1979

Farm (Soil Series)	pH (H_2O)		pH ($CaCl_2$)		Mn, Soil ($\mu g\ g^{-1}$)		Mn, Plant ($\mu g\ g^{-1}$)	
	W	NW	W	NW	W	NW	W	NW
1 (Aldbar)	6.3	6.6	5.6	6.0	2.4	2.0		
	6.0	6.3	5.4	5.6	4.0	2.2		
	6.2	6.5	5.5	5.8	2.8	1.8		
	6.3	6.4	5.7	5.8	2.0	1.6		
Mean	6.2	6.4	5.5	5.8	2.8	1.9		
2 (Humbie)	5.61	5.80	5.05	5.26	12.7	9.0		
	5.66	6.70	5.11	6.25	11.5	2.8		
Mean	5.64	6.25	5.08	5.76	12.1	5.9		
3 (Macmerry)	6.00	6.11	5.79	5.89	2.4	1.9		
	5.78	5.96	5.60	5.77	4.1	2.6		
	5.87	5.79	5.61	5.52	3.4	3.6		
Mean	5.88	5.95	5.67	5.73	3.3	2.7		
4. (Darleith)	6.17	6.28	5.90	5.92	1.7	1.6		
	6.23	6.44	5.97	6.03	1.6	1.2		
	6.17	6.33	5.96	5.92	1.8	1.6		
Mean	6.19	6.35	5.94	5.96	1.7	1.5		

Table 3.16 (Contd)

Farm (Soil Series)	pH (H_2O)		pH ($CaCl_2$)		Mn, Soil ($\mu g\ g^{-1}$)		Mn, Plant ($\mu g\ g^{-1}$)	
	W	NW	W	NW	W	NW	W	NW
5 (Carpow)	7.00	6.95	6.61	6.47	0.8	1.0		
	6.70	6.88	6.32	6.43	1.1	0.9		
	6.56	6.51	6.08	6.02	1.2	1.5		
Mean	6.75	6.78	6.34	6.31	1.0	1.1		
6 (Caprinton)	6.62	6.84	6.24	6.43	2.6	1.9		
	6.42	6.95	5.95	6.51	3.8	2.9		
	6.20	6.58	5.72	6.15	5.2	2.8		
Mean	6.41	6.79	5.97	6.36	3.9	2.5		
7 (Winton)	6.17	6.61	5.96	6.23	4.2	2.6	17.8	7.8
	6.17	6.40	5.90	6.03	4.1	3.4	15.0	13.2
	6.04	6.16	5.77	5.88	5.7	4.8	19.5	11.0
Mean	6.13	6.39	5.88	6.05	4.7	3.6	17.4	10.7

W = Wheel tracks

NW = Non-wheelings

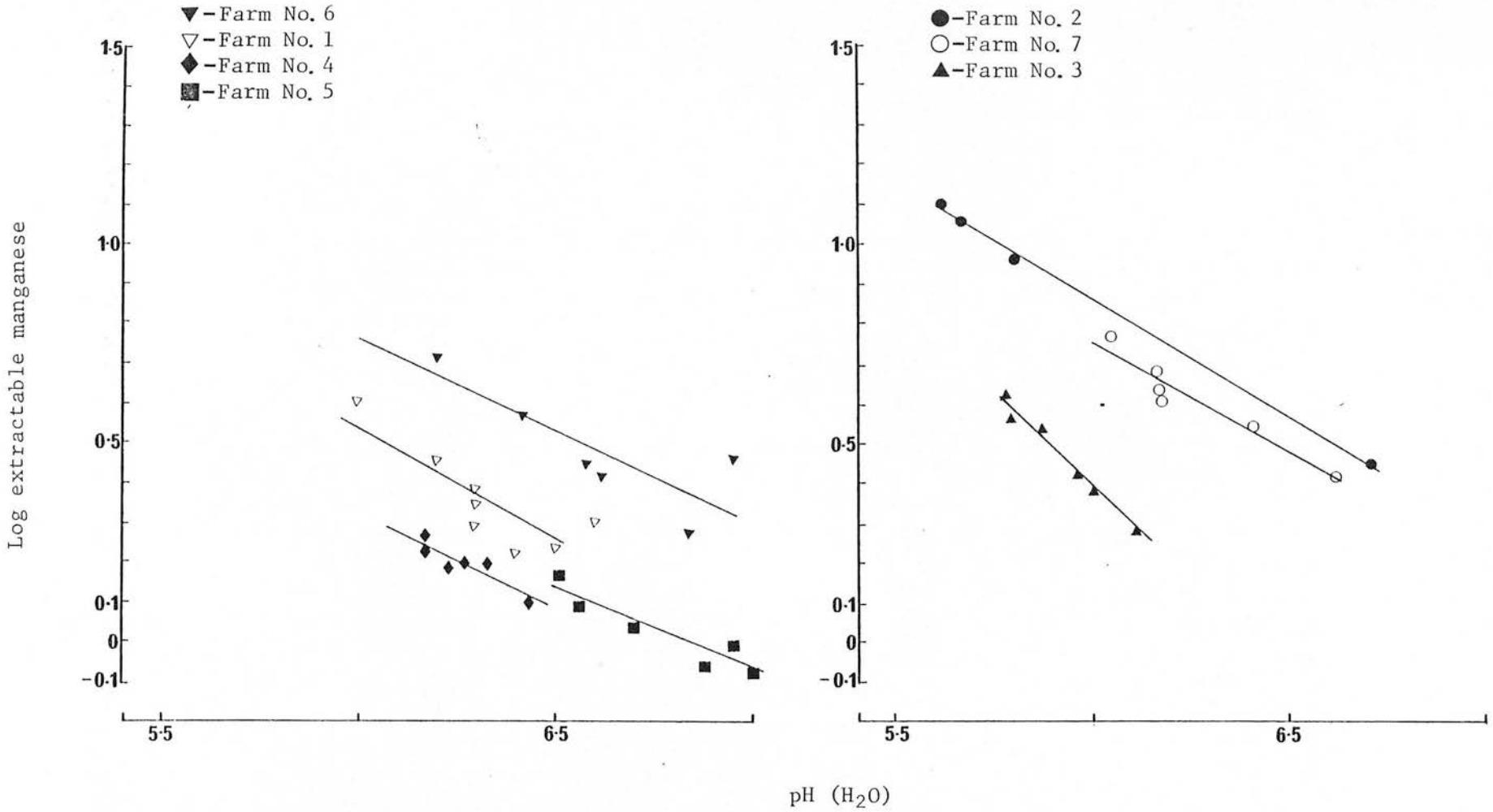


Figure 3.9 Relationship between pH (H_2O) and extractable manganese on the fields from the commercial farms, 1979.

application may play some role in bringing about the wheel-track effects.

3.4.3 Field Work, 1980

In the tillage/method of fertiliser application experiment on the Carpow soil in 1980, symptoms of very severe manganese deficiency developed in many of the plots very early on in the season. Differences in growth between wheeled and non-wheeled parts of the plots became very pronounced within 5 weeks of sowing and were maintained throughout the season (Plates 3.4 and 3.5).

The magnitude of these differences was clearly related to the mode of fertiliser application. Wheel-tracking effects were most pronounced in plots which had received the fertiliser as a broadcast onto the plough furrow prior to cultivation and drilling (treatment BB, similar to the application of fertiliser in cultivation NBB in the 1979 field trials). The BA treatment (fertiliser broadcast after drilling) plots showed a similar effect, but the relative differences between the wheel tracks and non-wheeled areas, although still very apparent, were not as pronounced as in the BB treatment. Those plots receiving fertiliser combined-drilled (C) showed only a very slight wheeling effect and symptoms of manganese deficiency were minimal.

The results of analysis of soil and plant samples taken 5 and 7 weeks after sowing are shown in Table 3.17. Tissue analysis showed that the concentration of manganese in the plant was correlated remarkably well with visible effects. The manganese concentration in the plants growing on the wheel tracks was greater in all treatments but the differences between plant manganese in the wheeled and non-wheeled areas decreased in the order BB>BA>C. The degree of significance followed the same trend with the BB and BA treatments showing significant differences ($p<0.001$ and $p<0.01$, respectively) on both sampling dates. Differences between wheeled and non-wheeled areas of plots receiving treatment C were significant ($p<0.05$) at the 7 week sampling only. Analysis of plant tissue from 6 plots (2 from each fertiliser application method) at final harvest still showed a marked difference in the BB treatment. The plants from the wheel-tracked and non-wheeled areas contained 7.6 and 5.0 $\mu g\ g^{-1}$, respectively. The corresponding figures for the BA and C treatments were 5.7 and 5.3 $\mu g\ g^{-1}$ and 9.7 and 9.3 $\mu g\ g^{-1}$.

3.4

3.5

Plates 3.4 and 3.5 Effect of tractor wheel compaction on barley growth and colour on individual plots at the field trial on the Carpow soil, 1980

Table 3.17 Soil pH and soil and plant manganese on the wheel-tracked and non-wheeled areas of plots as affected by 3 fertiliser application methods on the Carpow soil, 1980

Fertiliser Application Method	Area	Time of Sampling							
		5 weeks after sowing				7 weeks after sowing			
		pH (H_2O)	pH ($CaCl_2$)	Mn, Soil ($\mu g\ g^{-1}$)	Mn, Plant ($\mu g\ g^{-1}$)	pH (H_2O)	pH ($CaCl_2$)	Mn, Soil ($\mu g\ g^{-1}$)	Mn, Plant ($\mu g\ g^{-1}$)
BB	Wheel-tracked	6.67	6.38	0.9	19.1	6.61	6.28	1.3	27.2
	Non-wheeled	6.67	6.39	0.9	10.8	6.71	6.33	1.1	16.6
BA	Wheel-tracked	6.72	6.41	0.9	14.2	6.74	6.36	1.0	22.3
	Non-wheeled	6.73	6.42	0.9	10.6	6.74	6.36	1.1	16.4
C	Wheel-tracked	6.66	6.38	0.9	18.1	6.69	6.35	1.0	26.8
	Non-wheeled	6.60	6.33	1.0	16.7	6.68	6.32	1.2	22.4

BB = Fertiliser broadcast prior to cultivation and drilling.

BA = " " at drilling time.

C = " combine-drilled.

It is of interest of note the similarity of plant manganese concentrations between the non-wheeled areas of the plots receiving the fertiliser combine-drilled, and the wheel-tracked areas of plots receiving fertiliser as a broadcast. Soil pH and extractable manganese on the other hand were not significantly influenced on the wheeled and non-wheeled areas. However, a slightly depressed pH and higher extractable manganese were noted on the wheel tracks on the BB-treated plots after 7 weeks. It is also worthwhile to note that the 1980 trial was on the same farm (and same soil series) as one of the fields surveyed in 1979 (i.e. Farm No.5). On that occasion the higher extractable manganese in the wheel tracks was not associated with depressed pH at two of the three points from which samples were taken.

Effect of fertiliser rate

The higher fertiliser rate did not markedly influence soil pH and extractable manganese. This conforms with results found on the Giffordtown and Macmerry trials of 1978 (Table 3.18). Plant concentrations of manganese, although slightly higher, were not significantly affected by increasing the fertiliser application.

Effect of cultivation on soil and plant manganese

The effects of cultivation on soil and plant manganese on both sampling occasions are shown in Table 3.19. The overall seedbed consolidation treatments did not affect soil extractable manganese. However, a significant increase ($p<0.05$) was noted in plant uptake on the more consolidated treatments (CP1 and CP3) relative to the looser treatments (L and N) on both sampling occasions.

Effect of cultivation and manganese spray on yield

The data of Table 3.20 show that in the plots to which the fertiliser had been applied by broadcasting, without spray treatment, yields on the heavier consolidated seedbeds (CP1 and CP3) were significantly increased ($p<0.05$) over those of the looser seedbeds (L and N); no large differences were seen on plots with combine-drilled fertiliser. On the plots receiving manganese spray, it is readily apparent that yields were increased but the effects of fertiliser placement and consolidation, noted in the unsprayed plots, were diminished. This indicates that the yield response to placement and consolidation was governed at least in part by the plants ability to take up manganese.

Table 3.18 Effect of fertiliser application rate on soil pH and on soil and plant manganese on the Carpow soil, 1980

Time After Sowing	Fertiliser Application Rate	pH (H_2O)	pH ($CaCl_2$)	Mn, Soil ($\mu g\ g^{-1}$)	Mn, Plant ($\mu g\ g^{-1}$)
5 weeks	N1	6.66	6.36	0.9	14.3
	N2	6.70	6.40	0.9	15.5
7 weeks	N1	6.68	6.32	1.2	21.1
	N2	6.71	6.35	1.1	22.9

Table 3.19 Effect of seedbed consolidation on soil and plant manganese on the Carpow soil, 1980

Time After Sowing	Cultivation	Mn, Soil ($\mu g\ g^{-1}$)	Mn, Plant ($\mu g\ g^{-1}$)
5 weeks	L	0.9	12.6
	N	0.9	13.0
	CP1	0.9	17.4
	CP3	0.9	16.5
7 weeks	L	1.1	20.8
	N	1.2	20.0
	CP1	1.1	23.0
	CP3	1.1	24.0

Table 3.20 The influence of fertiliser placement, overall seedbed consolidation and manganese sulphate spray on grain yield on the Carpow soil, 1980 (t ha^{-1} at 15% moisture content)*

+/- Manganese Sulphate Spray	Fertiliser Application Method	Seedbed consolidation		Mean
		Loose (L+N)	Consolidated (CP1+CP3)	
- spray	BB	4.20	5.26	4.73
	BA	3.77	4.72	4.24
	C	5.80	5.72	5.76
+ spray	BB	5.35	5.36	5.36
	BA	5.12	5.42	5.27
	C	6.15	6.12	6.14

* Data supplied by Dr J C Holmes - Crop Production, Advisory and Development Department.

BB = Fertiliser broadcast prior to cultivation and drilling.

BA = " " at drilling time.

C = " combine-drilled.

Summary of 1980 field work

Results from this experiment showed that time and placement of fertiliser relative to the seed greatly influenced the relative differences in manganese uptake between the wheel-tracked and non-wheeled areas. Unlike the results of previous years, the distinct wheeling phenomenon was not accompanied by noticeably large changes in soil pH or extractable manganese. Manganese uptake was significantly enhanced in the heavily consolidated plots. Yield increases were also greatly improved but only on those plots where fertiliser had been broadcast. On the plots receiving manganese spray the yield responses as influenced by fertiliser placement and consolidation diminished considerably.

3.4.4 General Summary of Field Work Pertaining to Wheel-Track Effects

A striped pattern of differential growth and plant health was found on many of the individual plots in field trials, in 1978 and

1980 especially. Healthier plants grew down the length of each plot in areas corresponding to the tracks made by tractor wheels during the cultivation procedures.

Growth in the areas between the wheel tracks was poorer and symptoms of manganese deficiency much more in evidence. Similar effects were noted on a number of fields outwith the trial sites, indicating that the phenomenon was not an artefact of the experimental procedures used. Soil penetrometer readings taken on the wheel tracks and non-wheeled areas showed consistently higher measurements (greater soil resistance) on the former.

In many instances, particularly in the Giffordtown trial in 1978 (as well as on the Corby soil in the same year) and in the commercial farm survey in 1979, a lower pH and higher level of extractable manganese were found on the wheeled areas relative to the non-wheeled areas. This raises the possibility that the compaction somehow influenced soil pH which in turn affected extractable soil manganese. However, no large differences in pH and soil manganese were generally noted between wheel tracks and non-wheeled areas in the 1980 trial, although plant uptake in the former areas was twice as high in many cases.

Data from the 1980 field trial, (as well as some results from treatment NBB in the 1979 field trials) showed that the time and method of fertiliser application and placement relative to the sowing of the seed had a marked influence on the degree of the wheeling effect as well as on manganese uptake.

3.5 LABORATORY AND GLASSHOUSE (POT) EXPERIMENTS

With the exception of the first laboratory experiment (see 3.5.1 below) the following studies deal with possible mechanisms that could contribute to changes in soil pH and/or available manganese on wheel-tracked and non-wheeled soils observed in the field investigations.

3.5.1 The Effects of Modifying Soil pH on the Concentrations of Soil Manganese

Introduction

Results of the field experiments showed in many instances that pH had a marked influence on extractable manganese concentrations. The following experiment was preliminary in nature and was undertaken to investigate if similar relationships could be reproduced in the laboratory. The influence of pH on concentrations of soil solution manganese were also investigated since this form of the element would be expected to be the most readily available source to plants.

Materials and Methods

Samples (1600 g) of air-dried Giffordtown soil were amended as follows:-

A: - 2 g of $Ca(OH)_2$ (i.e. 54 milliequivalents) added as anhydrous powder.

B: - 4.5 ml of 6N HCl (i.e. 27 milliequivalents) added from an atomiser as a spray.

C: - Control

The amended soils were thoroughly mixed in an end-over-end shaker for 24 hours, transferred to cylindrical pots (1225 cm^3, 10.5 cm diameter), and brought to field capacity. The soils were then removed, thoroughly mixed and stored in a polythene bag.

After 1 and 3 weeks of storage at room temperature a portion of the soil from each bag was removed. Part of each sample was analysed in the moist state, the remainder was air-dried before analysis. The analyses carried out were:-

Moist soil

1. soil solution manganese
2. $CaCl_2$-extractable manganese
3. pH (H_2O)

Air-dried soil

1. $CaCl_2$-extractable manganese
2. pH (H_2O)

Results and Discussion

Results are shown in Table 3.21. Analysis of the soil after one week showed that the acid treatment decreased soil pH and produced a large increase in soil solution and extractable manganese. The increase in manganese in the soil solution in the base-treated soil could be explained by cation exchange brought about by excess calcium ions but this explanation cannot be applied to the increase in extractable manganese because the extractant is intended precisely for the purpose of releasing manganese from exchange sites. After 3 weeks of incubation however, soil solution and extractable manganese concentration decreased to levels less than that of the control.

Figure 3.10 shows results of pH and extractable manganese for the air-dried soils after the two incubation periods plotted with the regression line resulting from soil analysis from the 1978 field trial on the same soil. The effect of drying and remoistening and incubating the soil without amendment was very small as seen by the close proximity of the control soil to the line. The figure also clearly shows that the concentrations of extractable manganese in both acid and base-treated soils had been enhanced by the amendments, comparable with the values found in the field at equivalent pH's. A possible explanation is that manganese was released by the amendments from forms which are unaffected by the salt solution. Despite this occurrence, the concentrations of extractable manganese in the laboratory amended soils fell on a line parallel to that representing the field data (Figure 3.10).

Conclusions

The effect of changing pH on concentrations of both soil solution and extractable manganese agrees with the findings of other workers (e.g. Page, 1962). Laboratory alteration of the soil with acid or base maintains the relationship between pH and extractable manganese observed in the field.

Table 3.21 The effects of acid or base amendment on pH and extractable manganese of the Giffordtown soil incubated at field capacity for one and three weeks

Treatment	Initial Moisture Status	1 week of field capacity				3 weeks at field capacity			
		pH (H_2O)	pH ($CaCl_2$)	Mn_{ss}* ($\mu g\ g^{-1}$)	Mn_{ext}* ($\mu g\ g^{-1}$)	pH (H_2O)	pH ($CaCl_2$)	Mn_{ss}* ($\mu g\ g^{-1}$)	Mn_{ext}* ($\mu g\ g^{-1}$)
$Ca(OH)_2$	Field capacity	6.7	6.3	0.2	4.4	6.5	6.1	<0.02	0.4
	Air-dried	6.5	6.1	-	2.4	6.5	6.0	-	1.8
HCl	Field capacity	5.6	5.4	4.0	13.2	5.6	5.3	3.5	12.4
	Air-dried	5.6	5.4	-	13.5	5.6	5.3	-	14.2
Control	Field capacity	6.2	5.8	0.1	2.6	6.1	5.6	<0.02	0.6
	Air-dried	6.0	5.6	-	2.2	6.1	5.6	-	2.2

*Mn_{ss} = soil solution manganese; Mn_{ext} = $CaCl_2$-extractable manganese.

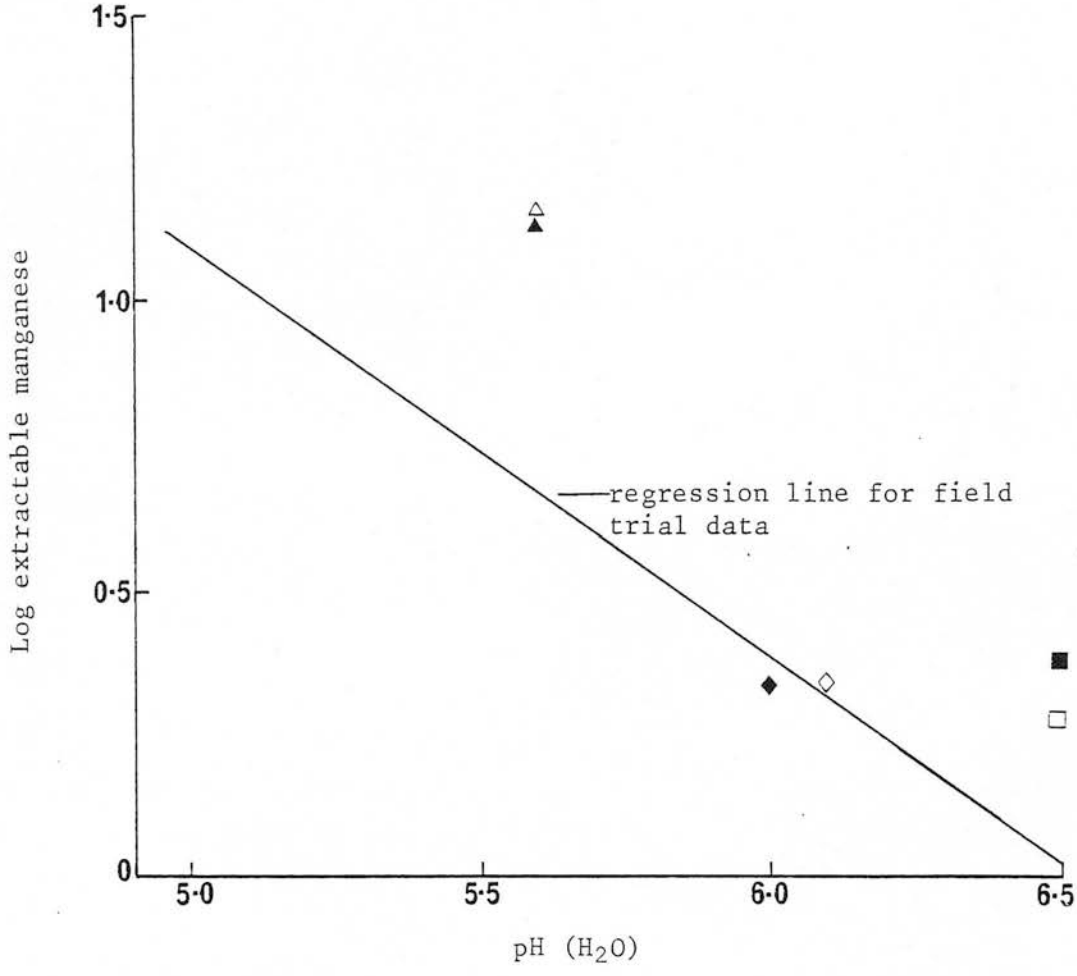


Figure 3.10 The effect of laboratory modification of pH on extractable manganese in the Giffordtown soil as compared with field samples.

■,▲,◆- after 1 week
□,△,◇- after 3 weeks
■,□- $Ca(OH)_2$
▲,△- HCl
◆,◇- control

3.5.2 The Effects of CO_2 on Soil pH and Extractable Manganese

Introduction

The relationship between soil pH and the partial pressure of CO_2 has been well documented. The pH of a calcareous soil is inversely related to the logarithim of the CO_2 partial pressure of the soil atmosphere (Bradfield, 1941); and more relevant to the present study, variations in CO_2 concentrations were found by Nichol and Turner (1957) to have a pronounced effect on the pH of near-neutral and acid soils that they investigated.

Measurements of CO_2 were not carried out under field conditions in this investigation but Bell (1980), in a study of Macmerry and Winton soils (the former being similar to the one of the 1978 field trial soil) found a greater accumulation of CO_2 in direct-drilled than in less consolidated shallow-ploughed plots. Given the possibility of higher CO_2 concentrations arising on wheel-tracked relative to non-wheeled soil, the following experiment was undertaken to determine the influence of CO_2 on soil pH and extractable manganese.

Materials and Methods

Three soils were used in this investigation:- the Giffordtown, Hexpath and Carpow soils from the field trials of 1978, 1979 and 1980 respectively.

Soil samples (150 g) were weighed into 220 ml capacity Buchner funnels previously lined with glass wool, brought to field capacity and stored for 48 hours. The Buchner funnels were covered with perforated aluminium foil to minimise evaporation.

Air-CO_2 mixtures containing 1, 5 or 10% CO_2 were passed through water in 500 ml Erlenmeyer flasks and then, via tubes attached to the stems of the Buchner funnels, through the soils. Normal air (containing 0.03% CO_2) was used as the control.

Passage through the water ensured that the gas stream was saturated and therefore would not alter the moisture content of the soils. It also allowed the flow rate to be determined by counting the number of bubbles per minute, after calibration with a soap-bubble flowmeter (Figure 3.11). The gases were slowly passed through

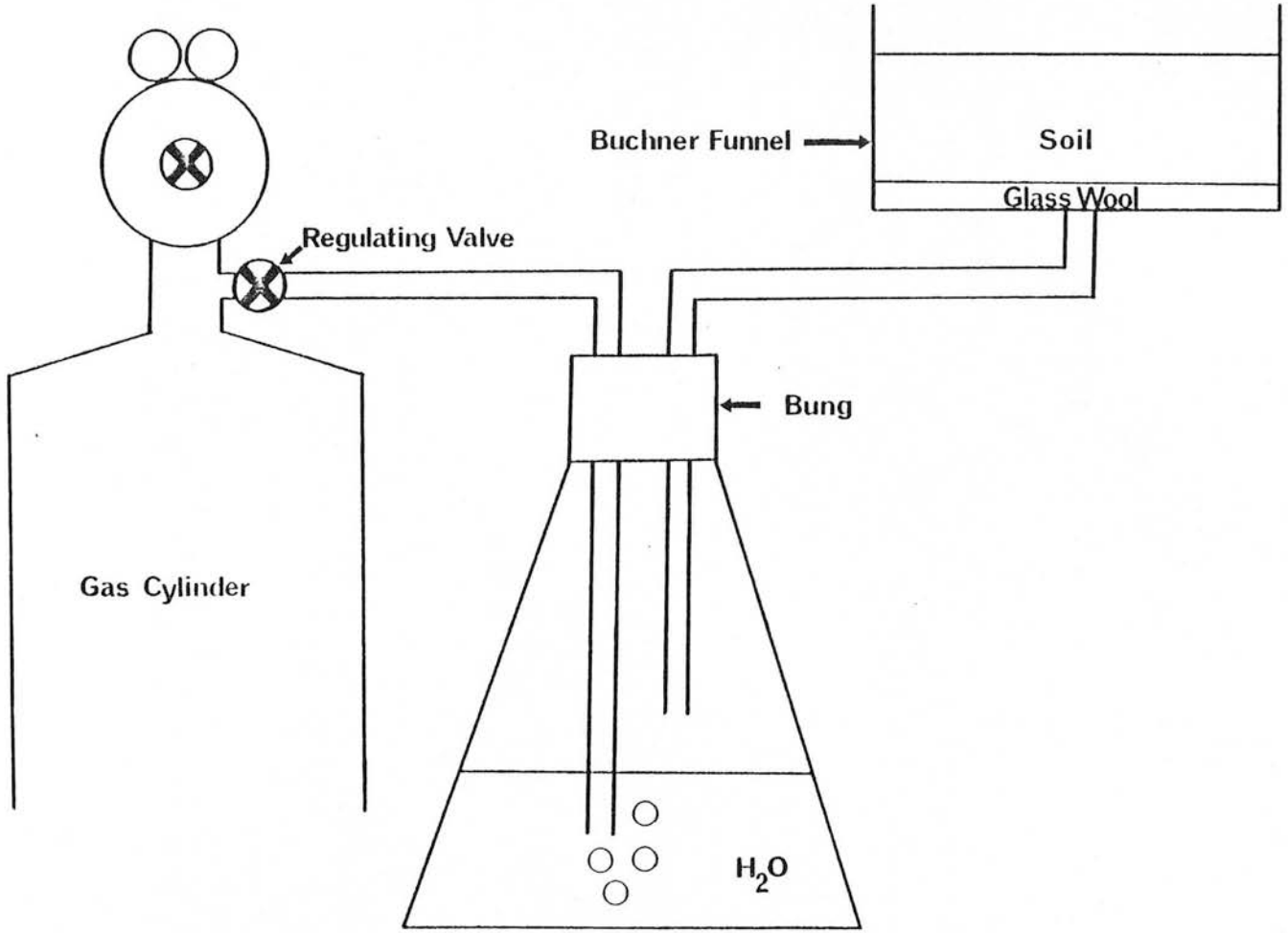


Figure 3.11 Apparatus for the perfusion of gas mixtures through soil

the soil at a rate of approximately 20 ml per minute. This was to ensure that the majority of the gas passed through the soil rather than the soil-funnel interface. The soil was then removed and air-dried prior to analysis.

The drying of the soil following the gas perfusion was justified on the basis that pH changes observed in various field soils persisted after disturbance and drying. Therefore it was necessary that the laboratory investigation be carried out treating the soil in the same manner.

Results and Discussion

The data in Table 3.22 show that the volume of gas that passed through the soil was sufficient to replace the soil atmosphere many times. It is readily apparent that the concentration of CO_2 had little effect on soil pH. A slight depression of pH was observed with the unamended Giffordtown soil at 1% CO_2 but this was thought to be due to experimental variability. Furthermore, the pH depression was not accompanied by a similar increase in extractable manganese.

Since the wheeling phenomenon was observed on fertilised, cultivated fields, it was thought desirable to see if any interaction between CO_2 and fertiliser produced the observed depression in pH. This was not the case as seen by the results in Table 3.22.

Although pH differences were not brought about by altering the CO_2 concentration, it should be noted that extractable manganese was greater in the fertilised Giffordtown soil than in the unfertilised sample. Air-dried storage of the soil between the two experiments was a probable explanation for this.

Increases in extractable manganese resulting from higher CO_2 concentrations were seen in the fertilised Giffordtown and unfertilised Hexpath soils (Table 3.22). Bolas and Portsmouth (1948) noted similar results as they extracted a soil with varying quantities of CO_2-saturated water, but offered no explanation for their findings. Changes in the soil redox potential as a result of the replacement of CO_2 with O_2 may be responsible for enhancing extractable manganese in the soils used in this experiment.

Table 3.22 Effect of CO_2 concentration on soil pH and extractable manganese

Soil (+/- fertiliser)	Gas Mixture	Volume of gas mixture (litres)	pH (H_2O)	pH ($CaCl_2$)	Mn^{-1} ($\mu g\ g^{-1}$)
Giffordtown (-)	Air	87	5.88	5.56	2.7
	1% CO_2	87	5.82	5.51	2.0
	5% CO_2	74	5.82	5.52	2.2
	10% CO_2	73	5.82	5.52	1.8
Giffordtown (+)	Air	102	5.72	5.65	5.3
	1% CO_2	103	5.76	5.62	6.2
	5% CO_2	106	5.83	5.63	6.8
	10% CO_2	107	5.78	5.61	6.8
Hexpath (-)	Air	100	6.20	5.64	4.3
	1% CO_2	101	6.23	5.77	4.3
	5% CO_2	94	6.23	5.75	4.4
	10% CO_2	113	6.21	5.74	5.0
Carpow (+)	Air	80	6.85	6.57	1.0
	1% CO_2	72	6.85	6.55	0.8
	5% CO_2	-	-	-	-
	10% CO_2	63	6.84	6.57	0.9

Conclusions

Results presented here show that the observed differences in pH and extractable manganese on the wheeled and non-wheeled areas in various fields cannot be explained in terms of an influence of CO_2 on soil pH. It is necessary to point out that these experiments were not conducted with compacted soils, and it is assumed that no interaction between the physical act of compaction and increased CO_2 concentration could occur that would affect pH.

3.5.3 Effects of Waterlogging on Manganese Availability

Introduction

It is well known that the compaction of medium to heavy-textured soils can impede water permeability and promote the occurrence of waterlogging. An obvious example would be standing water in wheel ruts, and this flooding could easily result in increases in manganese solubility (e.g. Ponnamperuma, 1972).

In sandy soils, waterlogging is generally restricted to those low-lying areas where high water tables can occur. The field investigations carried out as part of the present project were at sites whose topography made waterlogging unlikely. However, to get some general background information on the effect of the process in manganese availability where it does occur, the following experiment was set up.

Materials and Methods

Giffordtown soil samples (10 g) were weighed into plastic centrifuge tubes and submerged with 50 ml of distilled water for varying lengths of time (0-984 hours). Following the period of submergence, the soils were centrifuged and a determination of the manganese concentration in the supernatant carried out. The solution was then discarded and the remaining soil was left to air-dry prior to extractable manganese determinations.

Results and Discussion

Table 3.23 shows the concentration of manganese solublised following the flooding and air-drying process. Included with the extractable manganese is the quantity of manganese that passed into solution during flooding. The data show that a period of between 22

Table 3.23 Concentrations of extractable manganese following the air-drying of previously submerged Giffordtown soil

Time of submergence prior to air-drying (hours)	Extractable manganese ($\mu g\ g^{-1}$)
0	2.1
9	2.1
14	2.1
22	2.1
46	2.9
86	5.5
157	6.9
280	11.0
504	3.9
984	1.9

and 46 hours of submergence prior to drying was necessary for the manganese concentration to exceed that of soil not subjected to flooding. After approximately 12 days of submergence, extractable manganese decreased and continued to do so to a concentration slightly below the levels at the onset of the experiment.

3.5.4 The Effects of Fertiliser Amendments on Soil pH and Extractable Manganese

Introduction

Following the field investigations of 1978 and 1979 it was thought possible that the wheel-tracking effect could have been attributable to fertiliser accumulation in the wheel depressions. Mixed fertilisers containing ammonium compounds have an acidifying effect on soils, due to the reaction:-

$$NH_4^+ + 2O_2 \rightarrow 2H^+ + NO_3^- + H_2O$$

which is induced by the nitrifying microorganisms *Nitrosomonas* and *Nitrobacter*. Hydrogen ions so generated can then become adsorbed on soil colloids or react with other soil constituents. An experiment was undertaken to investigate the effects of nitrogen fertiliser on soil pH and extractable manganese.

Materials and Methods

Air-dried soil samples (20 g) from several field trial sites and fields of commercial farms were weighed into 250 ml Erlenmeyer glass flasks. Sixty grams of acid-washed sand (rinsed several times with distilled water to remove residual acidity) was mixed in with the soil. This was to facilitate adequate aeration of the mixture during the incubation process. Solutions (15 ml) prepared with distilled water containing various concentrations of nitrogen as Analar grade NH_4NO_3(N) or commercial NPK fertiliser (F) containing NH_4NO_3 and $NH_4H_2PO_4$ were added to the soil-sand mixture. The quantities added were equivalent to the following rates of application of nitrogen:-

Treatment	Application rate (kg N ha^{-1})
N_o	0
N_1	100
N_4	400
N_6	600
F_1	100
F_3	300

The contents of the flasks were weighed and thoroughly mixed with a glass rod and either air-dried immediately or incubated for three weeks prior to air-drying. Incubated flasks were lightly stoppered with cotton wool and stored at 30°C. Wet weights were maintained with applications of distilled water every second day.

Results and Discussion

The data in Table 3.24 show the effects of nitrogen fertiliser on pH and extractable manganese on soils incubated for 0 and 3 weeks prior to air-drying.

Changes in pH

The pH (H_2O) values of 3 of the 4 soils air-dried immediately - decreased as the nitrogen rate increased. Much of this was probably attributable to a salt effect resulting from the exchange of fertiliser ions for colloidally-bound H^+ ions, and not nitrification. This was most evident in the Carpow soil because pH ($CaCl_2$) values did not

differ to any great extent. The generation of H^+ ions by nitrification processes would be reflected in changes in pH ($CaCl_2$) because H^+ so generated would be displaced from the exchange complex by Ca^{2+} ions.

Table 3.24 Changes in pH and $CaCl_2$-extractable manganese in nitrogen amended soil

Treatment following N addition	Soil	Fertiliser Rate	pH (H_2O)	pH ($CaCl_2$)	Mn ($\mu g\ g^{-1}$)
Soils air-dried immediately	Giffordtown	N_0	5.72	5.46	2.4
		N^o_1	5.75	5.48	2.0
		N^1_4	5.70	5.50	2.2
	Dreghorn	N_0	6.76	6.52	0.9
		N^o_1	6.65	6.50	0.9
		N^1_4	6.60	6.41	1.0
	Winton	N_0	6.06	5.76	3.3
		N^o_1	5.98	5.72	5.1
		N^1_4	5.89	5.70	5.8
		N^4_6	5.86	5.68	6.6
	Carpow	N_0	6.34	6.03	1.4
		N^o_1	6.28	6.04	1.5
		N^1_4	6.24	6.04	1.6
		N^4_6	6.20	6.03	1.8
Soils incubated for 3 weeks prior to air-drying	Giffordtown	N_0	5.72	5.48	1.6
		N^o_1	5.60	5.40	1.9
		N^1_4	5.47	5.28	2.3
	Macmerry	N_0	5.75	5.34	2.3
		N^o_1	5.58	5.24	2.6
		N^1_4	5.44	5.14	3.6
		F^4_1	5.61	5.29	2.4
		F^1_3	5.50	5.21	3.1
	Caprington	N_0	6.64	6.36	1.5
		N^o_1	6.46	6.22	1.8
		N^1_4	6.02	5.88	2.8
		F^4_1	6.46	6.24	1.6
		F^1_3	6.29	6.12	2.1

Incubation of the soil for three weeks on the other hand, brought about quite substantial drops in pH regardless of the nitrogen source, masking any pure salt effect; the pH ($CaCl_2$) values decreased as the rate of nitrogen application increased. This was readily apparent with the Giffordtown soil incubated for 0 and 3 weeks. No significant differences in pH ($CaCl_2$) were observed with the former treatment, while in the latter a pH drop of 0.2 units was observed between the control and the

highest rate of fertiliser. Approximately a 0.2 unit drop and 0.5 unit drop in pH ($CaCl_2$) occurred on the Macmerry and Caprington soils respectively when treated in a similar fashion.

For the soils air-dried immediately after addition of fertiliser, all except the Giffordtown soil showed increases in extractable manganese with increasing rate of application, but only small changes in pH. However, after 3 weeks incubation there were increases in extractable manganese in all soils accompanied by drops in pH of a similar magnitude to those observed in the field.

Reasons for the observed increase in extractable manganese with increased nitrogen in the non-incubated soils, (especially in the Winton and Carpow soils where pH changes were minimal) are obscure because the Ca^{2+} ions in the extractant could be expected to displace manganese from exchange sites irrespective of whether the fertiliser had been added or not.

Conclusions

Results of this experiment have shown that nitrogen fertiliser can cause changes in pH, and hence extractable manganese, after 3 weeks' incubation; these changes are of a similar magnitude to those differences observed between wheel-tracked and non-wheeled areas in the field. However, the question of whether sufficient nitrification to bring about these effects would have occurred in the field by the time the first samples were taken cannot be answered in the absence of any direct observations.

3.5.5 Effects of Leaching on Nitrogen Transformation, Soil pH and Extractable Manganese under Different Soil Consolidation Treatments

The loss of nutrients through leaching is determined by both climatic factors and soil-nutrient interactions. Leaching in sandy soils will generally be greater than in finer textured soils not only because of greater macroporosity but also due to a lower cation exchange capacity. Since manganese dificiency is very often associated with light-textured soils, differential leaching of fertiliser nutrients could play a role in determining the severity of deficiency, or indeed its absence or presence.

The varying degrees of soil consolidation that are found on wheel-tracked and non-wheeled areas of light textured soils may influence the amount of leaching from the top soil. In relatively unconsolidated sandy soil such as that occurring in non-wheeled areas of a field, free space between individual soil particles may be of such magnitude as to allow the downward movement of water to proceed as a relatively uniform wetting front; this is commonly referred to as 'piston flow' movement. In this case, as shown in Figure 3.12a, dissolved fertiliser ions would be washed down the profile. Figures 3.12b and 3.12c on the other hand represent a situation that may possibly arise when layers of soil are consolidated by repeated passes of tractor wheels. In this instance, the heavy soil consolidation (Soane, 1973) could provide a barrier for free downward flow of fertiliser nutrients, by causing water to divert peripherally to the zone of compaction. Furthermore, the lateral placement of soil over the compacted layer may occur during cultivation thus bringing more fertiliser into the wheel mark (Figure 3.12c). Subsequent nitrification of retained ammoniacal fertiliser (see Table 3.24) would then create an acid residue on the wheel-tracked soil possibly affecting the solubility of soil manganese

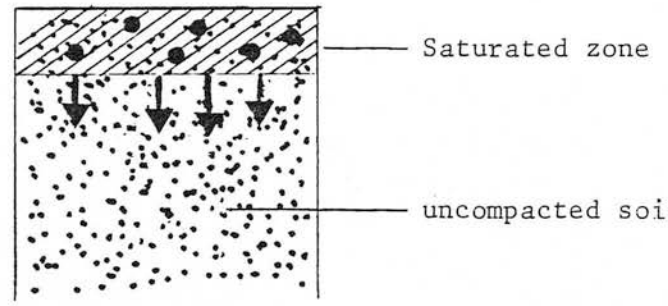
The following experiment was attempted in order to investigate the leaching characteristics and the subsequent behaviour of fertiliser material under different consolidation treatments.

Materials and Methods

Hexpath soil samples (1200 g) were added to each of 6 cylindrical plastic pots (1225 cm^3, diameter 10.5 cm) to give 2 pots at each of 3 levels of consolidation:- unconsolidated, surface compacted and compacted throughout. These levels of consolidation were carried out according to the procedure outlined in Section 3.5.6 below. A final 400 g amount of soil previously mixed with pulverised commercial fertiliser (25-10-10) was then added and the consolidation treatments completed. This was intended to simulate the distribution of a fertiliser in the field which had been worked into the surface following cultivation. Nitrogen amendments approximated to 167 $\mu g\ g^{-1}$ NH_4^+-N and 145 $\mu g\ g^{-1}$ NO_3^--N in the surface 400 g of soil. A total of 520 ml of distilled water was added in a dropwise fashion

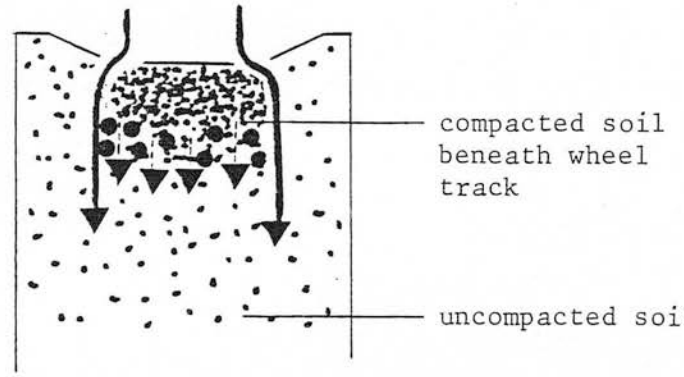
a) 'Piston Flow'

No preferential flow, therefore uniform leaching.



b) Flow in wheel track

Less flow under compacted zone, therefore less leaching.



c) Flow in wheel track

Less flow under compacted zone, therefore less leaching.

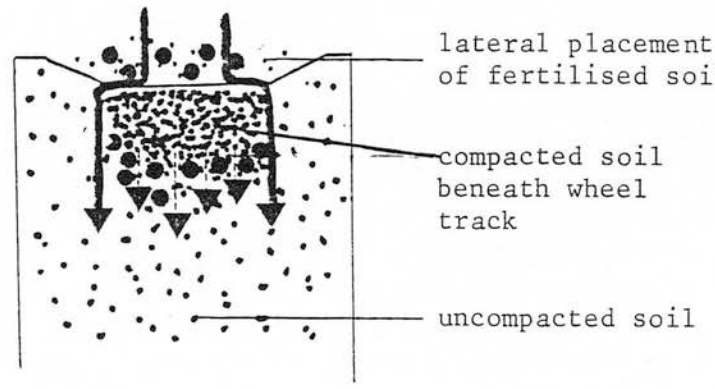
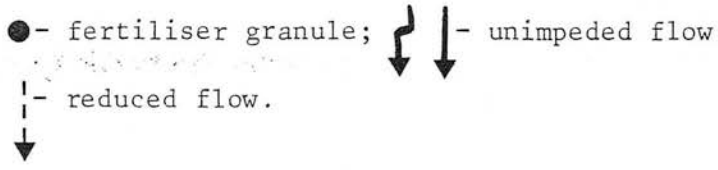


Figure 3.12 Idealised presentation of possible water flow in non-wheeled and wheel-tracked soil.



to each pot during a 21 day period (50-100 ml added intermittently throughout the period). Leaching out of the pots did not occur using this technique.

Following the 21 day period, the soil from one pot at each level of consolidation was partitioned into 4 horizons of equal depth. This was accomplished by extruding the soil from one end of the pot and slicing off smoothly with a large knife. Each layer was thoroughly mixed and a moist portion kept for dry weight and nitrogen analysis (NH_4^+-N and NO_3^--N). The remainder of the soil was air-dried for one week prior to the analysis of other soil parameters.

An additional 200 ml of distilled water was added to the remaining 3 pots in a similar fahsion to that previously described over an additional 21 day period. The soils were then removed and analysed as above.

Results and Discussion

Since the experiment was carried out in pots, the desired 'edge effects' such as those around a wheel mark could not be created. However, the experiment could indicate whether, even at high bulk densities water was able to move through the soil layer and would therefore be unlikely to bypass the wheel mark. Also it was hoped to show whether NH_4^+ ions would migrate under the various consolidation treatments. If not, physical compaction of the soil should not cause the number of H^+ ions resulting from nitrification to vary.

Changes in NH_4^+-N, NO_3^--N and pH at different depths over the course of the study are shown in Table 3.25. No measurements of NH_4^+ or NO_3^- concentrations in the unamended soil at the onset of the experiment were made. However, since the soil was collected in early spring from the surface 15 cm maximum leaching of any NO_3^- would probably have occurred. Although the subsequent drying of the soil in the laboratory could have converted some of the residual NH_4^+ to NO_3^-, such contributions undoubtedly would be small relative to that supplied by the fertiliser.

Table 3.25 Effect of leaching on nitrogen and soil pH under different consolidation treatment
pH ($CaCl_2$) at Day 0 = 6.40

Consolidation treatment	Depth in Pot (cm)	NH_4-N ($\mu g\ g^{-1}$)		NO_3-N ($\mu g\ g^{-1}$)		pH ($CaCl_2$)	
		21 days	42 days	21 days	42 days	21 days	42 days
Unconsolidated	0- 3.8	56.8	0.4	107.0	33.2	6.34	6.20
	3.8- 7.6	30.1	0.4	102.3	48.1	6.45	6.40
	7.6-11.4	9.2	0.4	77.4	73.7	6.66	6.51
	11.4-15.2	5.7	0.8	8.2	261.5	6.68	6.44
Surface compacted	0- 3.4	0.9	0.4	105.8	36.5	6.25	6.24
	3.4- 6.8	0.6	0.4	59.7	41.5	6.51	6.42
	6.8-10.2	4.6	0.4	63.5	83.4	6.66	6.52
	10.2-13.6	11.0	0.6	114.1	255.2	6.70	6.48
Compacted throughout	0- 3.1	0.1	0.5	80.8	16.2	6.25	6.25
	3.1- 6.2	0.1	0.4	64.6	21.7	6.52	6.42
	6.2- 9.3	0.9	0.4	77.9	37.8	6.68	6.52
	9.3-12.4	8.3	0.5	126.2	269.3	6.70	6.54

Ammonium-N

Concentrations of NH_4^+-N markedly decreased in the compacted treatments within 21 days. There was an increase in concentration with depth in these treatments but this could be due to slower nitrification processes (see below) rather than the downward migration of NH_4^+-N. With the unconsolidated soil, a substantial amount of the ion initially added remained in the NH_4^+ form especially in the surface layers.

Nitrate

Nitrate concentrations at 21 days were more variable with depth in the compacted soils, and in the unconsolidated soil they exhibited a similar trend to NH_4^+-N. In all treatments concentrations increased with depth at 42 days which may be explained by the susceptibility of NO_3^- ions to leaching.

Nitrification

Mean nitrate levels in the soils at 42 days - approximately 104 $\mu g\ g^{-1}$, 104 $\mu g\ g^{-1}$ and 86 $\mu g\ g^{-1}$ for the unconsolidated, surface compacted and compacted throughout treatments respectively far exceeded that introduced by the fertiliser (approximately 36 $\mu g\ g^{-1}$ on the basis of the entire quantity of soil per pot). These concentrations, and the virtual disappearance of NH_4^+ indicated that nitrification had taken place.

After 21 days, the pH ($CaCl_2$) values of the top layer for all treatments was lower than that of the soil at Day 0 (pH 6.40) indicating that the acidification process associated with nitrification had taken place. This acidification appeared to be greater in the compacted treatments and is in agreement with the aforementioned observation of the more rapid disappearance of NH_4^+-N in the compacted soils. This at first seems surprising since compacting the soil would be expected to decrease aeration below optimal levels for nitrifying organisms. Indeed, Whisler *et al* (1965) noted that even a slight compaction of the soil noticeably decreased NO_3^- production. It would appear, therefore, that moisture conditions in the consolidated soils were initially probably more favourable for nitrifying bacteria;

in the unconsolidated soil a greater percentage of larger pore space may have facilitated drying, to the detriment of the microorganisms. The fact that pH values in the unconsolidated treatment continued to decrease from 21 to 42 days to values similar to the compacted soils substantiates this view.

Phosphorus and Potassium

Table 3.26 shows data for the concentrations of phosphorus and potassium at the various depths for each consolidation treatment. Higher P levels were maintained in the top of the pots at both 21 and 42 days. This is to be expected since P is not very mobile in soil. Potassium, being a more leachable element displayed different trends between the two sampling periods. At 21 days, there was a higher concentration of K in the top horizon for all consolidation treatments, while at 42 days, distribution with depth for all the treatments was more uniform. The leachability of K did not appear to be influenced by soil consolidation.

Manganese

For the most part, manganese concentrations decreased in all consolidation treatments and at all depths between 0 and 21 days. The trend continued between 21 and 42 days and was most apparent in the unconsolidated soil and least so in the compacted throughout treatment (Table 3.27). Since there was no net increase in manganese concentrations in any treatment accompanying the nitrification and release of H^+, the differences between the unconsolidated soils and the others could indicate that the greater acidification initially in the compacted soils (Table 3.27) had partially offset a trend toward less available manganese. The uncertainties are too great however to make any definite conclusions.

Summary and Conclusions

Results of this experiment show decreases in pH ($CaCl_2$) values probably attributable to nitrification in the surface layers of the pot. Acidification rates were more rapid initially, in the compacted soils but values were more similar for all treatments at the end of the experiment. Water movement appeared to be unimpeded by consolidation since the variation of nitrate concentrations with

Table 3.26 Effect of leaching on phosphorus and potassium under different consolidation treatments

Consolidation treatment	Depth in Pot (cm)	P ($\mu g\ g^{-1}$)		K ($\mu g\ g^{-1}$)	
		21 days	42 days	21 days	42 days
Unconsolidated	0- 3.8	18.2	17.9	174	157
	3.8- 7.6	17.9	15.2	174	152
	7.6-11.4	15.7	15.5	142	136
	11.4-15.2	16.2	15.0	124	150
Surface compacted	0- 3.4	19.9	17.9	168	150
	3.4- 6.8	16.1	15.7	152	145
	6.8-10.2	15.6	15.7	133	139
	10.2-13.6	15.6	15.6	146	150
Compacted throughout	0- 3.1	19.5	19.3	166	148
	3.1- 6.2	16.1	15.7	149	148
	6.2- 9.3	15.7	15.7	136	141
	9.3-12.4	16.0	15.7	136	148

Table 3.27 Effect of leaching on extractable manganese under different consolidation treatments

Manganese (Day 0) = 2.4 $\mu g\ g^{-1}$

Consolidation treatment	Depth in Pot (cm)	Manganese ($\mu g\ g^{-1}$)		
		21 days	42 days	Δ Mn
Unconsolidated	0- 3.8	2.1	1.0	-1.1
	3.8- 7.6	2.2	0.9	-1.3
	7.6-11.4	2.4	0.9	-1.5
	11.4-15.2	1.8	1.0	-0.8
Surface compacted	0- 3.4	1.8	1.0	-0.8
	3.4- 6.8	1.2	0.8	-0.4
	6.8-10.2	1.6	0.8	-0.8
	10.2-13.6	2.1	0.9	-1.2
Compacted throughout	0- 3.1	1.7	1.0	-0.7
	3.1- 6.2	1.2	1.0	-0.2
	6.2- 9.3	1.2	1.1	-0.1
	9.3-12.4	2.3	2.0	-0.3

depth showed similar trends in all treatments after 42 days. Movement of potassium within the pots was apparently unaffected by consolidation and no relationship between differential leaching and subsequent changes in pH and extractable manganese could be detected. The artificial conditions of this experiment undoubtedly explain some of the anomalous results.

3.5.6 The Effect of Soil Consolidation on Soil and Plant Manganese

Introduction

Passioura and Leeper (1963) observed that oats growing in loose soils (light-textured and near-neutral) were very severely affected by manganese deficiency, while those on compacted soil were nearly cured of the problem. No differences in pH and EDTA-extractable manganese in the soil were detected between the loose and compacted treatments. The authors therefore concluded that the beneficial effects of compaction arose from an increase in surface area of the root mass that came into contact with reactive (potentially available) manganese oxides; absorption was then achieved by a contact reduction mechanism. No data on plant manganese content was included in their paper, however.

The possibility of a root contact mechanism accounting for the differences in plant uptake on differently consolidated soils is investigated in the following study.

Materials and Methods

The experimental methods described below apply not only to the consolidation study discussed in this section, but also to investigations described in Section 4 which involve tracer studies with radioactive manganese (^{54}Mn). For convenience and continuity of presentation, the procedures relating to the latter experiments are included here.

Soils

Three light-textured soils of the Eckford and Darvel associations - Giffordtown, Hexpath and Darvel series - were used for the pot experiments. Of the three soils, only the latter two were used in the present consolidation study, but all were used in experiments

with ^{54}Mn. The Giffordtown and Hexpath soils were taken from the sites of the experimental field trials in 1978 and 1979, respectively. The Darvel soil was taken from a manganese deficient field during the summer of 1980 and has not been previously described. Table 3.28 outlines some of the soil's parameters and for comparative purposes, the two field trial soils (from Table 3.1) are also included.

Table 3.28 Some properties of the soils used in the pot experiments

Soil Series	Texture	Organic Matter (%)	P	K	Mg	C.E.C. (meq/ 100g)	% B.S.*
			—(µg g^{-1})—				
Giffordtown	Sandy loam	7.17	4.4	140	127	22.0	84.1
Hexpath	Sandy loam	7.41	6.7	79	32	12.0	87.5
Darvel	Sandy loam	-	23.2	96	109	12.0	79.2

* % B.S. - % base saturation = T.E.B./C.E.C. x 100

Incorporation of ^{54}Mn in Soil

A quantity of soil (slightly air-dried to facilitate mixing) for each pot (1500 g) was spread evenly on a plastic sheet to a depth of approximately 1 cm. A known amount of activity (5 or 10 µCi ^{54}Mn) from the stock solution was sprayed evenly over the soil from an atomiser in several increments, with mixing of the soil between additions. The soil was then transferred to a 3 litre plastic jar and thoroughly mixed in an end-over-end shaker for 24 hours. Uniform distribution of the radioisotope was checked by counting the activity of 3 random 5 g soil samples from each jar. Further mixing was carried out if necessary to ensure uniform distribution of ^{54}Mn.

Fertiliser amendments

Analar grade NH_4NO_3, $NH_4H_2PO_4$ and KCl were used in order to avoid extraneous manganese addition. Fertiliser quantities per pot equivalent to 109 kg ha^{-1} N, 39 kg ha^{-1} P_2O_5 and 39 kg ha^{-1} K_2O were dissolved in a small quantity of distilled water (3 ml) and sprayed onto 50 g of soil. After air-drying, the soil was added to the 1500 g of soil labelled with ^{54}Mn and thoroughly mixed for several hours. It was hoped that the addition of fertiliser by this method would have little effect subsequently on ^{54}Mn equilibrium and distribution in soil.

Consolidation of soil in pots

Following the incorporation of ^{54}Mn the soils were transferred to 1225 cm^3 cylindrical pots made from 10.5 cm diameter plastic pipe, across the bottom of which nylon mesh was stretched. The soils were consolidated in several ways:-

unconsolidated - 1550 g soil added to pot without compaction. This was considered to be the control treatment.

surface compacted - 1550 g soil added to pot followed by compaction.

compacted throughout - 4 x 375 g increments added to pot, each amount compacted equally.

All consolidation treatments were carried out with a 4.2 kg Proctor Hammer falling from a height of 15-35 cm above the soil surface, the blows being uniformly distributed across the soil surface.

Storage of pots prior to sowing

Following compaction the soils were brought to field capacity by capillary action, covered with aluminium foil and stored for 3 weeks.

Germination, sowing of seeds and maintenance of pots

Barley seeds (varieties of Athos and Porthos prone to manganese deficiency problems) were germinated on moist tissue paper in the bottom of a petri dish and sown at a density of 7/pot. Once the seedlings were established, they were thinned to 5/pot. The soil surface was uniformly covered with 100 g acid washed sand to minimise evaporation. The soils were kept at 90% of field capacity throughout the course of the experiment by adding distilled water three times per week. Daylengths were 12-16 hours, artificial lighting (sodium lamps) being used to supplement natural light where required.

Determination of bulk density

Bulk densities of the consolidation treatments (unconsolidated, surface compacted and compacted throughout) were calculated from the volume of the pot occupied by a known amount of oven-dry soil.

Check on uniformity of bulk density

The procedure adopted for uniform compaction of the soil in layers was intended to result not only in homogeneous consolidation of the soil within pots, but also in the reproducibility of bulk density between replicate treatments.

Pots were set up containing non-radioactive soil which was compacted throughout the pots as outlined above and oven-dried at 105°C. The soil was removed from the pots and broken up by several random blows. Bulk densities of soil clods from different positions within each pot were determined using a modified paraffin wax bath technique (MAFF, 1974). Fairly uniform bulk density throughout the pots, and between replicate treatments was achieved in this manner (see Appendix B.6).

Analysis of soil and tissue

At the termination of the experiment, the soil from each pot was thoroughly mixed and air-dried prior to pH and manganese determinations.

The harvested tissue was dried overnight at 105°C, and finely ground. Known weights of tissue were counted for ^{54}Mn activity and compared against standards of known activity. Determinations of stable manganese as well as nitrogen were carried out following tissue digestion Methods 1 and 2 respectively (see Section 3.2.2).

Results and Discussion

Growth

Compacting the soil delayed emergence of the young seedlings by several days but after several weeks growth appeared similar in all treatments. The time of ear emergence was unaffected by the consolidation treatment.

Yield

Results of plant and soil analysis are shown in Table 3.29. Dry matter production was found to be significantly reduced by compaction; yields in the compacted throughout treatments were approximately 67% and 61% of those in the unconsolidated soils in the Hexpath and Darvel soils respectively. Surface compaction did not reduce yields by as

large a margin. These results disagree with those of Passioura and Leeper (1963) who found compaction to increase yields 2 to 3 fold in some cases. However, in the present study, manganese availability does not appear to be a limiting factor as evidenced by its concentration in the plant (Table 3.29). Thus any beneficial effects of compaction on yield as related to enhanced manganese uptake would not be realised in pot experiments using these soils.

The decrease in yield with compaction appeared to be unrelated to the plants ability to extract available nutrients from the soil. An inspection of the rooting system at harvest showed that, in all consolidation treatments, root exploration was unimpeded throughout the pot; no concentrations of roots at or near the soil surface in any of the pots were observed. The possibility arises that the yield differences may partially be related to nutrient availability which decreases with compaction. Analysis of plant nitrogen in the experiment with the Hexpath soil showed a significantly lower ($p < 0.05$) total uptake on both the compacted treatments relative to the unconsolidated pots. Total uptake amounted to 59 mg, 53 mg and 51 mg of N on a per pot basis for the unconsolidated, surface compacted and compacted throughout treatments respectively - an indication that soil compaction had hindered nitrogen mineralisation and hence the availability status of the element. This differs from the results of a previous experiment where less mineralisation was found initially in the unconsolidated soil (see 3.5.5). Differences in watering regimes between the two experiments may help explain the discrepancy.

Soil and plant manganese

Compaction significantly increased concentrations of extractable soil manganese in both experiments (Table 3.29). With the Hexpath soil, no associated changes in pH occurred, while with the Darvel soil, the inverse relationship between pH and extractable soil manganese was somewhat evident.

Manganese concentrations in the plant as well as total uptake were significantly enhanced by compaction in both soils. Surface compaction had the greatest influence in increasing total uptake, especially in the Hexpath soil.

Table 3.29 Results of soil and plant analysis on the Hexpath and Darvel soils

Soil	Consolidation	Soil Data				Plant Data		
		Bulk Density ($g\ cm^{-3}$)	pH (H_2O)	pH ($CaCl_2$)	Mn ($\mu g\ g^{-1}$)	Yield (g)	Mn uptake	
							Total µg	µg g^{-1}
Hexpath	Unconsolidated	1.10	7.11	6.66	1.2 a†	4.84 a	235.5 a	48.7 a
	Surface compacted	1.31	7.12	6.65	1.8 b	3.79 b	425.7 b	112.2 b
	Compacted throughout	1.40	7.09	6.63	1.9 b	3.24 c	292.4 a	90.2 b
Darvel	Unconsolidated	1.10	6.17	5.72	2.1 a	8.14 a	223.7 a	27.5 a
	Surface compacted	1.34	5.98	5.64	2.3 a	6.47 b	278.6 a	43.1 b
	Compacted throughout	1.40	5.90	5.60	3.0 b	4.94 b	246.0 a	49.8 b

† Means with the same letter within each experiment and each column are not significantly different at the 5% level.

When soil extractable manganese concentrations were plotted against manganese concentration in the plant, similar trends were observed, i.e. higher levels of soil manganese were reflected in the plant (Figure 3.13). It is of interest to note that the compaction treatments relative to the respective soil manganese concentrations, significantly enhanced manganese uptake in both the Hexpath ($p<0.001$) and the Darvel ($p<0.05$) soils. This was most evident with the surface consolidation treatments, especially in the experiment with the Hexpath soil. Mean ratios of $[Mn_{plant}] / [Mn_{soil}]$ for the unconsolidated, surface compacted and compacted throughout treatments were approximately 40, 62, 47 and 13, 19, 17 for the Hexpath and Darvel soils respectively.

It is doubtful that the increases in extractable soil manganese with compaction resulted from waterlogging. The watering regime was such as to avoid this possibility and impaired permeability would seem minimal in such light-textured soils. The plants showed no symptoms of waterlogging during the experiment and inspection of the soils following harvest showed no saturation at any depth in the pot.

The possibility of high CO_2 concentrations enhancing manganese solubility in the compacted treatments cannot be entirely ruled out since no measurements of CO_2 were carried out in the pots. As shown in a previous experiment, high concentrations of CO_2 (5 and 10%) increased manganese solubility of the Hexpath soil (see Table 3.22). It seems unlikely however that such levels of CO_2 would have accumulated within the pots without being deleterious to plant growth.

The action of root exudates on soil manganese minerals may explain the changes in extractable manganese between consolidation treatments. Barber and Gunn (1974) found that the growth of barley or maize roots through a solid medium (ballotini) caused the quantities of root exudates (amino acids and carbohydrates) to be increased relative to quantities exuded by roots growing in culture solution alone. Compact soil similarly may increase exudates relative to uncompacted soil. Bromfield (1958a, 1958b) actually showed that root washings dissolve γ-MnO_2 and that the amount brought into solution increased with the concentration of the washings.

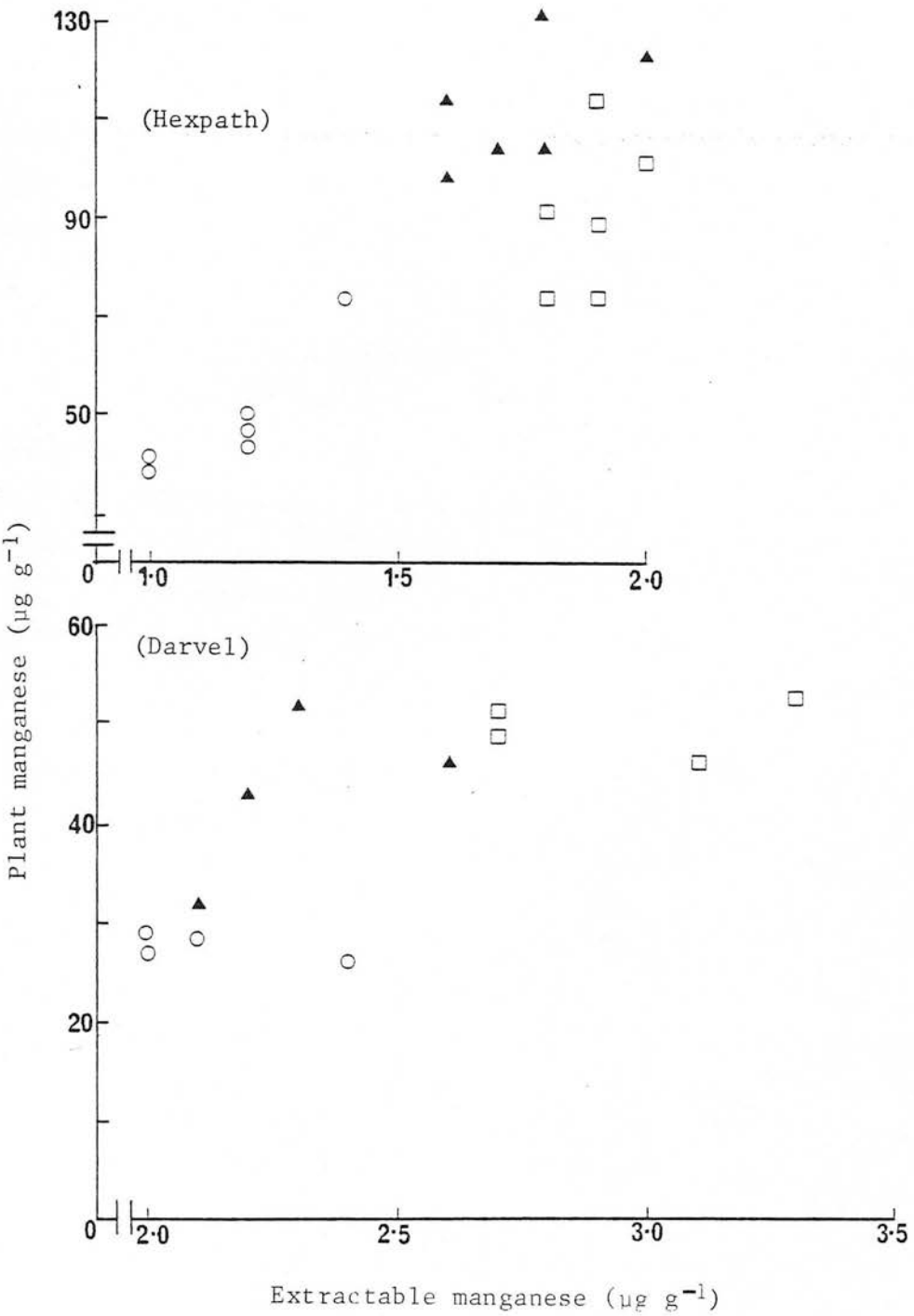


Figure 3.13 Relationship between extractable manganese and manganese content in the plant.

○ - light consolidation
▲ - surface consolidation
□ - uniform consolidation

Conclusions

The results of this experiment have shown that despite the fact that compaction reduced yield, consolidation enhanced the plant's ability to absorb manganese relative to levels of extractable manganese in the soil. This may imply that a contact reduction mechanism is in operation in which, unlike the situation found by previous workers (Passioura and Leeper, 1963), the solubility of soil manganese increased. The effect of consolidation in enhancing the quantity of root excretions may also be a prominent factor.

3.5.7 The Effects of Nitrogen Source, Soil Consolidation and Plant Presence on Soil and Plant Manganese

Introduction

Plant roots have the capability of either decreasing or increasing the pH of their growth medium, the direction and amount of change being influenced by differential rates of cation and anion absorption. Jackson and Adams (1963) found H^+ ions to be exuded from plant roots if excess cations were absorbed. This was found to occur particularly in a region 1 to 6 cm from the root tip. On the other hand, a net increase in OH^- or HCO_3^- ions were noted by Riley and Barber (1969) if anion absorption predominated.

In recent work by Clarkson and Warner, (1977) it was found that in nutrient solution, Italian ryegrass absorbed NH_4^+ ions in preference to NO_3^- ions at low temperatures. This observation could have considerable significance for crops in the field because of the low temperatures commonly occurring at sowing time. Ammonium ions from the fertiliser source could well be a major form of available nitrogen at this time. Low soil temperatures would hinder nitrification and other nitrogen transformations, and any nitrate initially present would be subject to leaching down the profile and possibly beyond the zone of early root development. The possibility arises that soil pH could be reduced and available manganese increased by root exudation of H^+ ions if NH_4^+ was the predominant nitrogen ion absorbed. The effect could be more pronounced in wheel-tracked soil where soil compaction around the seed may result in more efficient uptake of nutrients during early stages of growth, because of better root contact or a more satisfactory moisture regime.

An experiment was therefore designed to investigate the relative absorption of different nitrogen sources as affected by soil temperature and consolidation. As a consequence of this, the effects of plant presence on soil pH and extractable manganese were also investigated.

Materials and Methods

Twenty-four partially air-dried samples (1000 g) of Darvel soil (see Table 3.28) were spread evenly on plastic sheets to a depth of approximately 1 cm. To each of three groups of eight samples a solution of one of the following ^{15}N-labelled nitrogen compounds was added: NH_4NO_3 (5.2 atom per cent ^{15}N with only the ammonium ion labelled), $Ca(NO_3)_2$ (5.5 atom per cent ^{15}N) or $(NH_4)_2SO_4$ (5.4 atom per cent ^{15}N). The total quantity of nitrogen added was 44 μg g^{-1} soil. Incorporation of the various nitrogen compounds into the soil was carried out in the same manner as that outlined for incorporation of ^{54}Mn (Section 3.5.6). The soils were added to cylindrical plastic pots (1225 cm^3, diameter 10.5 cm). Four of the pots in each group were compacted (2 x 500 g amounts of soil, each compacted by 12 equally spaced blows of a Proctor hammer from a height of 25 cm) while the remaining soils received no consolidation. The pots were then brought to field capacity in a refrigerated room (4°C) with distilled water previously chilled to 4°C, and left to equilibrate for one week. All of the pots were placed in polystyrene boxes and packed to the top rim of the pot with insulating vermiculite material. Holes were made in the bottom of the boxes to allow for adequate drainage of the soils. Half of the compacted pots (two from each nitrogen source) and half of the non-compacted pots (two from each nitrogen source) were sown with barley at a density of 7 seeds/pot. The remainder were left fallow.

The 24 pots were placed outside on the 2nd May, 1980 under rainproof structures open at the sides and front to permit adequate ventilation. At emergence, the seedlings were thinned, if necessary, to 4/pot.

Fifty ml quantities of distilled water at 4°C were added to all the pots once per week during the six-week growth period. The plants were then harvested, dried for 24 hours at 105°C and finely ground.

The nitrogen and manganese content was then determined. The quantity of ^{15}N in the plant tissue was determined in an MS 10S mass spectrometer. The soils from the pots were thoroughly mixed and air-dried prior to analysis.

Results and Discussion

Soil temperature

The long-term mean soil temperature at 5 cm depth during the time of year (May) the experiment was conducted was about 10°C (Hay, 1976). In the absence of suitable temperature-controlled growth room facilities it was hoped that temperatures of this order would prevail during the experiment. However, soon after the start of the experiment, weather conditions became untypically warm with temperatures rising daily to >25°C over approximately a two week period. The insulating materials proved insufficient to maintain low soil temperatures. In both consolidation treatments soil temperatures rose to 21°C or greater within three weeks of sowing.

Seed germination and plant development

Germination of seed and growth was severely impaired in the compacted soils; in some cases only 2 plants/pot developed. In the unconsolidated soils, on the other hand, desired plant density (4/pot) was achieved and growth was much better. Examination of the soil at the conclusion of the experiment showed that compaction in this instance markedly inhibited root exploration of the soil. The bulk of the roots were found at the soil-pot interface. Root development and proliferation was more advanced in the unconsolidated soils. In view of the poor plant development on the compacted soils, only the data pertaining to the non-compacted treatment are dealt with in the present discussion.

Influence of fertiliser source on soil pH

The pH of the soils decreased in the order $Ca(NO_3)_2$ > NH_4NO_3 > $(NH_4)_2SO_4$ (Table 3.30) regardless of plant presence. These differences could be explained by the nitrification of NH_4^+ ions or in the case of the sulphate fertiliser, the biogenesis of sulphuric acid (Vavra and Frederick, 1952).

Influence of plant presence on soil pH

The presence of plants significantly increased soil pH as shown by the data in Table 3.30. The preferential absorption of NO_3^- resulting from high soil temperatures in this study could explain the pH increase and support the findings of Clarkson and Warner (1977). This becomes evident when a comparison is made of the relative contribution of nitrogen from the various fertiliser sources (Table 3.31). In the case of the NH_4NO_3- amended soil ^{15}N measurements showed that 18.4% of the plant nitrogen originated from the ammonium ion in the fertiliser. Uptake of what was originally NH_4^+-N on the $(NH_4)_2SO_4$ treatment - approximately 37% - was similar since twice the amount of NH_4^+ ion was present in this source. Since the quantity of available nitrogen was the same for both fertilisers, the uptake of NH_4^+-derived nitrogen had proceeded with equal effectiveness. With the $Ca(NO_3)_2$ source, on the other hand, a larger percentage of plant nitrogen originated from this source. Total nitrogen levels in the plants did not differ greatly between different fertiliser amendments (Table 3.31).

Soil manganese

Concentrations of extractable manganese did not differ appreciably between the planted and fallow soils, despite the effects of the plants in altering soil pH.

Conclusions

Results of this experiment have shown an apparent preferential absorption of NO_3^- ions relative to NH_4^+. This was probably attributable to the unavoidably high soil temperatures maintained during the course of the experiment. Absorption of NO_3^- by plants results in an increase in pH probably due to root exudation of OH^- or HCO_3^- ions (Riley and Barber, 1969). Extractable manganese was not significantly affected by the rise in pH, which is contradictory to the observations in other experiments. However, changes in manganese may be much more difficult to detect at pH's as high as those encountered here because of the low absolute values and the exponential relation between pH and soil manganese.

Table 3.30 The effect of plant presence and fertiliser source on soil pH†

+/- Plants	Fertiliser	pH (H_2O)	pH ($CaCl_2$)	Mn, soil ($\mu g\ g^{-1}$)
+ Plants	NH_4NO_3	6.78 ± 0.02	6.51 ± 0.02	1.1 ± 0
	$(NH_4)_2SO_4$	6.68 ± 0.02	6.46 ± 0.02	1.2 ± 0.1
	$Ca(NO_3)_2$	6.84 ± 0.01	6.58 ± 0	1.0 ± 0
- Plants	NH_4NO_3	6.60 ± 0	6.44 ± 0.02	1.0 ± 0.1
	$(NH_4)_2SO_4$	6.55 ± 0	6.40 ± 0	1.2 ± 0.1
	$Ca(NO_3)_2$	6.69 ± 0.01	6.50 ± 0	1.0 ± 0

† Mean of duplicate pots.

Table 3.31 The per cent nitrogen in the plant derived from the fertiliser source*†

Fertiliser	Plant Nitrogen (%)	Plant Nitrogen Derived from fertiliser (%)
NH_4NO_3	3.15 ± 0.07	18.42 ± 0.04
$(NH_4)_2SO_4$	3.21 ± 0.06	37.48 ± 0
$Ca(NO_3)_2$	3.40 ± 0.18	48.93 ± 0.86

* See Appendix B.7 for calculation.

† Mean of duplicate pots.

This experiment needs to be repeated under low temperature conditions to see whether, in fact, NH_4^+ is preferentially taken up. Unfortunately, there was no opportunity within the period of this project to carry out necessary work - the first available opportunity would have been the early spring of 1981.

3.5.8 Summary of Laboratory and Pot Experiments

Incubation of soil with acid or base resulted in appreciable shifts in soil pH and changes in manganese concentration. The quantities of extractable manganese were higher in the amended soils, relative to pH, than had been found in the field, but followed a similar trend with pH.

Perfusion studies with different CO_2 concentrations showed that the cause of pH depression on wheel-tracked soil was not attributable to changes in the partial pressure of CO_2 in the soil atmosphere. No pH depression was shown to occur despite CO_2 concentrations many times above that normally encountered in light-textured arable soils.

Waterlogging did not appear to play any role in producing the wheel-tracking effects in view of the texture of the soils under study, and the topography of the sites. A laboratory experiment showed that if in any circumstances soils of this type were subject to flooding then air-dried, manganese concentrations increased only after the soil had been submerged for at least 22 hours prior to drying.

Soil pH was found to decrease on nitrogen-amended soils, most likely as a result of nitrification processes. This was most evident in soils incubated for 3 weeks under conditions of aeration, moisture and temperature suitable for aerobic microbial activity. The pH decreased as the amount of added fertiliser increased; the associated changes in extractable manganese were of a similar nature to those observed in the field.

Simulated leaching of differently consolidated fertiliser-amended soils resulted in a more rapid acidification in the compacted treatments initially (at 21 days), but at the termination of the experiment no large pH differences between consolidation treatments

were evident. Extractable manganese concentrations decreased despite soil acidification. Determination of NO_3^- and K^+ at different depths did not indicate any differential leaching of these ions (with consolidation) at the termination of the experiment.

The effects of soil consolidation in enhancing manganese uptake was demonstrated on two soils. Compaction, although decreasing yields, was found to enhance significantly the uptake efficiency of soil manganese. This could have been attributable to changes in the concentration of root exudates and/or contact reduction mechanisms.

A pot experiment in which plants were grown in soils amended with different ^{15}N-labelled fertilisers had been intended to investigate possible enhanced uptake of NH_4^+ ions in cold conditions. In fact, it showed enhanced uptake of NO_3^- ions relative to NH_4^+. This preferential absorption was attributed to the high soil temperatures actually experienced. Plant presence increased soil pH, presumably because of OH^- and/or HCO_3^- exudation as NO_3^- was absorbed.

3.6 FINAL DISCUSSION

Consolidation of sandy soils, as a result of the passage of tractor wheels, has been clearly demonstrated to increase the availability of soil manganese. The effect was most noticeable - both on experimental sites and in the fields of commercial farms - when fertiliser was broadcast rather than combine-drilled. Furthermore, in the 1980 field trial, the plots receiving broadcast applications of fertiliser (on the plough furrow) prior to cultivation and drilling manifested the wheel tracking effect to a much greater extent than did those plots in which fertiliser was broadcast at drilling time (Table 3.17). The former treatment was included to allow broadcast fertiliser to fall into depressed wheel tracks, which would subsequently be filled in by tillage. In many instances, manganese uptake by the plants was related to a reduction in soil pH and an increase in the concentrations of extractable manganese in the soil.

Four mechanisms may be responsible (not necessarily simultaneously):- firstly, manganese availability could be enhanced as a result of the acidifying effect of the fertiliser, e.g. by the nitrification of NH_4^+ to NO_3^- in the zone of early root development. Secondly, the more readily available supply of fertiliser nutrients at the early stages of development would result in better plant growth, including a more prolific root system. This increase in rooting could be expected to result in increased uptake of all nutrient ions. The secretion of H^+ ions to balance the uptake of K^+ and NH_4^+ ions would depress the soil pH (i.e. increase acidity) and therefore increase manganese availability. In cold springs the possible favouring of NH_4^+ uptake relative to NO_3^- uptake would enhance this effect. Thirdly, an increase in manganese availability could result from greater exudation of compounds capable of dissolving insoluble forms of manganese in the soil. This process could be stimulated by compaction (Barber and Gunn, 1974) and could be expected to increase with increases in the total quantity of roots present. Fourthly, consolidation of the soil would result in increasing the area of root contact with reactive manganese oxides; this could lead to enhanced manganese uptake via a contact reduction mechanism.

Fertiliser-soil interaction

Changes in soil pH and extractable manganese were clearly demonstrated in the laboratory by incubating soil with nitrogen compounds (Table 3.24). However, it must be noted that nitrification took place under a temperature regime (30°C) that would maximise microbial activity. In contrast, soil temperatures in an area near the site of the Giffordtown trial (1978) rose slowly from approximately 7°C to 12°C during the 6-week period from the time of sowing to the first soil sampling*. Such temperatures, although not inhibitory to the nitrification process could nevertheless retard it to a considerable extent.

A further point to consider is the lack of effect on soil pH of different nitrogen application rates in the 1978 field trial on the Giffordtown soil. (These results as well as data on extractable manganese are shown in Table 3.32). It is evident that increasing the rate of fertiliser did not result in a reduction in pH or higher

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* Data supplied by Meteorological Office, Elmwood College, Cupar recording station.

Table 3.32 The effect of fertiliser application rate on soil pH and extractable manganese on the wheel tracks and non-wheeled areas of the plots on the Giffordtown soil (1978)*

Fertiliser Application Rate	Wheel tracks		Non-wheeled	
	pH ($CaCl_2$)	Mn, soil ($\mu g\ g^{-1}$)	pH ($CaCl_2$)	Mn, soil ($\mu g\ g^{-1}$)
N1	5.60	3.1	5.68	2.7
N2	5.78	2.2	5.89	2.0
N3	5.65	3.1	5.75	2.1

*Mean of 3 replications and 2 tillage treatments.

levels of extractable manganese, in contrast to what occurred in the laboratory experiment.

Despite such evidence to the contrary, the possibility of a soil-fertiliser interaction should not be eliminated at this time. Other workers have noted the beneficial effects of acid-producing fertilisers in alleviating symptoms of manganese deficiency. Randall *et al* (1975) showed that manganese deficiency in soyabeans could be eliminated with the use of NH_4^+-containing fertilisers. Similar results have been reported by Hoyt and Myovella (1979) concerning manganese deficiency in wheat.

More work is needed to investigate the process of nitrification, as affected by the interaction of soil temperature and the degree of consolidation, and its subsequent effects on soil pH and manganese availability. In this respect, leaching experiments under more natural conditions than those previously employed (Section 3.5.5) are needed. Studies with ^{15}N-labelled fertilisers in field conditions would certainly provide direct evidence of the fate of fertiliser applied to differently consolidated soils. Removal of intact soil cores from fields, followed by controlled leaching and sampling in the laboratory would be another useful alternative method of investigation.

Fertiliser-plant interaction

In the 1980 field trial on the Carpow soil, the combine-drilled treatments not only showed a decreased wheel track effect relative to the plots receiving fertiliser as a broadcast but also showed less severe manganese deficiency symptoms overall (Table 3.17). At harvest the combine-drilled plots yielded equally well regardless of consolidation treatment, whereas in the other fertiliser-applied treatments increased consolidation had a significant effect (Table 3.20). These observations stress the importance of early nutrient availability to barley on light-textured soils. It is worthwhile to note in this context that at the Giffordtown site in 1978, the commercial crop surrounding the experimental plots had been combine-drilled and, in contrast to the trial site, no wheel tracking effects were visible.

Significant changes in soil pH and extractable manganese between wheel-tracked and non-wheeled areas were not observed on the Carpow soil either in the commercial crop sampled in 1979 or in the 1980 field trial (Tables 3.16 and 3.17). This requires explanation, in view of the fact that other soils where wheel tracking was prominent manifested such changes. A possible cause could be that this soil had a higher buffering capacity, which would reduce the effect of root excretions on the pH of the rhizosphere. However, as the Carpow soil had a lower clay content and not an abnormally high organic matter content (Table 3.15), this possibility seems unlikely. It is worthwhile to note that of the three fertiliser application methods, the relationship between depressed pH and higher extractable manganese was most evident at the 7 week sampling for the BB treatment (fertiliser applied prior to cultivation and drilling) (Table 3.17). Although the differences in pH ($CaCl_2$) values between wheeled and non-wheeled areas were not quite significant at the 5% level, in no case was the pH lower in the non-wheeled areas. Likewise, manganese concentrations were always equal to or less than concentrations found in the wheel tracks. This is in contrast to the pH and manganese determination of the other treatments at the 5 and 7 week sampling periods. Further, it should be pointed out that any pH change at the root-soil interface could be masked by the bulking and mixing of the individual soil cores (during sampling) which consisted only partly of soil from

the immediate root zone. If the rhizosphere soil only had been sampled it is possible that the observed differences between wheeled and non-wheeled areas may have been greater.

3.7 CONCLUSIONS

The results of this study have shown that combine-drilling of fertiliser on light-textured, manganese deficient soils is apparently the most effective way of reducing manganese deficiency in barley and increasing yields. This has important implications not only concerning manganese availability but also in relation to other elements that may prove to be inadequate for normal plant growth. Deficiencies of copper, boron and zinc are being detected with greater frequency and the question of whether availability of these elements could also be influenced by better fertiliser uptake certainly provides scope for future work.

The change in the chemical composition of compound fertilisers in recent years is another factor that should be considered in relation to manganese availability; this concerns the present use of NH_4NO_3 in contrast to the once dominant $(NH_4)_2SO_4$ as the primary nitrogen source in compound fertilisers. The use of the sulphate fertiliser would result in greater acidification of the soil in at least three ways:- (1) enhanced nitrification, (2) a greater generation of exuded H^+ ions since NH_4^+ would be, at the time of application, the only nitrogen source readily available to the plant, (3) the synthesis of sulphuric acid. Field trials using different nitrogen sources as well as different fertiliser application methods should be undertaken to determine if in fact, the greater incidence of manganese deficiency in recent years can be partly explained by the change in fertiliser materials.

Varietal screening of barley is also of utmost importance, as emphasised by the lack of work carried out on this subject (Section 2.3). It would not be unreasonable to suppose that even on the most severely deficient soils, the use of a variety more resistant to deficiency, coupled with combine-drilling, would considerably alleviate or eliminate deficiency altogether.

SECTION 4

STUDIES WITH RADIOISOTOPIC MANGANESE (^{54}MN)

4.1 INTRODUCTION

The principles of radioisotopic exchange and isotopic dilution analysis have been extensively applied in soil research over the past 30 years. The pioneering work of McAuliffe *et al* (1948) and Fried and Dean (1952) with ^{32}P initiated a voluminous amount of research on factors affecting the availability of soil phosphorus, in particular. However, although suitable radioactive tracer isotopes have been available for many years for most micronutrients, it has only been in the past 10-15 years that radioisotopic techniques have been exploited to any extent in studying their forms and reactions in soil and their availability to plants.

The isotopes of an element differ in mass number but, with the exception of those of hydrogen, differ only to a very small extent in their chemical reactivity. The basic underlying principle (and assumption) in the application of isotopic exchange methods to soil research is that a radioisotope, if added to a soil solution in a sufficiently small amount so as not to disturb the existing equilibrium, will exchange with those forms of the element that are in kinetic equilibrium with that element in solution. If the stable (native) and active (radioisotopic) forms of the element are M and M*, respectively, then at equilibrium:-

$$\frac{[M^*_{surface}]}{[M^*_{solution}]} = \frac{[M_{surface}]}{[M_{solution}]}$$

The purpose of these studies was to apply the principles of radioisotopic exchange and investigate

(i) the kinetics of exchange reactions between native and radioactive manganese,

(ii) those fractions of soil manganese in equilibrium with solution manganese,

(iii) the effects of physical processes such as drying and heating on the distribution of native and radioisotopic manganese between the various soil fractions,

(iv) the measurement of isotopically exchangeable manganese using chemical equilibration and plant uptake.

4.2 MATERIALS AND METHODS

4.2.1 Radioactive Manganese

The manganese radioisotope, as carrier-free ^{54}Mn ($MnCl_2$), was obtained from the Radiochemical Centre, Amersham, England. The isotope is a high energy gamma emitter with a half-life >300 days. One hundred microcurie (μCi) amounts were diluted with 0.05M $CaCl_2$ to obtain activity concentrations of 1.0 μCi ml^{-1}. (A salt solution was used to dilute the isotope in order to inhibit absorption of ^{54}Mn on the sides of volumetric vessels).

4.2.2 Radioactivity Measurements

Activity of ^{54}Mn was assayed by counting in a NaI well-type crystal detector connected to a single-channel analyser (Panax Reigate Series) and comparing the activity with ^{54}Mn standards counted under the same conditions. To reduce the relative standard deviation to 1% for any one measured value the samples were counted for 10,000 recorded disintegrations. The counting rate (counts s^{-1}) for the sample was determined by subtracting a background count rate from the observed count rate.

4.2.3. Soil Studies

4.2.3.1. Soils

Ten soils exhibiting a wide range of characteristics (see Table 4.1) were used in these studies. Soils 2 and 10 originated from England; the remaining soils were collected in Eastern Scotland. Soils 3 and 4 were chosen because of their differences in organic matter amendments and cropping history. The relatively high organic matter content of the former soil, which was used for intensive vegetable production, arose from heavy applications of brewery industry wastes over the years; soil 4 had been used for cereals and other field crops and had not received large additions of organic matter. Both soils 1 and 7 were obtained from the field trial sites in 1979. Soil 6 (from the same series as soil 7) came from land used for rough grazing and consisted of the top 10 cm mineral horizon just below the peat layer.

4.2.3.2. Soil pH, organic matter and particle size analysis

Methods for these soil parameters have been previously described

Table 4.1 Characteristics of the soils used in the radioisotope incorporation studies

Soil No.	Soil Series	pH(H_2O)	Organic Matter (%)	Total Mn ($\mu g\ g^{-1}$)	Sand (%)	Silt (%)	Clay (%)	Soil Type*
1	Darvel	5.55	2.71	388	78	12	10	Brown forest soil
2	Denchworth	5.85	7.59	438	31	19	50	Surface water gley
3	Dreghorn 1	6.80	8.44	1275	66	24	10	Brown forest soil
4	Dreghorn 2	5.91	4.10	462	71	15	14	" " "
5	Giffnock	5.48	4.77	60	48	30	22	Non calcareous gley
6	Hexpath 1	3.70	16.50	49	66	21	13	Humus iron Podzol
7	Hexpath 2	6.68	7.41	400	81	12	7	" " "
8	Macmerry	6.25	5.67	525	51	26	23	Brown calcareous and brown forest soil
9	Stirling	5.90	6.44	322	9	58	33	Gleyed warp
10	Worcester	6.29	4.59	712	48	26	26	Gleyed brown earth

* Source - Soil Survey of Scotland

in section 3.2.1.

4.2.3.3. Equilibrium procedures for the determination of isotopically exchangeable manganese (Mn_E)

Two methods were employed to study isotopically exchangeable manganese in soils using $CaCl_2$ as the equilibrating medium. All equilibration experiments were carried out on duplicate 20 g soil samples and soil suspensions were shaken at 20±2^{0}C on an orbital shaker at 110 rpm.

Mn_E-$CaCl_2$

Method 1 was based on that used by McAuliffe *et al* (1948) for P and by Tiller *et al* (1969, 1972) for Co and Zn in which chemical equilibrium is achieved prior to the addition of isotopic tracer. Soil samples were added to 200 ml 0.05M $CaCl_2$ with 0.5 ml chloroform (to minimise microbial activity) in 380 ml capacity polypropylene centrifuge bottles and shaken continuously for 5 days. Preliminary experiments showed that chemical equilibrium was achieved at this time. Also, chloroform was found to have no effect on soil manganese. As a further precautionary measure, the centrifuge bottles were opened daily for several minutes to minimise the decrease in aeration that could occur in sealed containers. After equilibration was achieved between the native forms of soil and solution manganese the suspensions were centrifuged at 3000 rpm for 5 minutes. A tracer quantity (1.0 µCi) of ^{54}Mn was added and shaking immediately resumed.

Method 2 was based on that of Scott Russell *et al* (1954) for P and Tiller *et al* (1969, 1972) for Co and Zn in which tracer was added to the soil-$CaCl_2$ system and chemical and isotopic equilibrium achieved simultaneously. Soil samples were added to 200 ml 0.05M $CaCl_2$ with 0.5 ml chloroform and centrifuged at 3000 rpm for 5 minutes. A tracer quantity (1.0 µCi) of ^{54}Mn was added and shaking immediately begun.

Mn_E-DTPA

The determination of Mn_E values using diethylenetriaminepentaacetic acid (DTPA) solution (Lindsay and Norvell, 1969) was also carried out in a similar manner to Method 1 except that the shaking time after ^{54}Mn introduction was limited to 48 hours.

4.2.3.4. The rate of disappearance of ^{54}Mn from soil solution

At specific time intervals the soil suspensions prepared according to Methods 1 and 2 were centrifuged at 3000 rpm for 5 minutes. A 5 ml aliquot from the supernatant was pipetted into a 10 ml vial and the activity of ^{54}Mn remaining in the solution phase was determined. The contents of the vial were returned to the centrifuge bottles and shaking was immediately resumed. The process was repeated intermittently until the establishment of equilibrium between the radioactive and native manganese, at which time the concentration of native manganese was determined in a small quantity of the supernatant.

4.2.3.5. Fractionation of ^{54}Mn and native soil manganese

Fractionation schemes are invaluable in studying the soil chemistry of micronutrients and provide useful information on elemental retention, or availability for plant uptake. Comprehensive sequential schemes have been employed for other micronutrients, e.g. for soil copper (McLaren and Crawford, 1973) but few attempts have been made to quantitatively determine the various fractions of manganese that can exist in available, potentially available and unavailable forms. Furthermore, the effects of changing physical parameters of the soil on manganese distribution between different fractions have been given scant attention. The use of radioactive tracers is a sensitive tool for assessing the fate of manganese in soil - either added as a fertiliser nutrient, or arising from weathering of organic or inorganic constituents.

Procedure

The isotopically equilibrated soils of Method 1 ($CaCl_2$-equilibrated) were rapidly filtered by suction through 9.0 cm GF/A glass microfibre filter paper. Fifty ml of 0.01M $CaCl_2$ was used to rinse the soil into the filtering apparatus. The use of the dilute salt solution was necessary to maintain the soil in a flocculated state.

The moist soil pad was mixed thoroughly with a stainless steel spatula and divided into 3 portions. One portion was maintained in the moist state at 1^0C while a second was air-dried for 5 days at

16^{0}C; the final portion was oven-dried for 24 hours at 105^{0}C.

Replicate 2 g samples from each portion were extracted sequentially with 5 different reagents to determine the quantities of native and radioisotopic manganese in a corresponding number of soil fractions. Extractions on the moist, air-dried and oven-dried portions were carried out simultaneously.

As mentioned in the literature, manganese is found in the soil either as a water-soluble ion or bound to various organic and inorganic constituents. These different forms of manganese are separated and defined by various chemical extrants. Some of the solutions and procedures used below have been developed by previous workers, and extract a particular form of manganese. Other reagents are known to react with various soil constituents, and hence it is hoped that associated manganese would be released by them. The fractionation scheme employed in this study is shown in Figure 4.1.

Extract 1 - Water-soluble + 'exchangeable' manganese - extracted by 0.05M $CaCl_2$

Many extractants have been employed to estimate exchangeable manganese and the use of a 0.05M $CaCl_2$ solution is purely arbitrary. However, since $CaCl_2$ solution extracts less organic matter than other extractants frequently used (e.g. ammonium acetate) and has less effect on the normal pH of the soil, it was thought to give a better estimate of that fraction of soil manganese associated with exchange sites. Ghanem *et al* (1971) found 70-95% of ammonium acetate extractable manganese to be in a chelated form.

Twenty ml of reagent were added to 2 g soil and shaken for 2 hours at 22^{0}C. The suspension was then centrifuged at 7000 rpm for 10 minutes and a portion of the supernatant (5 ml) was kept for determinations of active and native soil manganese. The remaining supernatant was discarded and the soil was washed twice with two 20 ml aliquots of 0.01M $CaCl_2$. (Calcium chloride was used as the washing medium in order to maintain the soil in a flocculated condition).

Extract 2 - Organically bound maganese - extracted by ethylenediamine tetra-acetic acid (EDTA)

The washed residue was extracted with 20 ml of EDTA solution for 2 hours according to the method of Beckwith (1955a). Both

active and native manganese were determined in the supernatant after centrifuging, and the residue washed twice with 0.01M $CaCl_2$. Preliminary investigations in the laboratory confirmed Beckwith's findings, by showing that the reagent had a minimal solubilising effect on oxide materials (commercial grade MnO_2 and Mn_3O_4 as well as a laboratory-prepared oxide, K14, birnessite*). Since the reagent is specific for divalent manganese only, it was assumed that any Mn(II) remaining in the soil following extraction in $CaCl_2$ (as outlined above for Extract 1) would be associated with the organic matter fraction. Another reagent thought to be specific for organically bound nutrients - pyrophosphate (McLaren and Crawford, 1973) - was found to attack the oxide materials, solubilising large quantities of manganese, and could therefore not be used in this investigation.

Extract 3 - Easily reducible manganese minerals (oxides) - extractable by ammonium acetate and hydroquinone (AA-Hq)

The washed residue was shaken for 8 hours with 20 ml of a buffered solution of neutral, normal ammonium acetate containing 0.2% hydroquinone. The suspensions were centrifuged and the supernatant collected. An additional 10 ml of the solution were used to wash the soil and combined with the initial supernatant (Leeper, 1934). Active and native soil manganese were determined in the combined extracts. The residue was then washed in the usual manner with 0.01M $CaCl_2$.

Extract 4 - Resistant manganese minerals (oxides) - extracted by acid oxalate and ultraviolet light (AO-UV)

The procedure was carried out according to the method of McLaren and Crawford (1973). The residue was transferred to a 100 mm evaporating basin and evaoporated to dryness on a boiling water bath. One hundred ml of acid oxalate reagent (0.1M oxalic acid, 0.175M ammonium oxalate at pH 3.25) were added. (Le Riche and Weir (1963) found that this extractant dissolved 84 to 95% of the soil manganese). The residue was then extracted on the water bath for $2\frac{1}{2}$ hours under ultraviolet light; distilled water was periodically added to maintain

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*prepared according to McKenzie (1970)

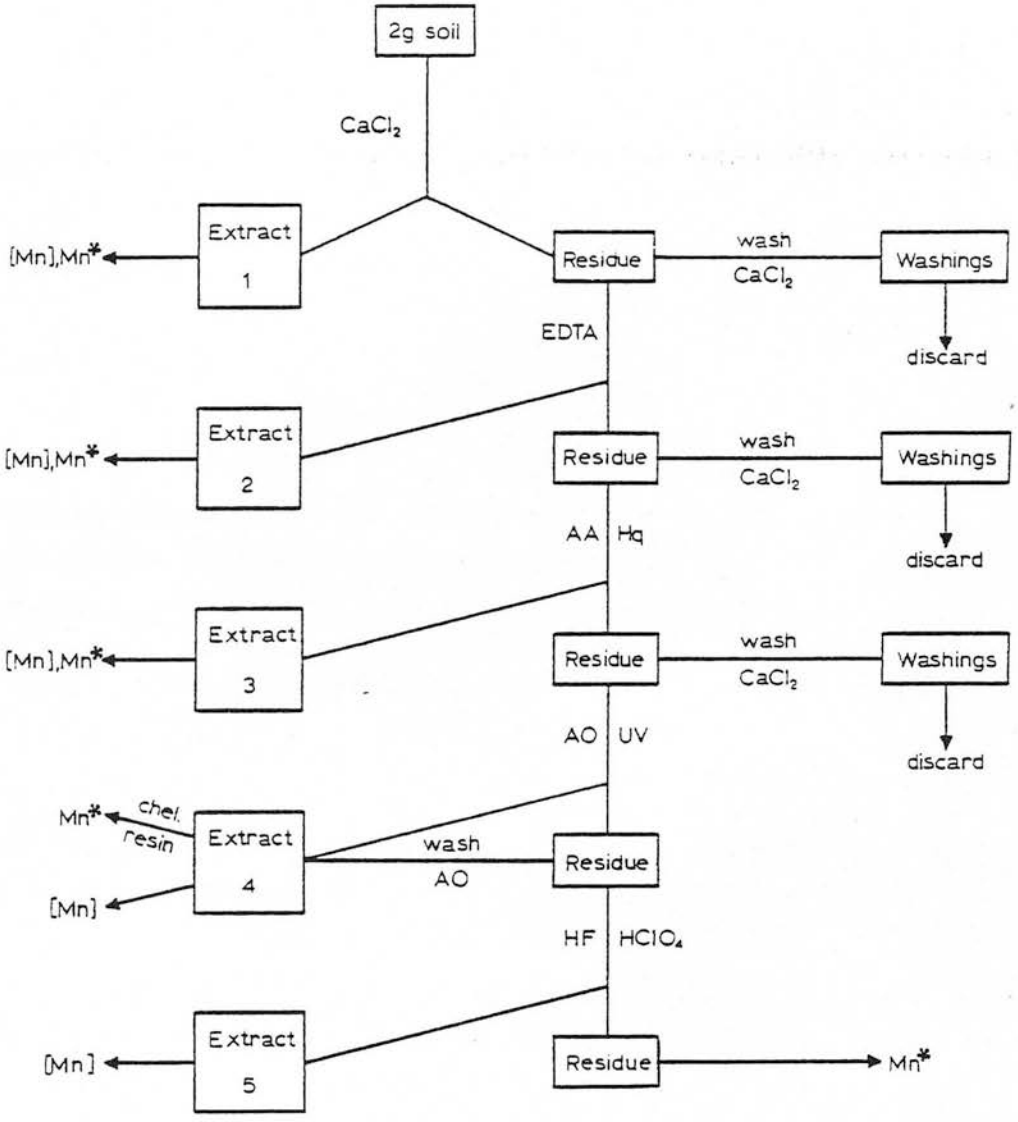

Figure 4.1 Flow diagram for the fractionation of native and radioactive manganese from isotopically equilibrated soil

[Mn] - concentration of native manganese

Mn* - radioactive manganese

AA, Hg - ammonium acetate, hydroquinone

AO, UV - acid oxalate, ultraviolet light

the liquid level in the basin. The entire contents of the basin were then filtered through Whatman No. 42 filter paper. The residues were washed with a further 50 ml of the acid oxalate reagent and the combined filtrates were brought to the 200 ml mark in volumetric flasks. The solution was then analysed for native manganese.

Concentration of ^{54}Mn from the acid oxalate extract

Due to the large dilution of the oxide fraction it was necessary to concentrate the ^{54}Mn present in the extract to ensure high enough counting rates for accurate determinations. One hundred ml of the acid oxalate filtrate were adjusted to pH 7 with a solution of NH_3 (33% w/w). Two grams of Bio Rad analytical grade chelating resin (Chelex 100) were added and the solution was shaken overnight. The resin was filtered through Whatman No.42 filter paper, counted, and compared with ^{54}Mn standards prepared in a similar manner. Isotope recovery was >95% using this method.

Extract 5 - Residual manganese (native) - extracted by hydrofluoric and perchloric acids ($HF-HClO_4$).

The residues from the acid oxalate extraction were dried overnight at 105^0C and finely ground (<0.1 mm). Subsamples (0.1 g) were digested with HF and $HClO_4$ acids according to the method outlined by Pratt (1965). This procedure is known to dissolve silicate minerals thereby releasing any manganese bound within them. (Total manganese in the soils (see Table 4.1) was also determined using this technique).

Residual manganese (radioisotopic)

A 1.0 g sample of the residue was weighed into a counting vial and the remaining activity determined by comparison with standards which were prepared by 'spiking' similarly treated inactive soil with known amounts of activity.

4.2.4. Plant Studies

The materials and methods for these investigations have been previously outlined in Section 3.5.6.

4.3 RESULTS AND DISCUSSION

4.3.1 Equilibration of ^{54}Mn in soil suspensions

Plots of the proportion of ^{54}Mn remaining in solution as a function of time (employing Method No. 1 described in Section 4.2.3.3) are shown in Figures 4.2 and 4.3 for soils 1-5 and 6-10 respectively. The most rapid disappearance of ^{54}Mn occurred within the first 30 minutes after its addition to the soil-$CaCl_2$ system. For most of the soils, 60-90% of the total incorporation of the radioisotope took place prior to 3 hours; thereafter the rate of isotopic incorporation decreased.

The overall time period for the establishment of isotopic equilibrium varied considerably between soils. Equilibrium conditions were achieved within 30 minutes for soils 5 and 6, over 80 hours for soil No. 7, and within 24-48 hours for the remainder. Equilibrium values of solution activity, once established did not deviate significantly. The maximum variations observed (±1-2%) were within experimental errors. Thus, there appeared to be no involvement of microbial activity or other extraneous factors influencing the equilibrium values over the shaking period.

The total quantity of ^{54}Mn taken up by the soil at the termination of the shaking period varied markedly between soils. Isotope incorporation ranged from approximately 5% for soil 5 to over 80% for soil 10 and was significantly correlated with pH ($r = -0.79$, $p<0.01$). This agrees with the findings of many other workers (e.g. Page, 1962) concerning the effect of pH on solution manganese. Organic matter content on the other hand had little effect on the total radioisotopic incorporation.

4.3.2. The Forms of Soil Manganese in Equilibrium with Solution Manganese

On replotting the data presented in Figures 4.2 and 4.3 as the logarithms of solution activities versus time, curvilinear plots were initially obtained (see example in Appendix C.1). Since isotope exchange reactions follow first-order reaction kinetics (Mackay, 1938), this may indicate that the disappearance of ^{54}Mn from solution involved a number of exchange reactions occurring simultaneously between the active and native forms of the element.

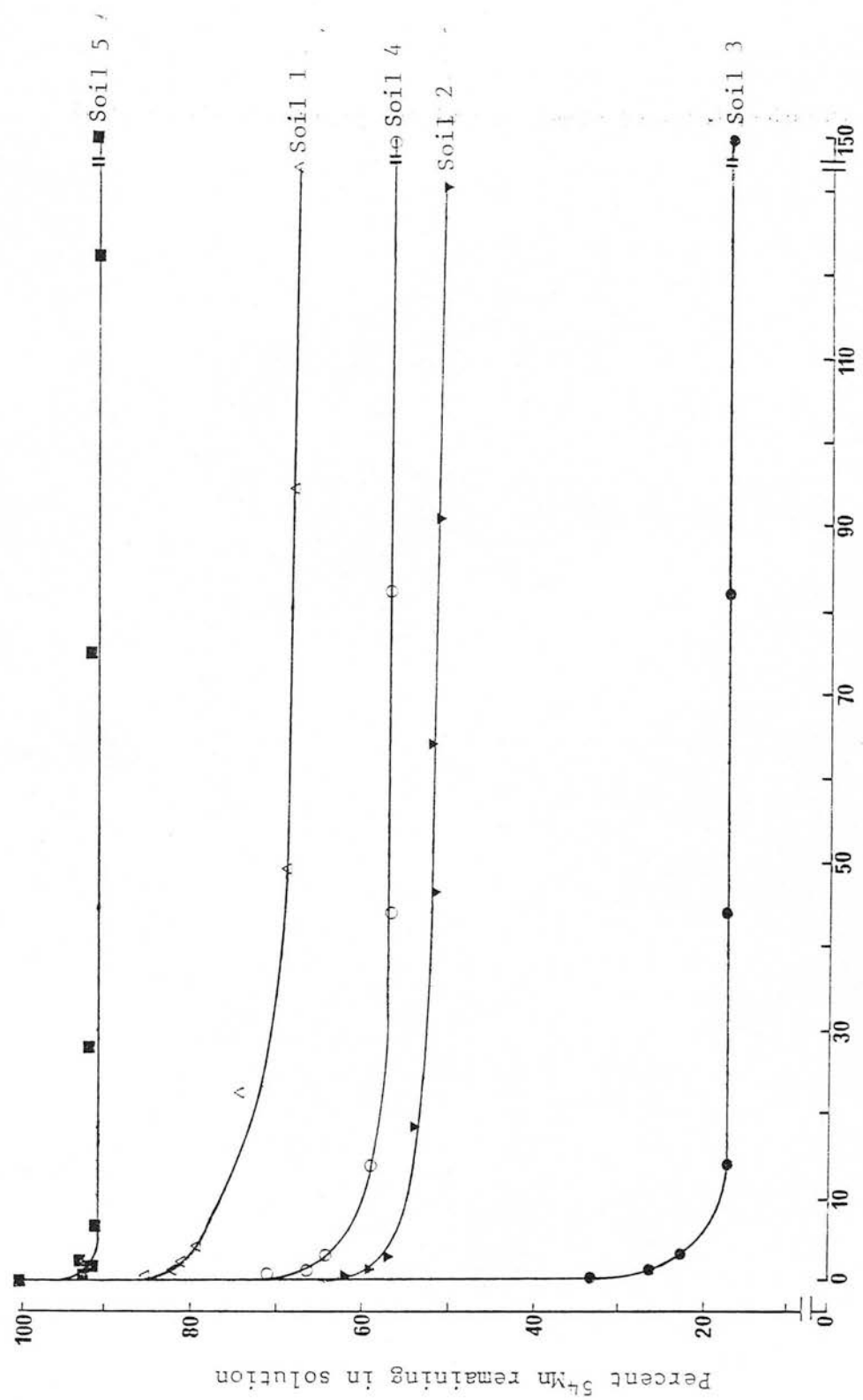

Figure 4.2 The percentage of activity of ^{54}Mn remaining in solution with time for soils 1-5

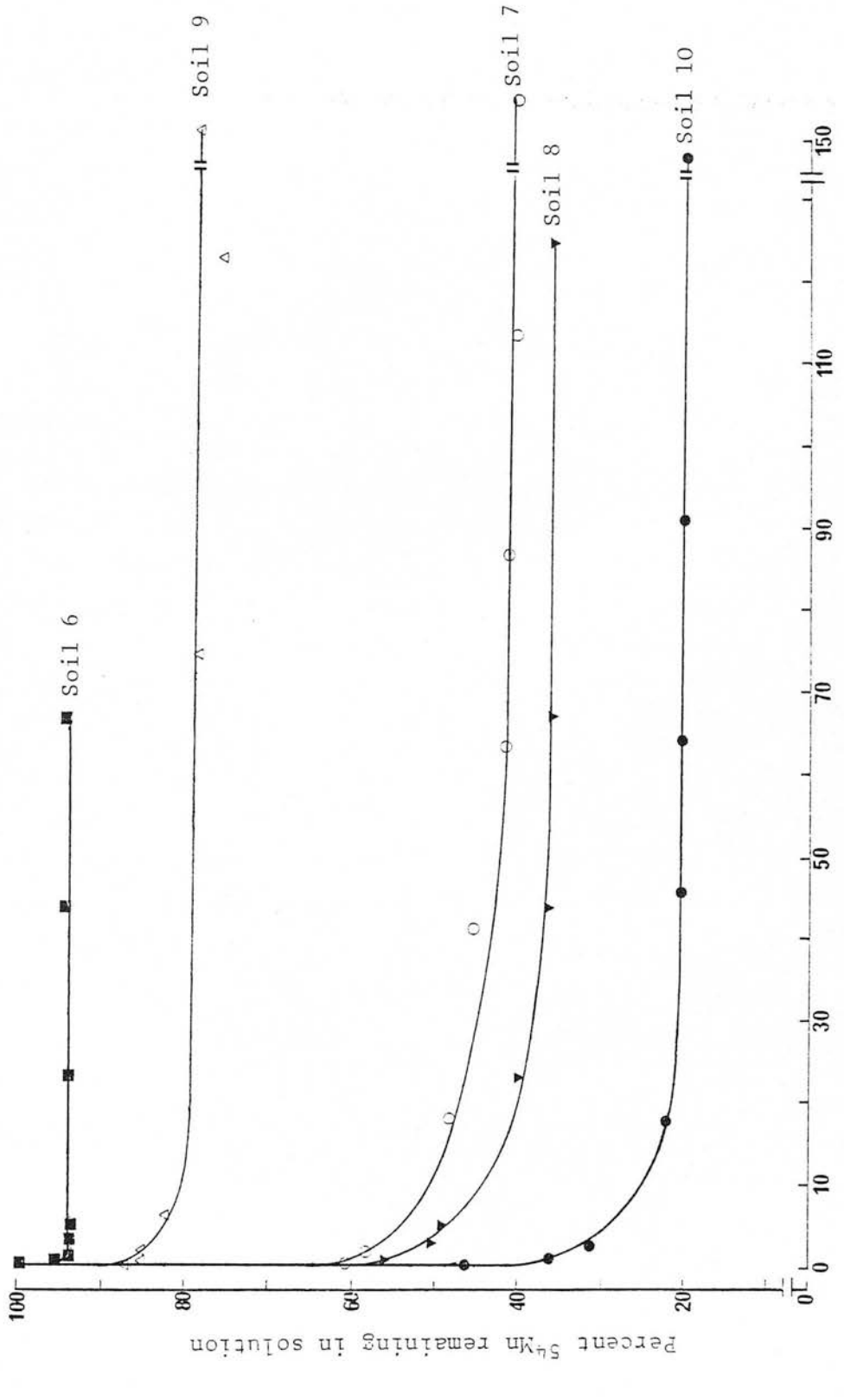

Figure 4.3 The percentage of activity of ^{54}Mn remaining in solution with time for soils 6-10

These observations are similar to those of Weir and Miller (1962) and Reid and Miller (1963) who reported that at least two first-order exchange reactions occurred between soil and solution manganese. The authors thought that these reactions corresponded to different chemical forms of manganese in the soil with differing surface properties.

Table 4.2 shows data representing the native and associated radioactive manganese from the fractionation of the moist soils prepared according to the procedure described in Section 4.2.3.5. The quantity of ^{54}Mn recovered from each fraction was calculated as the percentage of the total activity incorporated in the soil at isotopic equilibrium. The relative distribution of ^{54}Mn in each fraction as well as the non-recoverable portion is also shown in Figure 4.4.

Total recovery of the isotope from the various fractions was usually well over 90%. Failure to achieve complete recovery is not surprising given the complexity of the fractionation scheme. The initial rinse of the soil into the filtering apparatus with 0.01M $CaCl_2$ probably leached some very weakly incorporated native manganese and associated ^{54}Mn. The summation of the experimentally determined native manganese content of each fraction usually exceeded the value obtained for a single determination of native manganese in the whole soil (compare the total manganese concentration in Table 4.1 with the sum total of manganese removed in the individual soil fractions in Table 4.2). Large dilution factors arising from sample preparation for atomic absorption analysis, most notably for the resistant and residual fractions, probably accounted for this.

Radioactive manganese labelled all the soil manganese fractions determined. Approximately 80% of the incorporated isotope was extracted from the first three fractions (water-soluble + exchangeable, organically bound and easily reducible) in most of the soils. The relative distribution of ^{54}Mn with each soil fraction varied greatly between the soils. Generally the easily reducible fraction contained the highest percentage of activity. With the exception of soils 5 and 6, 25-55% of the isotope was extracted from this fraction alone. Extractions of ^{54}Mn from the water-soluble + exchangeable manganese fractions varied from 6 to 85% of the total incorporated, while values for organically associated ^{54}Mn were generally within

Table 4.2 The fractionation of radioisotopic and native manganese from the isotopically equilibrated soils

Soil No.	Water-soluble + exchangeable		Organically bound		Easily reducible		Resistant		Residual		Loss ^{54}Mn (%)	Sum total native Mn of individual soil fractions ($\mu g\ g^{-1}$)*
	^{54}Mn (%)	Native Mn ($\mu g\ g^{-1}$)	^{54}Mn (%)	Native Mn ($\mu g\ g^{-1}$)	^{54}Mn (%)	Native Mn ($\mu g\ g^{-1}$)	^{54}Mn (%)	Native Mn ($\mu g\ g^{-1}$)	^{54}Mn (%)	Native Mn ($\mu g\ g^{-1}$)		
1	22.0	4.4	7.2	1.3	52.3	142	12.6	210	1.2	55	4.7	446
2	31.4	11.4	19.3	6.1	24.5	84	11.9	235	2.4	160	10.5	536
3	8.4	11.2	11.0	10.8	55.4	436	19.2	616	3.0	196	3.0	1296
4	31.7	14.2	11.1	3.7	36.8	105	16.1	250	2.4	51	1.9	484
5	76.3	0.7	9.4	0.1	2.7	0.4	5.4	11.0	1.4	42	4.8	67
6	88.0	0.3	T	T	T	0.4	T	5.2	T	32	12.0	39
7 †	-	-	45.9	3.4	27.3	49.2	12.1	151	5.8	186	8.9	396
8	18.9	4.1	10.6	1.9	53.3	126	13.8	328	2.7	106	0.7	578
9	30.2	3.9	4.7	0.1	41.0	89	13.2	168	3.3	102	7.6	400
10	6.3	6.4	14.8	16.5	54.7	270	17.1	245	3.0	180	4.1	746

* - Included in the sum total is the concentration of soil manganese extracted by the 0.05M $CaCl_2$ solution at the termination of the isotopic equilibration procedure of Method 1.

T - Trace (for ^{54}Mn, count rate was only slightly above background; for native manganese, concentrations were less than 0.05 $\mu g\ g^{-1}$).

† - Water-soluble + exchangeable manganese was not fractionated separately for soil No.7 and is assumed to be included in the organically bound fraction.

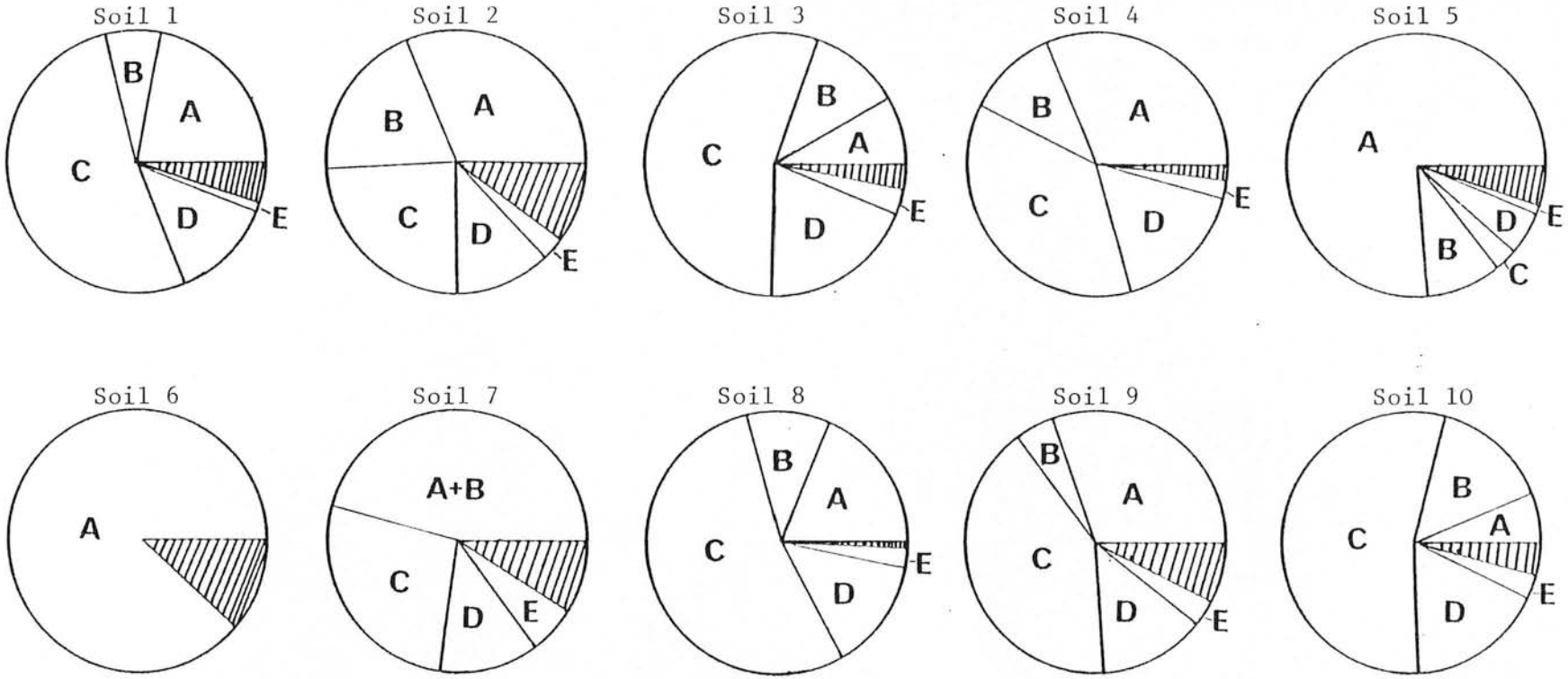

Figure 4.4 Relative activity of ^{54}Mn (as percentages of total incorporated) resulting from the fractionation of the ten soils.

A - Water-soluble + exchangeable; B - Organically bound; C - Easily reducible; D - Resistant,

E - Residual; ◁ - non recoverable.

the 5 to 20% range.

Surprisingly, substantial amounts of ^{54}Mn were removed from the resistant oxides and residual manganese fractions; from 12 to 19% and from 1 to 5% of total isotope incorporated were recovered from the former and latter fractions respectively. The work of Sims *et al* (1979), whose paper appeared after this study began, contains similar findings. Other workers (Weir and Miller 1962; Reid and Miller 1963), however, found virtually 100% of equilibrated ^{54}Mn was extractable with pyrophosphate or $ZnSO_4$ and hydroquinone solutions - much weaker solvents than acid oxalate and hydrofluoric-perchloric acid solutions used in the present study. These conflicting results cannot be satisfactorily resolved given the limited amount of work of this nature reported in the literature.

4.3.3. Specific Activity

The specific activity of manganese (the amount of ^{54}Mn per unit weight of native manganese) indicating the reactivity of the various fractions is presented in Table 4.3. Results show the specific activity of water-soluble + exchangeable and organically bound manganese far exceeded that of easily reducible, resistant and residual manganese. This may imply that a greater proportion of native manganese atoms in the water-soluble + exchangeable and organically bound fractions participated in isotopic exchange reactions. However, care must be exercised in such interpretation since it cannot be proven positively that the incorporation of the radioisotope in each fraction resulted from simple isotopic exchange as opposed to surface adsorption of the added tracer. The ease with which ^{54}Mn can exchange with native manganese will be dependent on the energy required to desorb the element from the different sites on surfaces to which it is attached. Manganese simply bound electrostatically on the soil exchange complex would be expected to exchange quite readily with the radioisotope; exchange in the organic fraction would be dependent on the nature of the linkages between the native element and the organic molecules. Exchange in oxide minerals cannot be ruled out since the substitution of lower valency manganese for Mn(IV) commonly occurs in higher manganese oxides (Wadsley, 1952). However, it would seem unlikely that the detection of ^{54}Mn in the residual fraction (extracted with hydrofluoric and perchloric

Table 4.3 The specific activity of manganese in the 5 soil fractions*

Soil Number	Water-soluble + exchangeable	Organically bound	Easily reducible	Resistant	Residual
1	0.83	0.92	0.061	0.010	0.004
2	0.69	0.79	0.073	0.013	0.004
3	0.31	0.43	0.053	0.013	0.006
4	0.46	0.62	0.073	0.013	0.010
5	3.71	3.50	0.27	0.018	0.001
6	5.18	-	-	-	-
7	†	2.79	0.12	0.017	0.006
8	1.37	1.66	0.13	0.013	0.008
9	0.82	3.85	0.049	0.008	0.003
10	0.39	0.36	0.081	0.028	0.007

* - Values presented are (μCi ^{54}Mn/μg native manganese) x 1000.

† - Specific activity of water-soluble + exchangeable manganese is assumed to be included in the organically bound fraction.

acid) would have resulted from the exchange of the radioisotope with native manganese firmly bound in mineral lattices.

4.3.4. Practical Considerations

As previously stated (Section 4.2.3.5.) the distribution of ^{54}Mn provides an indication of the possible fate of fertiliser manganese applied to soil or manganese released by natural processes. Except for the most acid soils investigated (e.g. 5 and 6) most of the isotope (usually well over 60%) was found to be in non-exchangeable (i.e. not extractable in $CaCl_2$) forms. It is worthwhile to note in this context that Mn(II) ions are known to oxidise rapidly in soils with a pH >5.5 (Bromfield 1958a). Recently, Shuman (1979) found fertiliser manganese mainly associated with the exchangeable and organic matter fractions, but as he used H_2O_2 as the extractant for manganese in the latter fraction, this would, as he himself admitted, also have dissovled any MnO_2 present. The present work shows that the proportion of manganese in the easily reducible oxide fraction is generally high, over a wide range of soil types (Table 4.2 and Figure 4.4), thus it must be concluded that any scheme using H_2O_2 cannot be relied upon to indicate the size of the organically bound fraction and Shuman's results do not therefore, necessarily conflict with those reported here.

4.3.5. Effects of Drying on Radioisotopic and Native Manganese in the Soil Fractions

Results of the distribution of radioactive and native manganese between the different fractions, as affected by the moisture state, are presented in Figure 4.5.

Recoveries of ^{54}Mn ranged from 83% to over 99% with means in excess of 90% for each drying treatment, but no trends in overall recovery between moist, air-dried and oven-dried samples were evident (Table 4.4). However, agreement between duplicate determinations of radioisotope extracted from any one fraction was very good in most instances, regardless of the moisture state of the soil. Therefore, losses of ^{54}Mn were probably random between fractions. It was thus decided that the effects of drying on the distribution of manganese between fractions could best be compared as averages over the range of soils. Values are presented in Table 4.5 as the percentage of

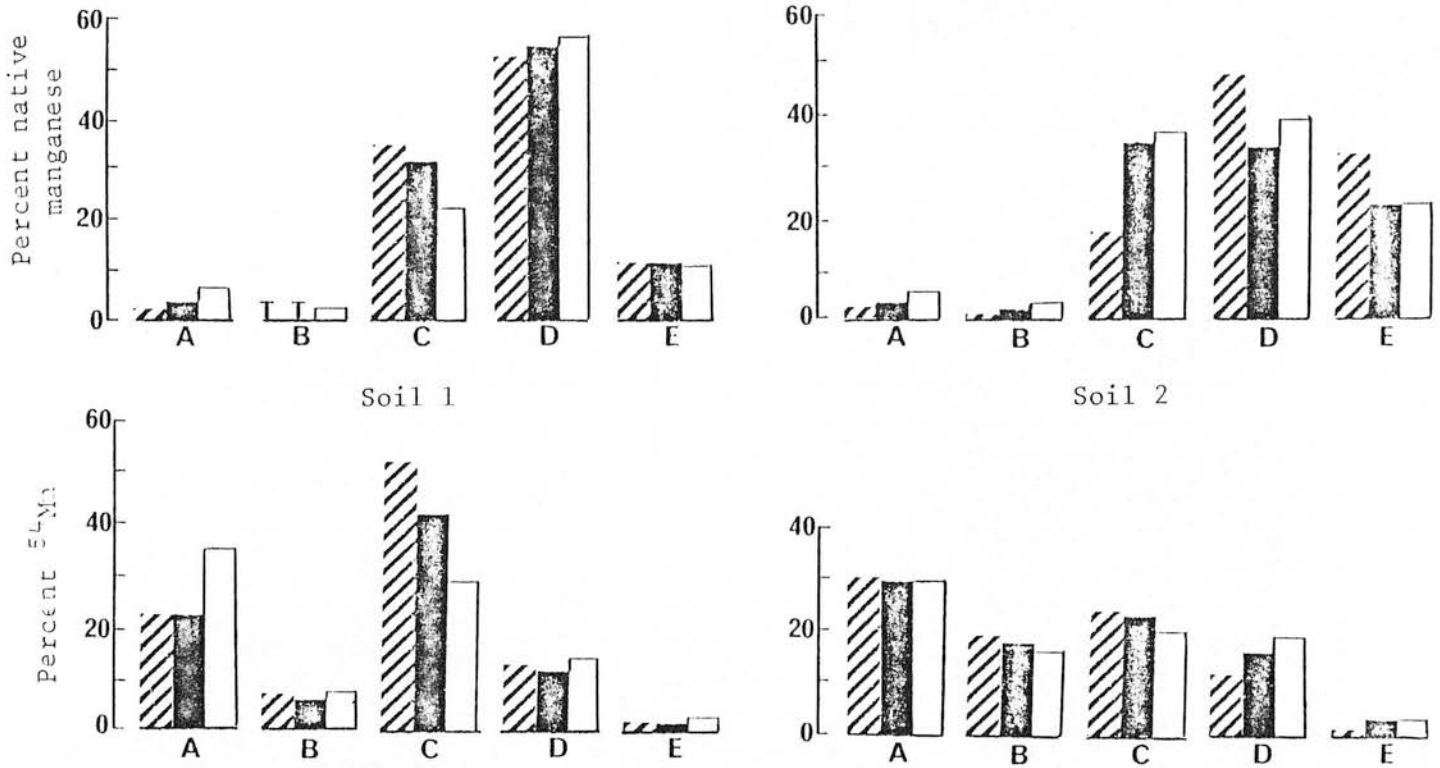

Figure 4.5 Percent native manganese and percent ^{54}Mn in the five soil fractions as affected by drying.

T - trace

A - water-soluble + exchangeable; B - organically bound; C - easily reducible; D - resistant;

E - residual

▨- moist; ■- air-dried; □- oven-dried

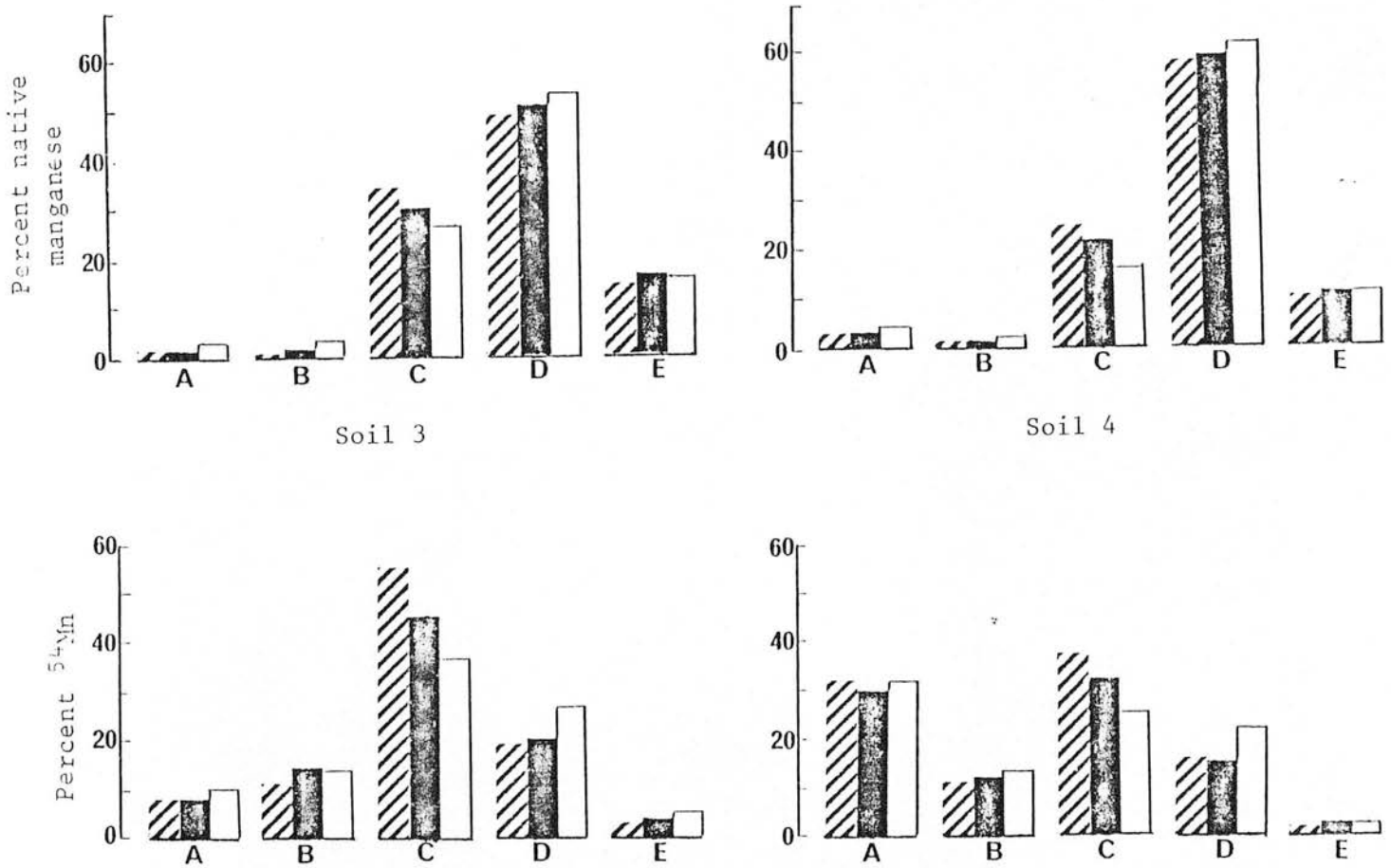

Figure 4.5 continued

A - water-soluble + exchangeable; B - organically bound; C - easily reducible; D - resistant; E - residual

▨- moist; ■- air-dried; □- oven-dried

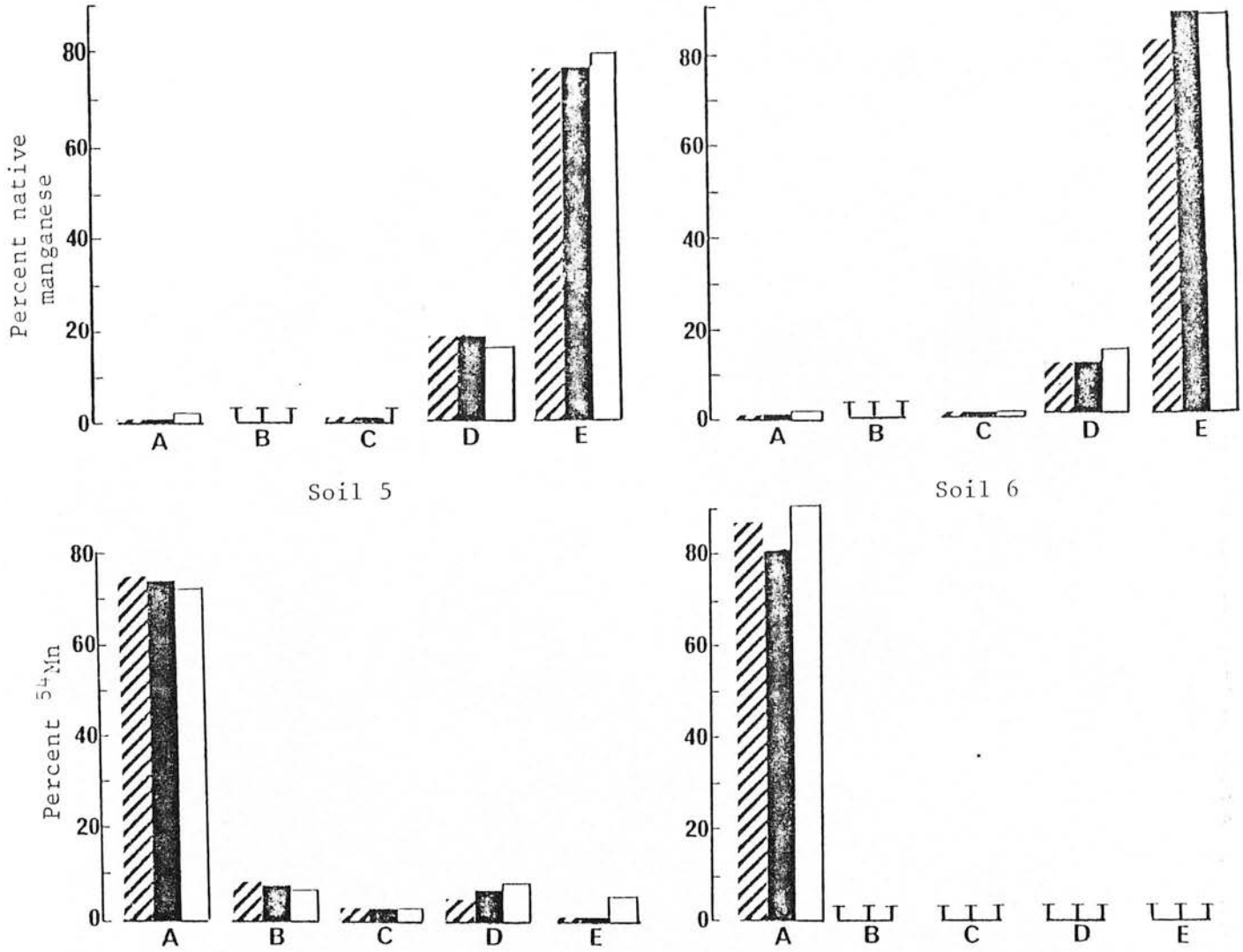

Figure 4.5 continued T - trace

A - water-soluble + exchangeable; B - organically bound; C - easily reducible;
D - resistant; E - reducible

▨- moist; ■- air-dried; □- oven-dried

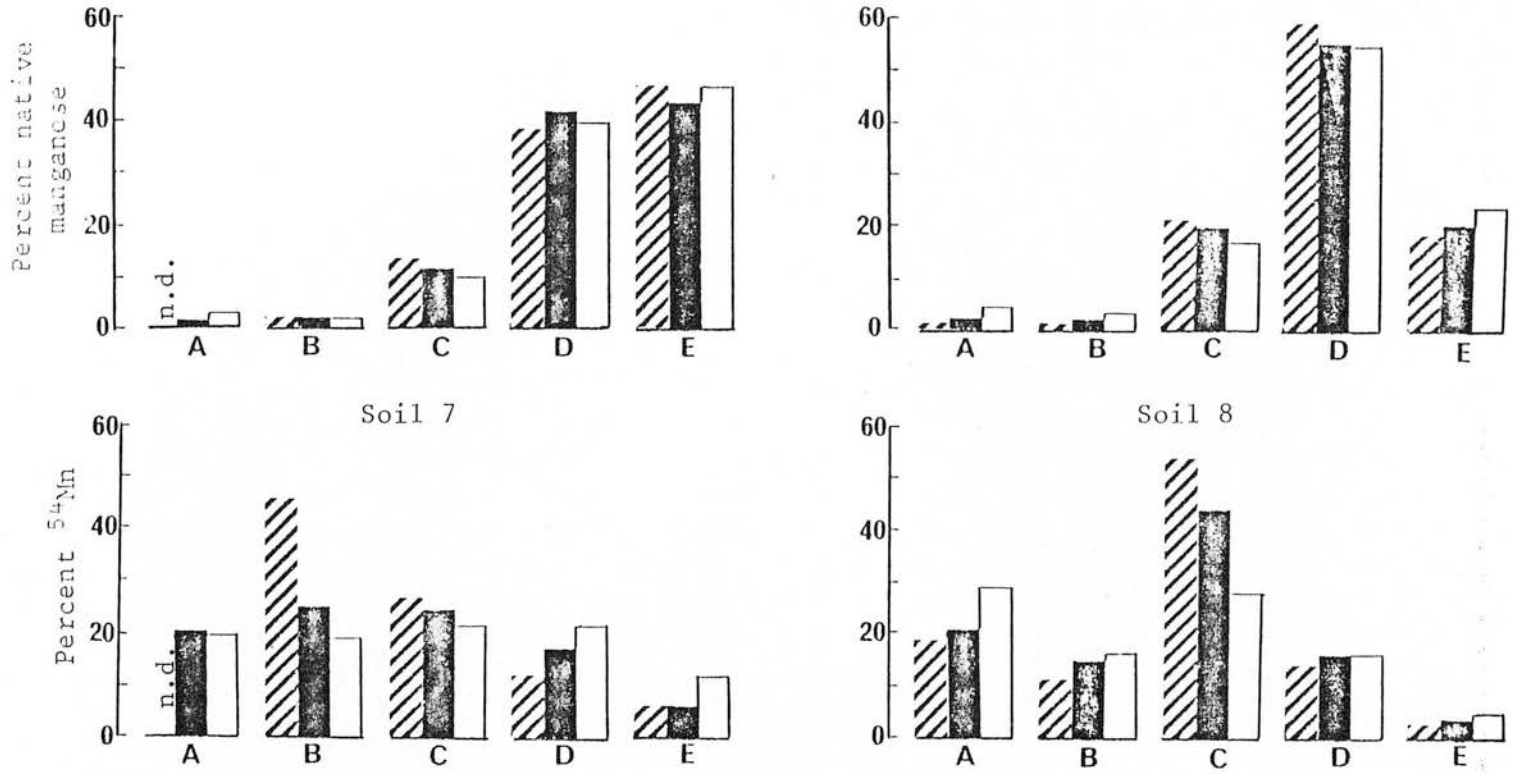

Figure 4.5 continued A - water-soluble + exchangeable; B - organically bound; C - easily reducible
D - resistant; E - residual
n.d. - not determined
▨- moist; ■- air-dried; □- oven-dried

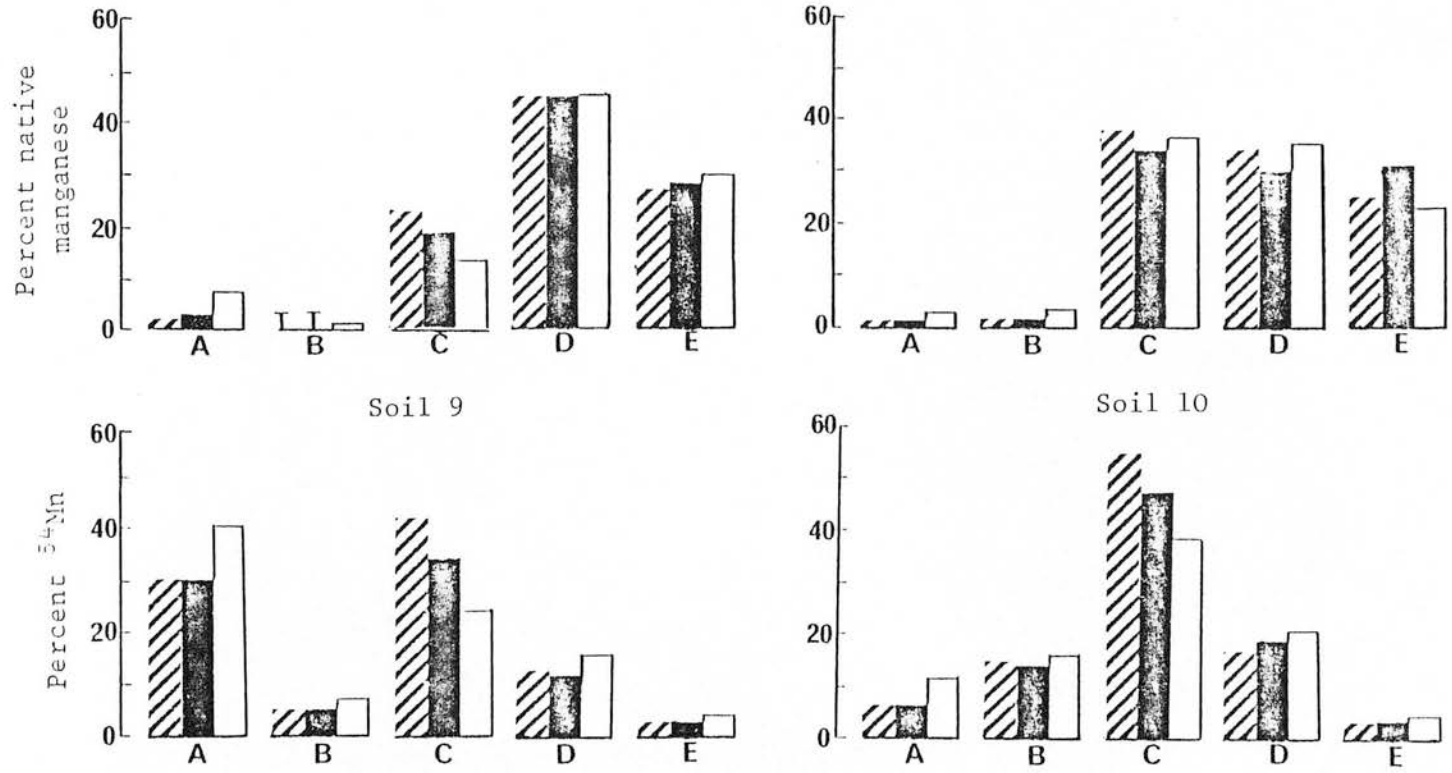

Figure 4.5 continued T - trace

A - water-soluble + exchangeable; B - organically bound; C - easily reducible; D - resistant; E - residual

▨-moist; ■- air-dried; □- oven-dried

Table 4.4 Per cent recovery of isotope from the fractionation of the moist, air-dried and oven-dried soils

Soil	Per Cent Recovery ^{54}Mn		
	Moist	Air-dried	Oven-dried
1	95.3	83.3	86.4
2	89.5	89.9	87.8
3	97.0	90.9	90.2
4	98.1	92.1	96.5
5	95.2	95.6	98.5
6	88	83	97
7	91.1	93.4	94.1
8	99.3	99.0	94.4
9	92.4	84.0	89.8
10	95.9	89.7	91.2
Mean:	94.2	90.5	92.6

Table 4.5 Effect of three moisture regimes on incorporated ^{54}Mn and native manganese in the five soil fractions (expressed as percentages of the total)*

Manganese	Fraction	Treatment		
		Moist	Air-dried	Oven-dried
Radioisotopic	Water-soluble + exchangeable	28.2	27.8	32.3
	Organically bound	11.2	11.2	11.9
	Easily reducible	40.1	33.4	25.3
	Resistant	13.6	14.8	18.1
	Residual	2.4	3.0	4.2
Native	Water-soluble + exchangeable	1.4	1.7	4.2
	Organically bound	0.8	1.0	1.8
	Easily reducible	24.4	24.9	21.4
	Resistant	45.8	43.9	44.9
	Residual	27.8	28.5	27.6

* Each value represents the mean of 8 soils.

the total recovered for ^{54}Mn and native manganese, respectively. Omitted from the calculations were soils 6 and 7. Soil 6 differed greatly from the others in being a very acid (pH 3.60) hill soil and Figure 4.5 shows a very different distribution of manganese between the fractions from the general picture. Soil 7 (moist) was not extracted for separate determinations of water-soluble + exchangeable manganese and the organically bound fraction.

In the easily reducible oxide fraction, ^{54}Mn showed a large reduction on drying which was accompanied by a smaller reduction of the native manganese (Table 4.5). In contrast, the water-soluble + exchangeable and organically bound fractions showed 3-fold and approximately 2-fold increases in native manganese respectively which were accompanied by much smaller relative changes in ^{54}Mn.

Net gains of ^{54}Mn were observed in the resistant and residual manganese fractions as the moist soils were dried, presumably due to occlusion or oxidation of ^{54}Mn in these fractions. Inspection of Figure 4.5 shows this to be the case for all the soils. Native manganese appeared to be little affected by the drying treatments, but the complexity of the fractionation scheme and the large dilution factors employed could be responsible.

It is not surprising that ^{54}Mn would be more affected by drying than native manganese because the radioisotope is inevitably concentrated on or near surfaces whereas much of the stable element is contained within minerals. However, trends of ^{54}Mn and native manganese reinforce each other.

Although oven-drying is a very artificial process, the results are no more drastic than prolonged air-drying. Figure 4.5 emphasises this observation. Furthermore, as such a small fraction of total soil manganese is usually available for plant uptake, the 3-fold increase in the water-soluble + exchangeable fractions observed is significant from this point of view.

The increases of manganese noted from the water-soluble + exchangeable and organically bound fractions with drying is in agreement with the work of other investigators e.g. Fujimoto and Sherman (1945), Boken (1952) and Zende (1954). The cause of the

observed increases in exchangeable forms of manganese has been the subject of debate by these and other workers as discussed in Section 2.6.5. The results presented here show that the relative increases in the water-soluble + exchangeable and organically bound fractions upon drying are accompanied by decreases in the easily reducible oxide fraction. This is especially evident with soils 1, 3, 4, 8, and 9 (Figure 4.5). Therefore, the possibility that the increase in water-soluble + exchangeable manganese upon drying may result from the dehydration of oxide material (Fujimoto and Sherman, 1945) or oxide reduction by organic matter (Zende, 1954) cannot be excluded here.

4.3.6. The Manganese Labile Pool

The uptake of a nutrient by the plant occurs from the soil solution, and the rate is dependent on the concentration or intensity factor of the element in the solution phase. This in turn is controlled by the equilibrium with the exchangeable nutrient on the solid phase of the soil, or the capacity factor. As nutrient is removed from solution by the plant, it is replenished from the solid phase, and the total quantity potentially available for plant uptake during the growing season is referred to as the labile pool (Lopez and Graham, 1970).

Radioisotopic techniques have proved to be very useful in determining the labile pools of many plant nutrients in soil. The labile pool is determined by measurements of both the native element and its radioisotope that are exchangeable with each other. Two techniques for the determination of isotopically exchangeable manganese - one involving chemical equilibration and the other involving plant uptake - were investigated as described below.

4.3.6.1. Mn_E Values (chemical equilibration)

If the assumption is made that all ^{54}Mn remains isotopically exchangeable during the equilibrating process and that all forms of manganese contributing to chemical equilibrium have the same specific activities, then at equilibrium:-

$$Mn_E = \frac{\text{wt. of Mn in extract } (\mu g) \div \text{soil wt. } (g)}{\text{fraction of Mn* in extract}} \qquad (1)$$

where Mn = native manganese

Mn* = radioactive manganese

Two alternative methods of determining Mn_E values have been described in section 4.2.3.3. Results obtained by the two methods (Table 4.6) were not significantly different for 8 out of the 10 soils. For the remaining two soils (soils 2 and 9), Method 2 gave values just over 80% of those obtained by Method 1.

Comparative plots of the activity of ^{54}Mn in solution versus time obtained from both the equilibration procedures are shown in Figures 4.6 and 4.7 for soils 1-4 and 7-10 respectively. Both methods yielded identical results for soils 5 and 6 and these results are therefore not shown. For the remainder, the addition of ^{54}Mn prior to chemical equilibrium (Method 2) resulted in its greater adsorption by the soil. This is especially evident for soils 3 and 4, where solution activities were nearly 20% less (i.e. 20% greater incorporation of ^{54}Mn) after 30 minutes of shaking than those obtained utilising Method 1. A net desorption of the radioisotope was noted, however, as shaking continued in soils 2, 3, 4 and 9. Desorption was most marked in soils 3 and 4 and the activity in solution was approaching equilibrium values similar to those obtained in Method 1 by the time shaking ceased. For the remaining soils equilibrated according to Method 2, isotopic equilibrium apparently was established at solution activities several per cent lower than those obtained according to the first method. However, this reduction was cancelled out by lower concentrations of stable manganese in solution, thus giving the generally good agreement between the two sets of results shown in Table 4.6.

The cause of the relatively poor agreement between the methods for soils 2 and 9 is difficult to diagnose. Both methods are subject to certain limitations as discussed by various workers (e.g. Scott Russell *et al*, 1954) in relation to determinations of the labile pools of other plant nutrients.

In utilising Method 1 the possibility arises that chemical equilibrium previously established will be disturbed once the radio-

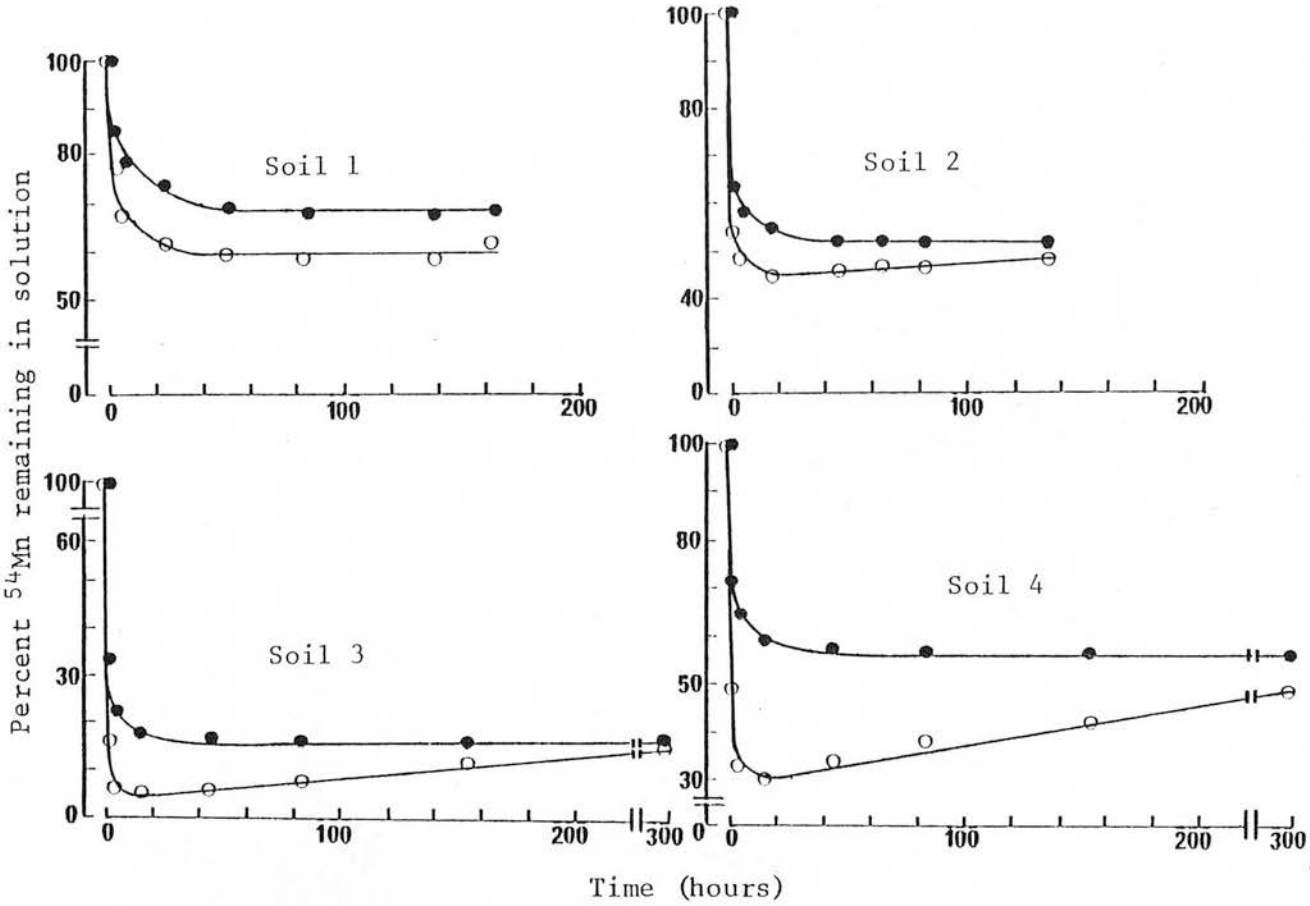

Figure 4.6 Equilibration of ^{54}Mn in soil suspensions

● - Method 1; O - Method 2

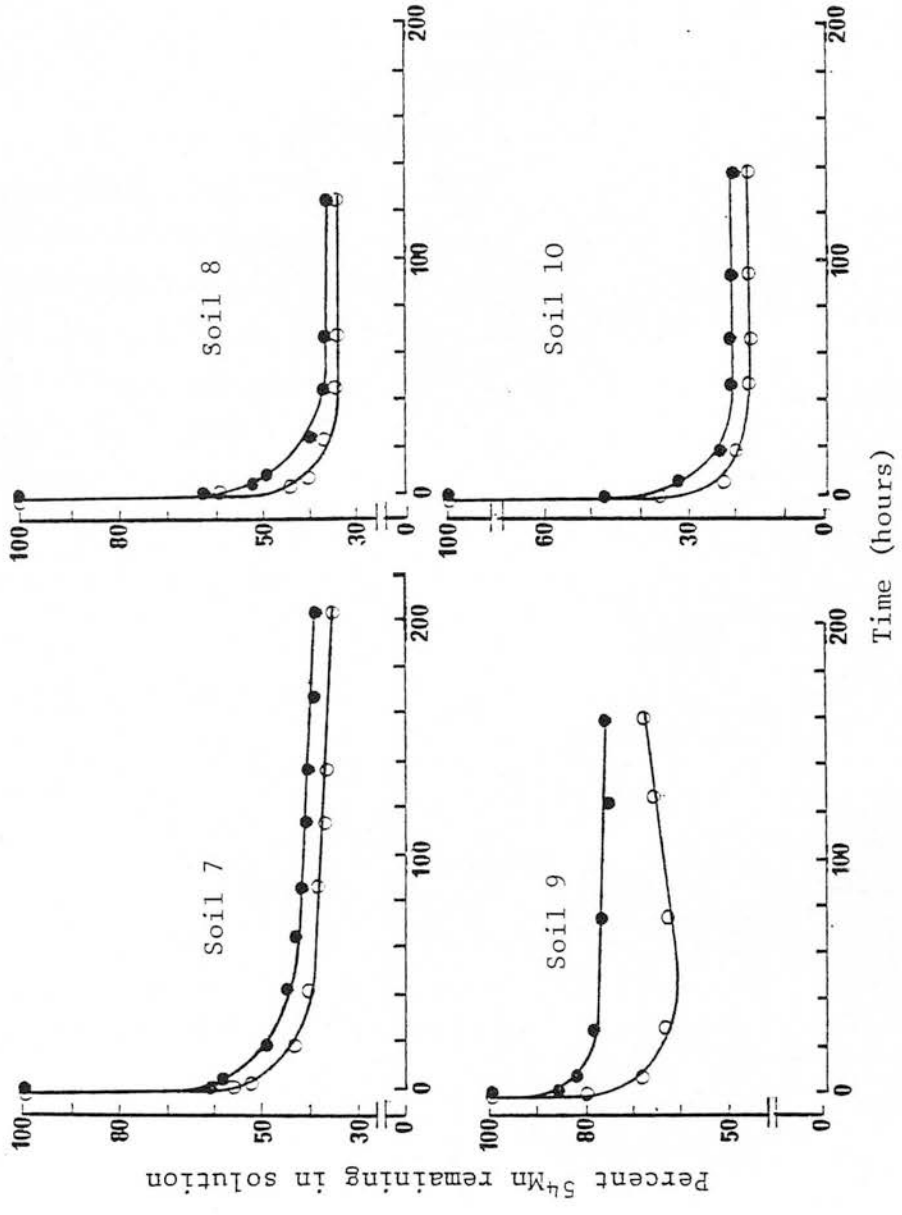

Figure 4.7 Equilibration of ^{54}Mn in soil suspensions

● - Method 1; O - Method 2

isotope is introduced. However, in the present study the small quantity of radioactive manganese (<1 ng) in 1 ml of solution added to such large quantities of soil and $CaCl_2$ (20 g, 200 ml) would have an insignificant effect on equilibrium conditions. If irreversible binding of ^{54}Mn at unoccupied sites occurs when the radioisotope is introduced before chemical equilibrium is achieved (Method 2) then the Mn_E values obtained by the latter method will be greater than those obtained by Method 1; i.e. the reverse of the result reported here.

The extent to which ^{54}Mn could be desorbed from the soil was investigated by the following procedure:-

Soils 2,3,9, and 10 were equilibrated with ^{54}Mn according to the procedure outlined for Method 1. The supernatant was counted for ^{54}Mn activity, discarded and replaced with solution (0.05M $CaCl_2$) containing 250 $\mu g\ g^{-1}$ manganese as a carrier*. The samples were reshaken for 48 hours, after which they were centrifuged and aliquots taken for measurement of solution activity. The supernatant was discarded and replaced with a fresh portion of manganese solution and shaking resumed. Counting of the supernatant followed after a further 48 hour shaking period. This process was repeated four times in total over an 8 day period.

Table 4.7 shows the desorption of ^{54}Mn from the soils following the extraction with each new batch of carrier solution. Activity in the supernatant decreased with each successive extraction and did so proportionately (by approximately half) in soils 2 and 10. Total desorption of the incorporated isotope was accomplished with soil 9 after 3 extractions, while a 4th extraction virtually desorbed all the ^{54}Mn from soil 2. Had further extraction with fresh solution been made of soils 3 and 10, there is no reason to suspect that desorption would not continue, albeit at slower rates.

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*a carrier is defined as the native element in a measurable amount which carries the radioisotope with it through a chemical or physical process (Comar, 1955).

Table 4.6 Comparison of Mn_E values determined according to Methods 1 and 2*

Soil No.	Mn_E ($\mu g\ g^{-1}$)	
	Method 1	Method 2
1	51.1 ± 0.1	52.3†
2	77.8 ± 1.1	64.1 ± 1.7
3	164.8 ± 4.0	165.4 ± 3.5
4	103.1 ± 2.6	98.5 ± 5.8
5	13.5 ± 0.1	13.5 ± 0
6	1.5 ± 0.1	1.5 ± 0
7	16.5 ± 0.1	16.2 ± 0.1
8	30.4 ± 1.2	31.8 ± 1.8
9	47.2 ± 1.2	38.0 ± 0.6
10	135.9 ± 0.5	134.6 ± 1.4

* Mean of duplicate samples.

† Single determination only.

Table 4.7 Desorption of ^{54}Mn from soil using carrier solution

Soil	^{54}Mn adsorbed by soil at equilibrium (μCi)	^{54}Mn desorbed (μCi)				Per Cent ^{54}Mn desorbed
		Manganese solutions				
		Batch 1	Batch 2	Batch 3	Batch 4	
2	1.97	0.99	0.52	0.26	0.13	96.4
3	3.33	1.25	0.79	0.43	0.22	80.8
9	1.37	0.92	0.35	0.13	-	100
10	2.92	1.52	0.72	0.29	0.13	91.1

Since the easily reducible forms of manganese contributed most to radioisotopic incorporation (Table 4.2 and Figure 4.4) it is not surprising that the stable manganese concentration in this fraction was significantly correlated ($r = 0.89$, $p < 0.001$) with Mn_E values. Inclusion of water-soluble + exchangeable and organically bound manganese only marginally improved the correlation ($r = 0.90$).

Mn_E-DTPA

The use of the complexing agent DTPA to predict micronutrient availability in soil has been investigated by several workers in recent years. This reagent is considered to have a similar capacity to form complexes with micronutrient ions to the compounds present in root exudates which are known to dissolve these ions by chelation or complex formation (Bromfield, 1958a, 1958b; Godo and Reisenauer, 1980).

Unlike the previous experiment (in which $CaCl_2$ was used as the extracting medium), checks of the supernatant solution at 0.5, 24 and 48 hours revealed that ^{54}Mn activity remained relatively constant throughout the equilibration period. Most of the added ^{54}Mn (95-100%) was recoverable from the supernatant at all times. Therefore, Mn_E values subsequently calculated (Table 4.8) were virtually based on DTPA-extractable manganese. Comparison of Table 4.8 with Table 4.6 shows that the magnitudes of the labile pools determined with DTPA were much greater than those determined with $CaCl_2$. Since DTPA is a powerful complexing agent, it is not surprising that added ^{54}Mn would be retained in solution and prevented from exchanging with the native soil manganese.

The results are somewhat similar to those of Lopez and Graham (1970). They used a modified DTPA solution for predicting labile pools of micronutrients, and found them to be dependent on the composition of the extractant and the pH of the soil suspension. The ability of DTPA solutions to complex maganese increases with rising pH (Norvell, 1972).

General applicability of isotopic exchange methods involving chemical equilibration

The range of pH values found in the soils used in this study

(with the exception of soil 6) corresponds with that commonly encountered on arable land. At higher pH values however, further chemical and microbiological effects (e.g. oxidation, organic matter complexing) may complicate the application of isotopic exchange methods. Tiller *et al* (1972) found that irreversible fixation of radioisotopic zinc in alkaline soils complicated the validity of Zn_E determinations and more work is needed to investigate whether comparable effects occur for manganese also.

Comparison of Mn_E values with $CaCl_2$-extractable manganese concentrations

It should be kept in mind that when measuring Mn_E values by equilibration of a soil with a solution containing ^{54}Mn, the value obtained will differ with the chemical nature of the solution; this is because different solutions will result in different equilibria being established with the complex mixture of substances in the soil. In the presence of excess Ca^{2+} ions, as is encountered with the 0.05M $CaCl_2$ solution used in the present study, the laws of cation exchange specify that virtually all exchange sites non-specific for manganese would be occupied by Ca^{2+} ions, thus displacing virtually all the manganese ions into solution. If ^{54}Mn tracer used in the Mn_E value determinations all remained exchangeable, at equilibrium, the results obtained should agree with simple $CaCl_2$ extraction of exchangeable soil manganese. However, since the apparent concentration of isotopically exchangeable manganese is greater than that determined by simple $CaCl_2$ extraction for most of the soils used in the laboratory (and pot) studies (Table 4.9), then sorption of ^{54}Mn must have occurred in the Mn_E value determinations, thus giving artificially high values. This view is substantiated by the detection of ^{54}Mn in fractions unaffected by $CaCl_2$ (Table 4.2 and Figure 4.4). The use of a complexing agent such as DTPA gives enhanced Mn_E values (compare Table 4.6 with Table 4.8)for a different reason, namely that soil manganese is dissolved to a much greater extent (see Equation 1).

4.3.6.2 Mn_L values (Plant uptake)

Another radioisotopic technique employed in the determination of nutrient labile pools concerns the growing of plants in pots containing radioactively labelled soil and determining the active and

Table 4.8 Mn_E values obtained with DTPA equilibriation for five soils*

Soil No.	Mn_E ($\mu g\ g^{-1}$)
3	264 ± 4
4	157 ± 5
7	35.0 ± 1.0
9	106 ± 1
10	327 ± 3

* Mean of duplicate samples.

Table 4.9 Manganese concentrations as determined by $CaCl_2$ extraction and isotopic exchange (Mn_E)

Soil	Manganese ($\mu g\ g^{-1}$)	
	$CaCl_2$-extraction*	Mn_E
1	37.1	51.1
2	51.1	77.8
3	37.9	164.8
4	75.2	103.1
5	13.1	13.5
6	1.8	1.5
7†	6.4	16.5
8	15.7	30.4
9	40.9	47.2
10	34.1	135.9
11†	18.0	30.7
12†	11.8	18.8

* Included (with the exception of soils 7, 11 and 12) is the water-soluble + exchangeable manganese from the fractionation of the moist soil (Table 4.2, column 2).

† Soils used in the pot experiments
7 - Hexpath
11 - Darvel
12 - Giffordtown

native quantities of the element in the plants at harvest. The value obtained, Mn_L, is conceptually similar to the Mn_E value. Plant uptake of native and active manganese in determining the Mn_L value is analogous to the extractant's ability to solubilise soil manganese in Mn_E determinations. For both techniques, the radioisotope is assumed to exchange with the total amount of the potentially available native forms of the element. Therefore,

$$Mn_L = \frac{\text{Mn in plant (µg)} \div \text{wt. of soil in pot (g)}}{\text{Fraction of Mn* in plant}} \qquad (2)$$

where Mn = native manganese
Mn* = radioactive manganese.

Pot experiments for Mn_L determinations

Pot experiments were set up in an attempt to measure the labile pool of the soil as determined by plant uptake. The effects of different levels of soil consolidation on Mn_L determinations were also studied since soil compaction can, under some circumstances, affect levels of soil and plant manganese in field conditions (See Section 3). Some characteristics of the soils have been previously outlined in Table 3.28.

In a preliminary experiment, a soil of the Giffordtown series was used, and plants were grown for 21 days before harvesting for the analysis of stable manganese and ^{54}Mn. Two further experiments were later carried out using soils of the Hexpath and Darvel series. These experiments were similar to the earlier one except that the period of plant growth was much longer, the barley being harvested after ear emergence. It should be noted that the Darvel soil used in the pot experiment was collected from a different site than the Darvel soil described in Table 4.1.

Equilibration of ^{54}Mn with soil for pot experiments

Since a precondition for valid Mn_E determinations involves the establishment of equilibrium conditions between ^{54}Mn and native manganese, a similar situation must be met for Mn_L determinations for meaningful comparisons to be made. Therefore, the radioisotope should be taken up by the plants in a proportion equivalent to that

of the stable isotope. The establishment of equilibrium conditions, however, would vary not only between elements but amongst soil types as well. A review of the literature has revealed that equilibrium between radioactive and native forms of the element is usually assumed to have occurred without direct experimental evidence, and equilibration times vary from 1 week (Tiller *et al*, 1972) to 1 month (Rule and Graham, 1976) prior to sowing.

In order to investigate the matter, 5 μCi of ^{54}Mn was incorporated into 2000 g of soil (Giffordtown) in the manner outlined in Section 3.5.6. The soil, after being brought to field capacity, was removed from the pot and stored in a polythene bag at room temperature. Random samples were taken periodically and extracted (10 g shaken in 50 ml 0.1M $CaCl_2$ for 30 minutes), and 5 ml aliquots of the extract were counted for ^{54}Mn activity. Results (Figure 4.8) show that within 5 hours (following polythene bag storage), only 32% of ^{54}Mn was recoverable from the soil. Extractable ^{54}Mn continued to decline, so that <5% was extractable after 180 hours, and equilibrium was established after 16 days, when 2.5% remained extractable. Therefore, for the pot experiments, the soil was stored for 3 weeks prior to sowing to allow this equilibrium to become established.

The effects of soil consolidation on Mn_L values

Results for the three trials are shown in Tables 4.10-4.12. Included are Mn_L values obtained under different consolidation treatments and the corresponding Mn_E values for $CaCl_2$ and DTPA equilibrations. The precision of the replicate determinations was quite satisfactory in most cases, given the nature of the experimental conditions (see Appendix C.2).

With the exception of pot experiment No. 1, consolidating the soil significantly affected Mn_L values ($P < 0.01$) in experiments 2 and 3. This was especially evident in the second experiment, while in the third study, Mn_L values for the unconsolidated and surface compacted soil did not differ significantly at the 5% level (Tables 4.10-4.12). Since Mn_L values varied with different consolidation treatments it would therefore be difficult to achieve a satisfactory reference procedure against which to test possible extractants.

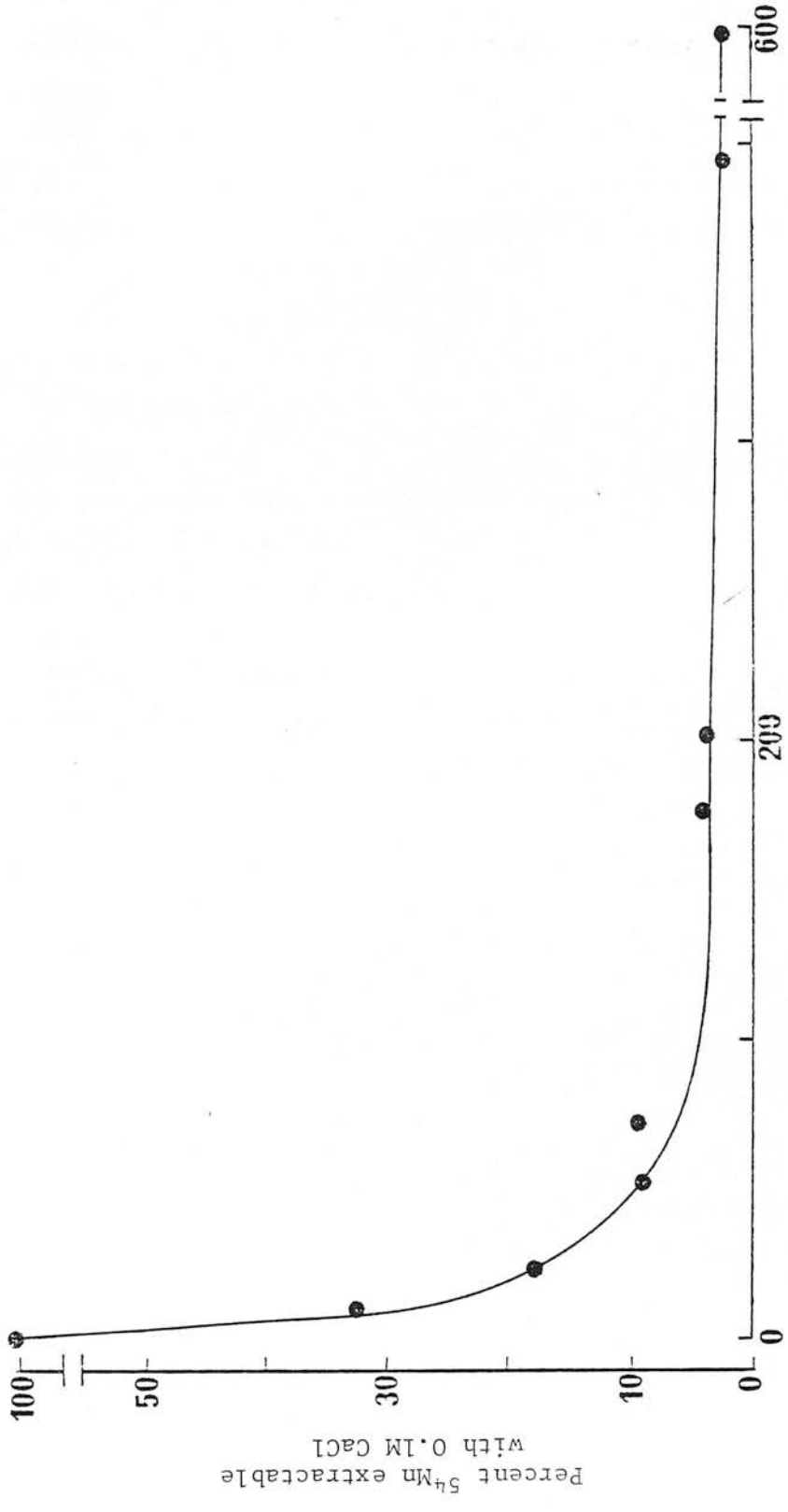

Figure 4.8 Extraction of ^{54}Mn from soil after radioisotopic equilibration (following polythene bag storage).

4.3.6.3 Comparison of Mn_L with Mn_E values

In the first pot experiment (Table 4.10) results show that Mn_L values closely agreed with the results for Mn_E ($CaCl_2$) determinations. This indicates that the same manganese labile pool which is extractable in the Mn_E value determination is available to the plant over this period of time. Pot experiment 2 (Table 4.11) resulted in values for the labile pool which were much higher than the Mn_E values determined in $CaCl_2$ or DTPA. Similarly, Mn_L values were greater than the Mn_E values for either extractant in the third pot experiment (Table 4.12) but the Mn_E (DTPA) and Mn_L values were closer than in the second study (Table 4.11). The fact than Mn_L values exceeded Mn_E values could have been attributable to 2 processes:-
(1) that much of the ^{54}Mn had become fixed once it was added to the soil and was therefore unavailable for plant uptake and/or (2) in addition to some sorption of ^{54}Mn, some isotopically non-exchangeable soil manganese came into solution during plant growth and was taken up. Work by Bromfield (1958a, 1958b) and more recently by Godo and Reisenauer (1980) on the solubilising power of root exudates indicates the likely contribution of the latter process to the higher Mn_L values. This could be proven more conclusively if the total quantity of manganese extracted by the plant had exceeded that solubilised by $CaCl_2$ or DTPA. However, results in Table 4.13 show that total manganese uptake by the plant was a small fraction of the total amount of manganese extracted by either $CaCl_2$ or DTPA. Therefore it is not possible to distinguish conclusively between the two processes.

4.4 SUMMARY AND CONCLUSIONS OF THE RADIOISOTOPIC STUDIES

The principles of radioisotopic exchange and isotopic dilution analysis have been applied to the study of soil manganese. A wide range of soil types was used and the fate of ^{54}Mn, when added to soil-salt suspensions, was investigated. The time taken for attainment of isotopic equilibrium varied greatly between the soils from less than 30 minutes to over 80 hours. The curvilinear relationship of the log of ^{54}Mn activity in soil solution versus time could have been an indication that the isotope had exchanged with different forms of native manganese with varying desorption energies.

Table 4.10 Results of pot experiment 1 on Giffordtown soil and corresponding Mn_E values

Consolidation	Bulk density ($g\ cm^{-3}$)	Mn_L ($\mu g\ g^{-1}$)*	Mn_E ($\mu g\ g^{-1}$)	
			$CaCl_2$	DTPA
Unconsolidated	1.15	16.1	18.8	34.8
Intermediate compaction throughout	1.31	17.0		
Heavy compaction throughout	1.38	17.1		

* Mean of 3 replicate pots.

Table 4.11 Results of pot experiment 2 on Hexpath soil (soil No.7, Table 4.1) and corresponding Mn_E values

Consolidation	Bulk density ($g\ cm^{-3}$)	Mn_L ($\mu g\ g^{-1}$)*	Mn_E ($\mu g\ g^{-1}$)	
			$CaCl_2$	DTPA
Unconsolidated	1.10	55.5 a†	16.4	35.0
Surface compacted	1.35	103.3 b		
Compacted throughout	1.45	79.9 c		

* Mean of 6 replicate pots

† Means with different letters are significantly different at the 1% level.

Table 4.12 Results of pot experiment 3 on Darvel soil and corresponding Mn_E values

Consolidation	Bulk density ($g\ cm^{-3}$)	Mn_L ($\mu g\ g^{-1}$)*	Mn_E ($\mu g\ g^{-1}$)	
			$CaCl_2$	DTPA
Unconsolidated	1.10	71.4 a†	30.7	60.0
Surface compacted	1.35	79.3 a		
Compacted throughout	1.40	108.0 b		

* Mean of 4 replicate pots.

† Means with the same letter are not significantly different at the 5% level.

Table 4.13 Total quantity of manganese extracted by the plant and by $CaCl_2$ and DTPA solutions

Soil/Consolidation		Manganese extracted per pot (mg)		
		By plant*	By $CaCl_2$†	By DTPA†
Hexpath	Unconsolidated	0.24)	9.9	52.7
	Surface compacted	0.43)		
	Compacted throughout	0.29)		
Darvel	Unconsolidated	0.22)	27.9	89.9
	Surface compacted	0.28)		
	Compacted throughout	0.25)		

* Data obtained from Table 3.30.

† Values calculated by multiplying concentrations of $CaCl_2$-extractable manganese (Table 4.9) and DTPA-extractable manganese by the quantity of soil used for each pot (1550 g).

A sequential fractionation procedure was employed in an attempt to isolate the various soil fractions with which ^{54}Mn was incorporated at isotopic equilibrium. For most of the soils, the radioisotope labelled all the soil manganese fractions, with the majority of ^{54}Mn associated with the water-soluble + exchangeable, organically bound and easily reducible oxide fractions; substantial portions of ^{54}Mn were also found in the resistant and residual fractions as well. However, whether actual isotopic exchange or simple adsorption of the isotope into these fractions occurred, cannot be ascertained.

The effects of drying on radioisotopic and native manganese were investigated. Both forms in the water-soluble + exchangeable, organically bound and easily reducible fractions behaved in similar fashion with drying. However, the changes in the native manganese content were proportionately greater than ^{54}Mn in the former two fractions, while in the easily reducible fraction the reverse was true. Changes in ^{54}Mn were also noted in the resistant and residual fractions without apparent effect on the native content. Concentration of the radiosotope on the oxide and other mineral surfaces could explain this difference. The decrease in native manganese in the easily reducible fraction suggests that this may have been the source of the increase in exchangeable manganese upon drying.

Two radiosotopic techniques were employed for the estimation of the available (labile) pool of manganese in soil, namely the determination of E and L. The former (Mn_E) was determined in the laboratory by using chemical extractants ($CaCl_2$ or DTPA) and measuring radioactive and native manganese at equilibrium, while the latter (Mn_L) entailed the determination of both forms of manganese taken up by the plant.

Good agreement between Mn_E determinations using two different equilibration procedures in $CaCl_2$ solution was obtained in most of the soils investigated. However, it would appear that the use of $CaCl_2$ in assessing the labile pool of manganese is of limited value due to the sorption of ^{54}Mn, irrespective of the equilibrating procedure used. The determination of Mn_E using DTPA solution gave much higher values than those obtained in $CaCl_2$. This was due to the greater solubilising power of the complexing agent rather than to sorption of ^{54}Mn during the equilibration process. Unlike the equilibration in $CaCl_2$, sorption of the radioisotope in DTPA was

minimal; therefore the resulting Mn_E values differed little from those obtained by simple extraction in that medium.

Labile pool determinations as measured by plant uptake (Mn_L) showed them to be similar to Mn_E values determined with $CaCl_2$ in the short-term pot experiment only. In two long-term pot experiments (where the plants were grown to ear emergence) on the other hand, the Mn_L values exceeded Mn_E-$CaCl_2$ and Mn_E-DTPA determinations, indicating that either native manganese not in isotopic equilibrium with ^{54}Mn was taken up by the plant or that the radioisotope was incorporated in forms unavailable for plant use. These uncertainties, and the further variability resulting from the effects of consolidation make Mn_L values unreliable as a reference method to which routine soil tests could be related.

SECTION 5

THE RELEASE OF MANGANESE IN FLOODED SOILS

5.1 INTRODUCTION

The release of Mn(II) ions under anaerobic soil conditions is brought about by chemical and biological mechanisms (Ponnamperuma, 1972; Alexander, 1977). Although the role of microorganisms has been widely considered, a review of the literature has revealed that minimal work has been attempted to estimate the magnitude of Mn(II) release affected by chemical mechanisms (e.g. reduction of manganese oxides by organic matter itself, enzymatic reactions, exchange or dismutation reactions). Given the large environmental fluctuations of temperate regions and the susceptibility of the microbial balance in soil to various physical factors, a knowledge of the relative chemical and microbiological contributions to Mn(II) mobilisation would be useful. Also important would be a better understanding of the kinetics of manganese release following the flooding of soils which, unlike tropical rice-paddy soils, are not normally in a waterlogged, anaerobic state.

5.2 MATERIALS AND METHODS

5.2.1 Soils

The four soils used in this study - Macmerry, Stirling, Dreghorn and Darvel - are described in Section 4, Table 4.1 (soils 1, 4, 8 and 9). Finely ground (<0.2 mm) samples were used to ensure a more homogeneous system.

5.2.2 Sterilisation

5.2.2.1 Soils

Effective sterilisation was achieved by irradiating air-dry 200 g soil portions in 350 ml polythene containers with a dose of 3.0 Mrad from a ^{60}Co gamma-ray source (located at the Scottish Universities Research and Reactor Centre, East Kilbride, Glasgow). Following irradiation the soils were thoroughly mixed in an end-over-end shaker for 16 hours. Sterility was checked by inoculating

1 g soil samples in nutrient broth (Oxoid No. 2) or onto soil extract agar (Allen, 1950)

5.2.2.2. Materials

Spatulas, centrifuge tubes and weighing boats were individually wrapped in aluminium foil amd steam-sterilised at 121°C for 15 minutes.

Fifteen ml aliquots of distilled water were measured into sterilising bottles and autoclaved in similar fashion.

5.2.3 Incubation and Extraction

5.2.3.1 Initial procedure

Three gram portions of the sterile soil were aseptically weighed into sterile 30 ml capacity 'Quickfit' glass centrifuge tubes. The control soil samples (non-sterile) were weighed out in a similar manner under non-sterile conditions.

Prior to submergence, the pre-weighed soils and pre-measured distilled water solutions were stored for 24 hours at either 1°C, 12°C or 30°C to allow for temperature equilibration before incubation at these temperatures.

5.2.3.2 Submergence and extraction

Fifteen ml of water was added to each tube, to submerge the soil. Following submergence, the centrifuge tubes were immediately sealed with fitted ground glass stoppers. Release of manganese was simultaneously monitored on the sterile and non-sterile soils at pre-designated time intervals (0-28 days) in the following manner. Fifteen ml non-sterile aliquots of 0.1M $CaCl_2$ (temperature equilibrated) were added to the soil-water system. The resulting extracting solution was therefore equivalent to 0.05M $CaCl_2$. Soil extraction was accomplished by rotary shaking for 10 minutes at 110 rpm and the suspension was immediately filtered through Whatman No. 42 ashless filter paper. The temperature of the system did not change significantly during the extraction period. Filtrates were stored at 1°C for later analysis.

5.3 RESULTS AND DISCUSSION

5.3.1 Grinding

The grinding of a soil (or other reactive substances) increases surface area and hence reactivity. In order to investigate the effects of smaller particle size on the manganese release, an experiment was carried out in which non-sterile sieved (≤2.0 mm) and ground (≤0.2 mm) Macmerry and Dreghorn soils were incubated under flooded conditions. The procedure was similar to that outlined in Section 5.2.3. Although the rate and magnitude of release of manganese was greatly increased in the ground soil samples, overall trends between the sieved and ground soil were essentially similar (Figure 5.1). Thus, the nature of the chemical reactions in the soil associated with manganese were probably unaffected by the grinding process. The greater exposure of soil surfaces, which promotes cation exchange and reducing processes, probably accounted for the observed differences between the sieved and ground soils.

As is evident from Figure 5.1, the change in manganese solubility between sieved and ground samples was much more pronounced in the Dreghorn soil than the Macmerry. Peak values of manganese solubilised were approximately 2.8 times greater after grinding in the former soil but differed by only about 25% at the termination of the experiment in the latter. This discrepancy between two soils may partially be explained by comparing the particle size distribution (Table 5.1).

Table 5.1 Particle size distribution of the Dreghorn and Macmerry soils

Soil	Sand (%)	Silt (%)	Clay (%)
Dreghorn	70.6	9.8	19.6
Macmerry	51.0	26.5	22.5

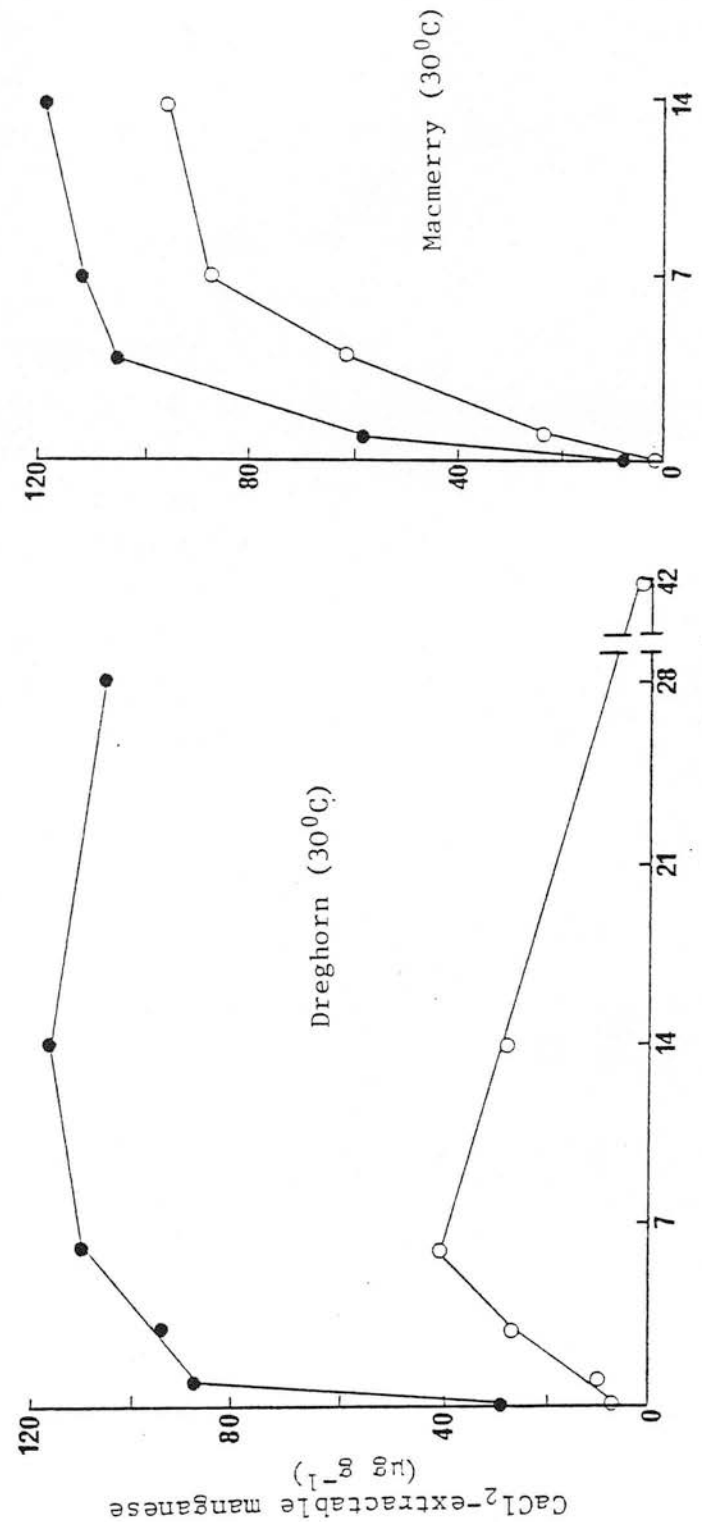

Figure 5.1 Changes in concentrations of $CaCl_2$-extractable manganese in flooded Dreghorn and Macmerry soils of differing sieve size.

○ ⩽ 2.0 mm; ● ⩽ 0.2 mm

Since the Dreghorn soil had a greater proportion of coarse particles (2.0 to 0.05 mm), the act of grinding would have a greater influence in increasing surface area. However, it should be kept in mind that some other factors may be operating, since the sand fraction alone would not be expected to be a reservoir of highly reactive or exchangeable manganese. Possible factors might be relative aggregate size and the occurrence of concretionary material which might break down to give a much increased active surface area. Further investigations here would be useful.

5.3.2 Sterilisation

According to McLaren (1969), an irradiated soil is described as being sterile if no microbial growth can be demonstrated in a sterile medium following its inoculation with the irradiated soil. Plate 5.1 shows the effects of a 3 Mrad dose of gamma radiation on the Macmerry soil incubated in the Oxoid nutrient broth. Unlike the non-sterile sample (C) the sterilised soil (S) revealed no microbial influences (as evidenced by a clear solution) after 1 month's incubation at 30°C. As expected, no microbial proliferation occurred in those samples incubated at 1°C (sterile and non-sterile). These results are in agreement with those of Salonius *et al* (1967), and Jackson *et al* (1967) who found that 3 Mrad (gamma radiation) consistently proved sufficient to sterilise their soils. At the intermediate incubation temperature (12°C) microbial growth was observed after 1 week in the non-sterile soils.

Periodic checks of the supernatant solution of the sterile, flooded soils by inoculation of soil extract agar (Allen, 1950) or Oxoid broth showed that contamination was apparently non-existent during the course of the incubation.

5.3.3 Effects of Ionising Radiation on some Soil Manganese Fractions

The effects of ionising radiation on the physical and chemical properties of soil has been given wide consideration by a number of workers. Bowen and Cawse (1964), for example, found that the bulk of carbon, nitrogen and inorganic elements liberated by irradiation probably arose from the lysis of microbial cells. However, increases observed in levels of soluble

manganese after soil irradiation were too large to have arisen from microbial lysis *per se*. It was thought that the organic matter solubilised by the irradiation technique reduced manganese dioxide. Indeed, a 'sweet' odour apparent in the four irradiated soils used in this study was thought to be indicative of the presence of recently solubilised organic matter.

The effects of a 3 Mrad dose of gamma radiation on the more reactive soil manganese fractions as well as on soil pH is shown in Table 5.2. Determinations of exchangeable, organically bound and easily reducible manganese were carried out according to the procedure outlined in Section 4.2.3.5. The exchangeable and organically bound manganese fractions in the sterile soils increased slightly at the expense of the easily reducible oxide fraction. The action of solubilised organic matter upon the latter fraction could account for these findings. Also, the degradation of previously stable organic molecules containing manganese could release some of the element. However, relative to the total amount of manganese present in the three fractions, changes observed when the soils were irradiated were slight. The fact that the soils had been stored several months in an air-dried state prior to gamma irradiation may account for the small differences observed between the two treatments. Salonius *et al* (1967) found that the change in level of soluble organic matter was much greater in irradiated moist soil than in irradiated dry soil. They also noted that after prolonged air drying, soils did not change so drastically during irradiation (equal quantities of air-dried and irradiated soil extracts gave similar respiration curves when inoculated with soil microorganisms) and therefore postulated that the effects of soil irradiation are similar to those occurring during prolonged storage of air-dry soil.

5.3.4 Temperature Effects

The changes in concentration of $CaCl_2$-extractable manganese in the Macmerry and Darvel soils during the first seven days of soil submergence are shown in Figures 5.2 and 5.3 respectively. The effects of temperature on the release of the divalent ion is readily apparent in both the sterile and non-sterile system.

Plate 5.1 Macmerry soil after 28 days of incubation in nutrient broth

From left to right:-

C - non-sterile soil at 30°C

S - sterile soil at 30°C

C - non-sterile soil at 1°C

S - sterile soil at 1°C

Table 5.2 Concentrations of manganese in several fractions in sterile and non-sterile soil

Soil	Treatment	Manganese ($\mu g\ g^{-1}$)* Exchangeable	Organically bound	Easily reducible	pH†
Macmerry	Sterile	13.7 ± 0.2	17.0 ± 0.2	102.5 ± 0.5	6.10
	Non-sterile	12.2 ± 0.4	16.3 ± 0	103.0 ± 3.0	6.15
Stirling	Sterile	58.0 ± 0	32.3 ± 1.6	29.5 ± 0.5	5.43
	Non-sterile	58.5 ± 0.5	30.0 ± 0.7	32.0 ± 0	5.41
Dreghorn	Sterile	53.5 ± 0.5	91.1 ± 3.0	25.5 ± 2.5	6.12
	Non-sterile	47.0 ± 0	87.1 ± 1.0	31.0 ± 2.0	6.16
Darvel	Sterile	56.5 ± 0.5	30.5 ± 0.5	62.5 ± 1.5	5.94
	Non-sterile	55.0 ± 0	27.5 ± 0.5	66.0 ± 1.0	5.97

* - mean of duplicate samples
† - single determination only.

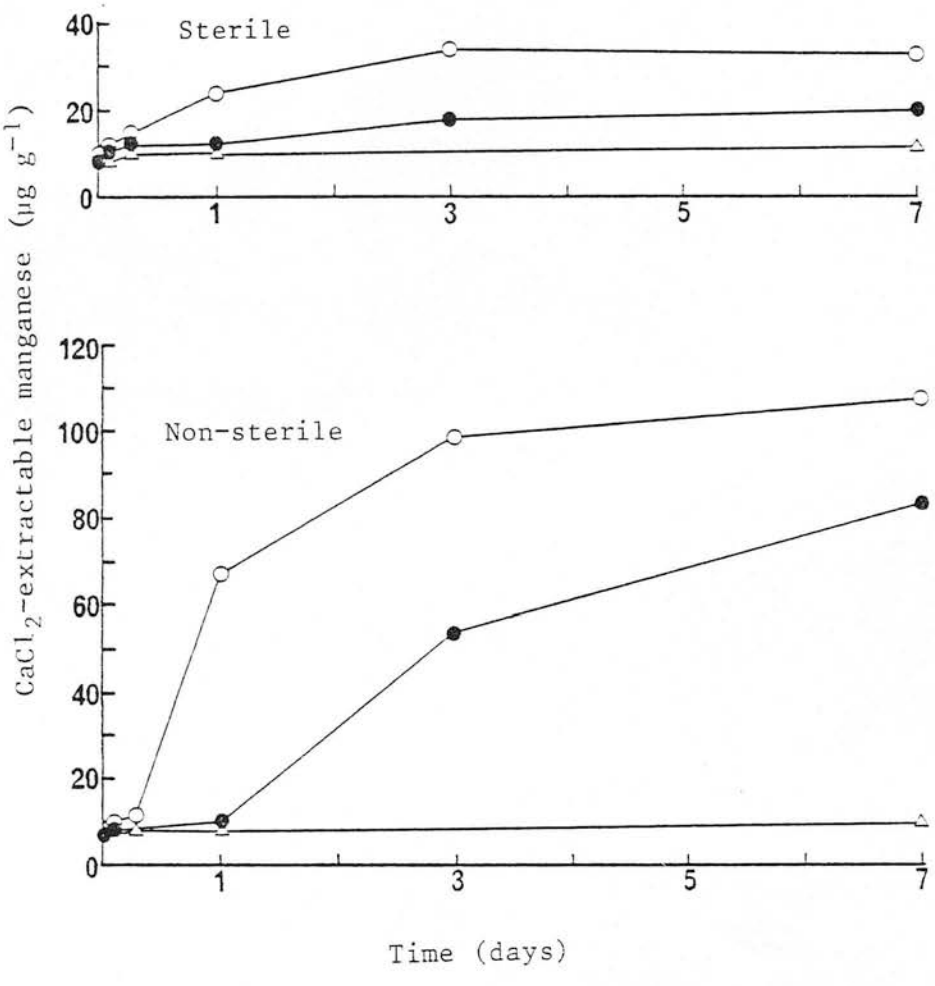

Figure 5.2 Changes in concentrations of $CaCl_2$-extractable manganese in sterile, flooded and non-sterile, flooded Macmerry soil at three temperatures during the first week of submergence.

O-30^{0}C; ●-12^{0}C; Δ-1^{0}C

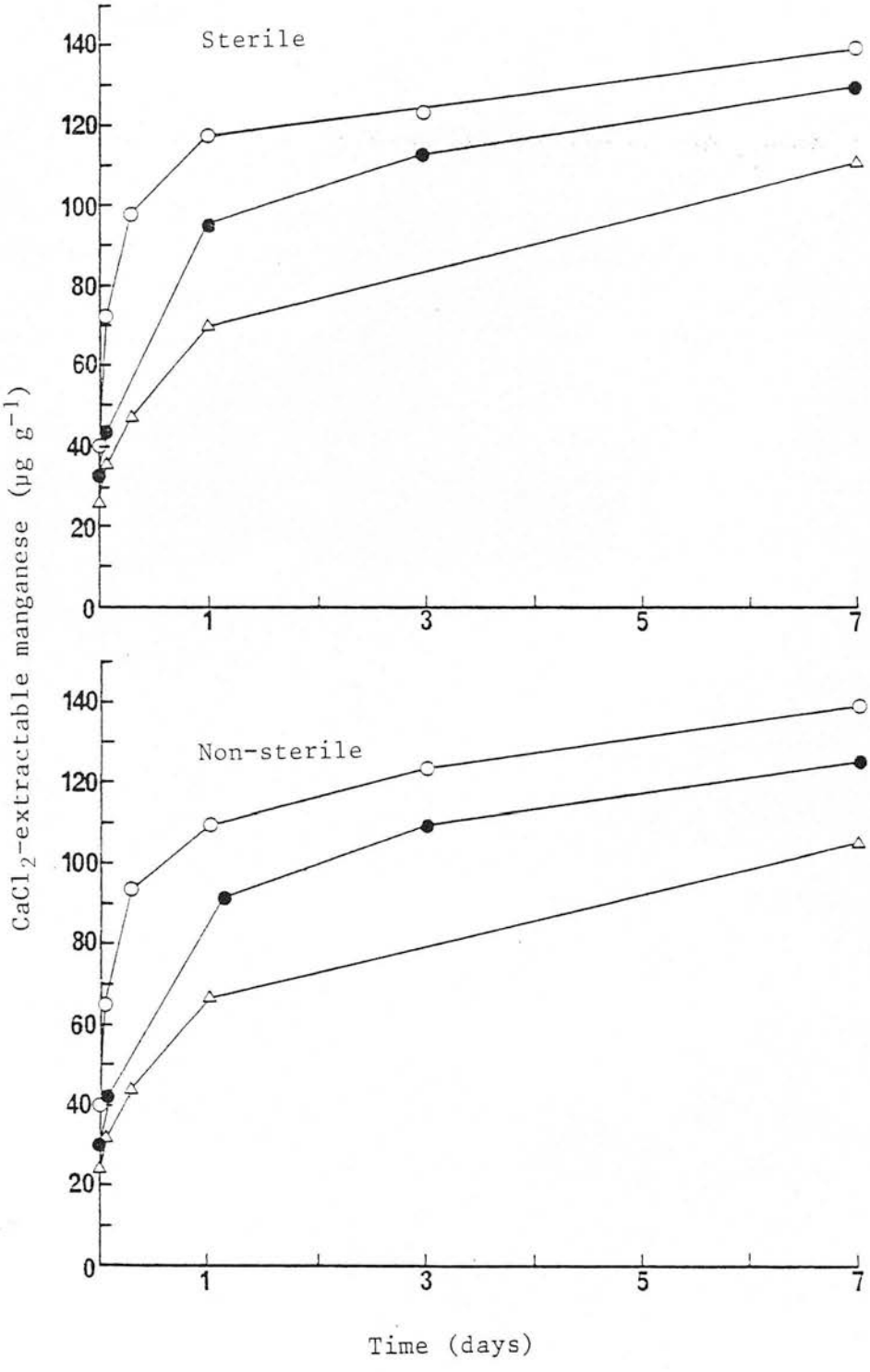

Figure 5.3 Changes in concentrations of $CaCl_2$-extractable manganese in sterile, flooded and non-sterile, flooded Darvel soil at three temperatures during the first week of submergence.

O-30^{0}C; ●-12^{0}C; Δ-1^{0}C

Results for the Stirling and Dreghorn soils were similar. The well known effects of temperature on both microbial activity (Alexander, 1977) and chemical reaction rate (Gregg, 1963) readily explain these observations.

Data for the sterile Dreghorn and Darvel soils (Figure 5.4) showed manganese concentrations at the three incubation temperatures to be approaching somewhat similar equilibrium values with time. However, different results were obtained with the sterile Macmerry and Stirling soils, in which the equilibrium Mn(II) concentration appeared to be more temperature-dependent (Figure 5.5). The possibility arises that reducing agents present in a system where higher valency manganese is reduced (e.g. according to the reaction $MnO_2 + 4H^+ + 2e^- \rightleftarrows Mn(II) + 2H_2O$) are altered or inactivated at lower temperatures. This effect occurs with enzymes which, according to Alexander (1977), each have a range of pH values and temperatures within which they are active, as well as an optimum pH and temperature for activity.

5.3.5 Liberation of Manganese in Sterile and Non-sterile Flooded Soils

Figures 5.6-5.9 represent the data obtained throughout the 28 day incubation period at the two temperature extremes (1^oC and 30^oC) for the four soils. Replication between duplicate samples was extremely good in most instances. Data for the intermediate incubation temperature (as well as the 1^oC and 30^oC temperatures) can be found in Appendix D.

5.3.5.1 Stirling (Figure 5.6)

The kinetics of manganese release for the flooded Stirling soil indicated that a chemical contribution of manganese reduction appeared to predominate during the period of soil submergence. At peak values for both the sterile and non-sterile soils incubated at 30^oC, the presence of microbial activity was associated with a difference of approximately 16% of the total concentration of $CaCl_2$-extractable manganese. Equilibrium manganese concentrations attained relatively stable values after 3 days of flooding in the sterile soil, whereas a steady decrease in manganese was observed after 7 days in the non-sterile sample. At 1^oC, extractable manganese reached similar

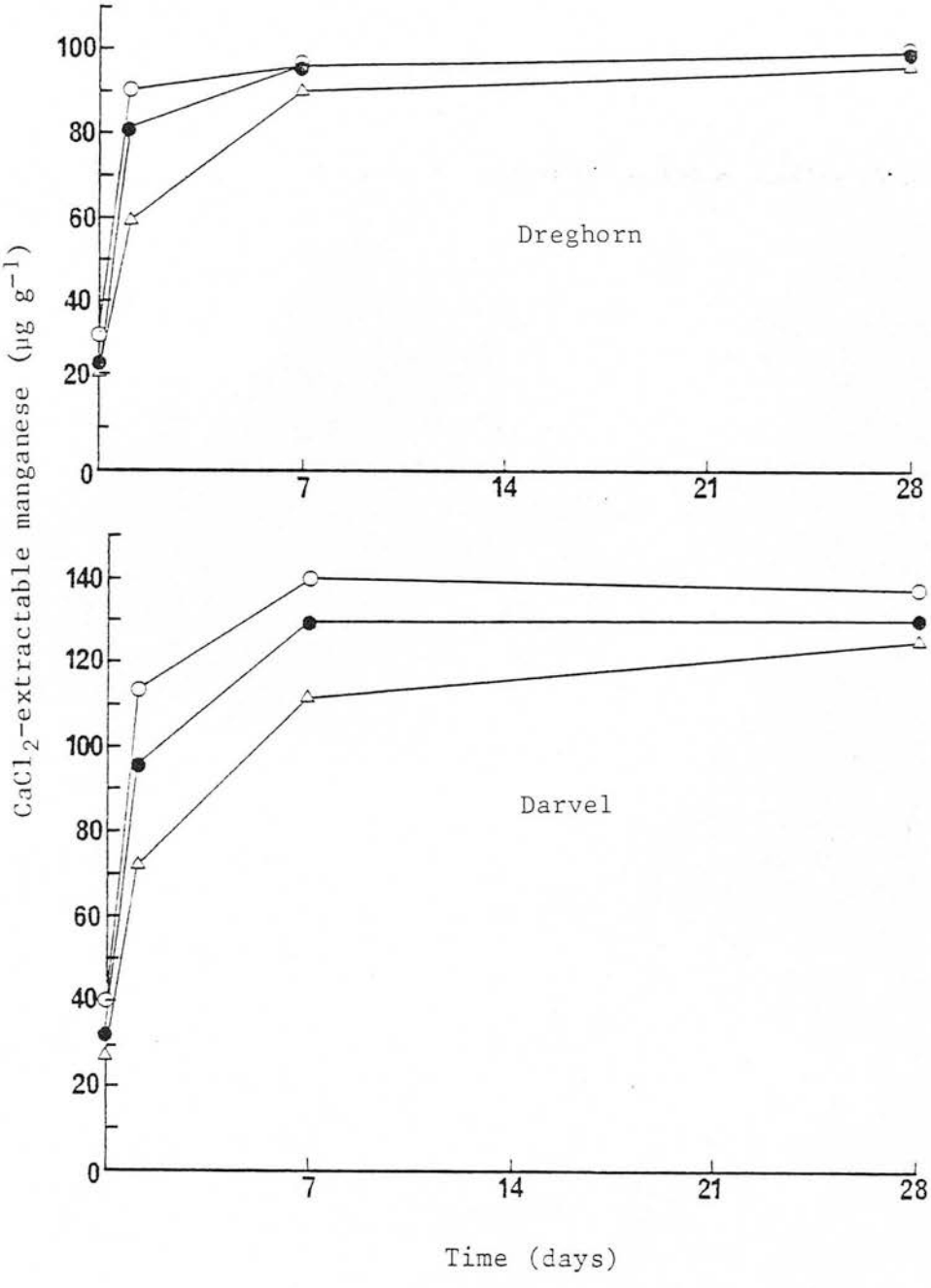

Figure 5.4 Changes in concentrations of $CaCl_2$-extractable manganese in sterile, flooded Dreghorn and Darvel soils at three temperatures.

O-30^{0}C; ●-12^{0}C; Δ-1^{0}C

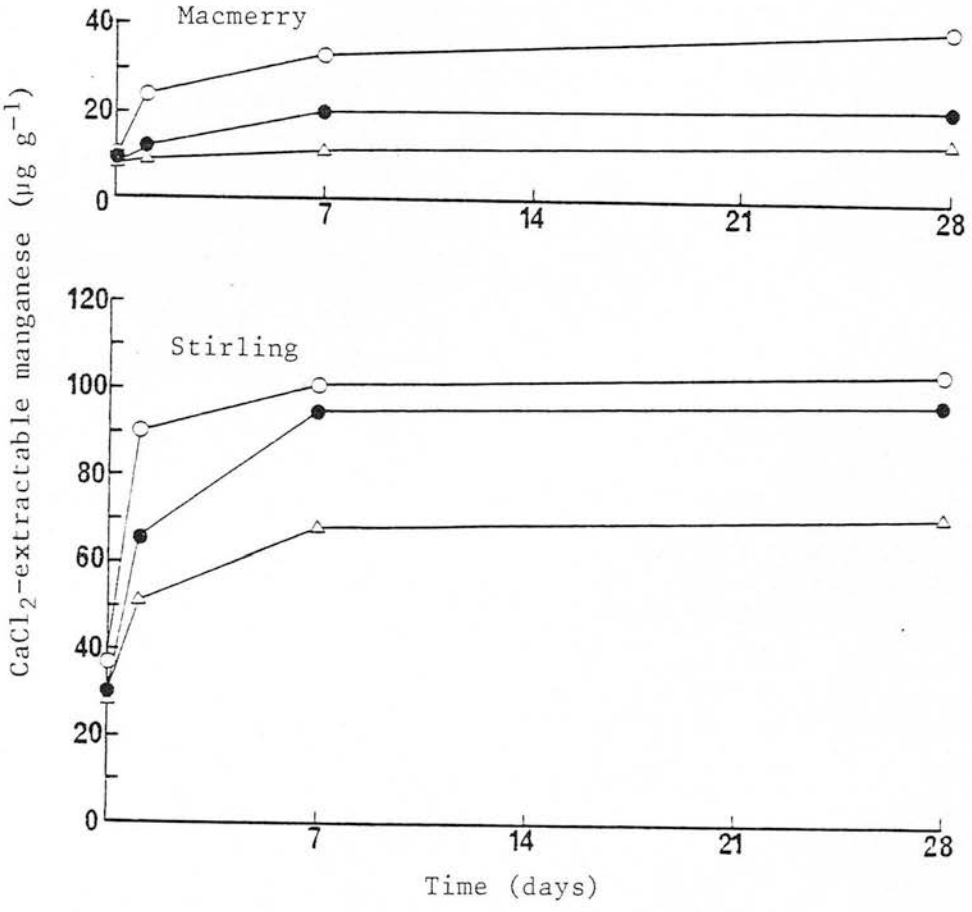

Figure 5.5 Changes in concentrations of $CaCl_2$-extractable manganese in sterile, flooded Macmerry and Stirling soils at three temperatures.

O-30^0C; ●-12^0C; Δ-1^0C

maximum concentrations after 7 days for both sterile and non-sterile soils. Chemical release of manganese at the low incubation temperature amounted to over 50% of the maximum solubilised at 30°C under non-sterile conditions.

5.3.5.2 Dreghorn (Figure 5.7)

Results for the Dreghorn soil were similar to those of the Stirling soil. Equilibrium in the sterile soil incubated at 30°C was virtually achieved after 1 day and concentrations of manganese released during this time were similar to that of the non-sterile counterpart. Thereafter, manganese concentrations increased in the non-sterile soil, presumably when microbial activity had built up, followed by a slight decline after 14 days. At 30°C the chemical contribution to $CaCl_2$-extractable manganese at peak values accounted for nearly 87% of the total manganese liberated. At 1°C, there were no large differences between the sterile and non-sterile soil, both reaching equilibrium after 7 days.

5.3.5.3 Darvel (Figure 5.8)

The quantities of manganese released in both sterile and non-sterile soils incubated at 30°C were not significantly different during the first 7 days of submergence, indicating the predominence of chemical mechanisms during this time.

For the non-sterile soil incubated at 30°C a sharp decrease in $CaCl_2$-extractable manganese was observed after 7 days of submergence. Indeed, concentrations eventually decreased to values well below those observed in the sample incubated at 1°C. At the low temperature, equilibrium was not achieved during the 28 day submergence period.

5.3.5.4 Macmerry (Figure 5.9)

The flooding of the non-sterile soil at 30°C initiated nearly a 3 fold increase in $CaCl_2$-extractable manganese over its sterile counterpart within 24 hours after submergence. The maximum concentration of extractable manganese was observed at 14 days of submergence for the non-sterile 30°C incubated soil; thereafter, manganese concentrations declined. In the sterile soil (30°C) only a slight increase in manganese was noted after 3 days. Chemical contribution

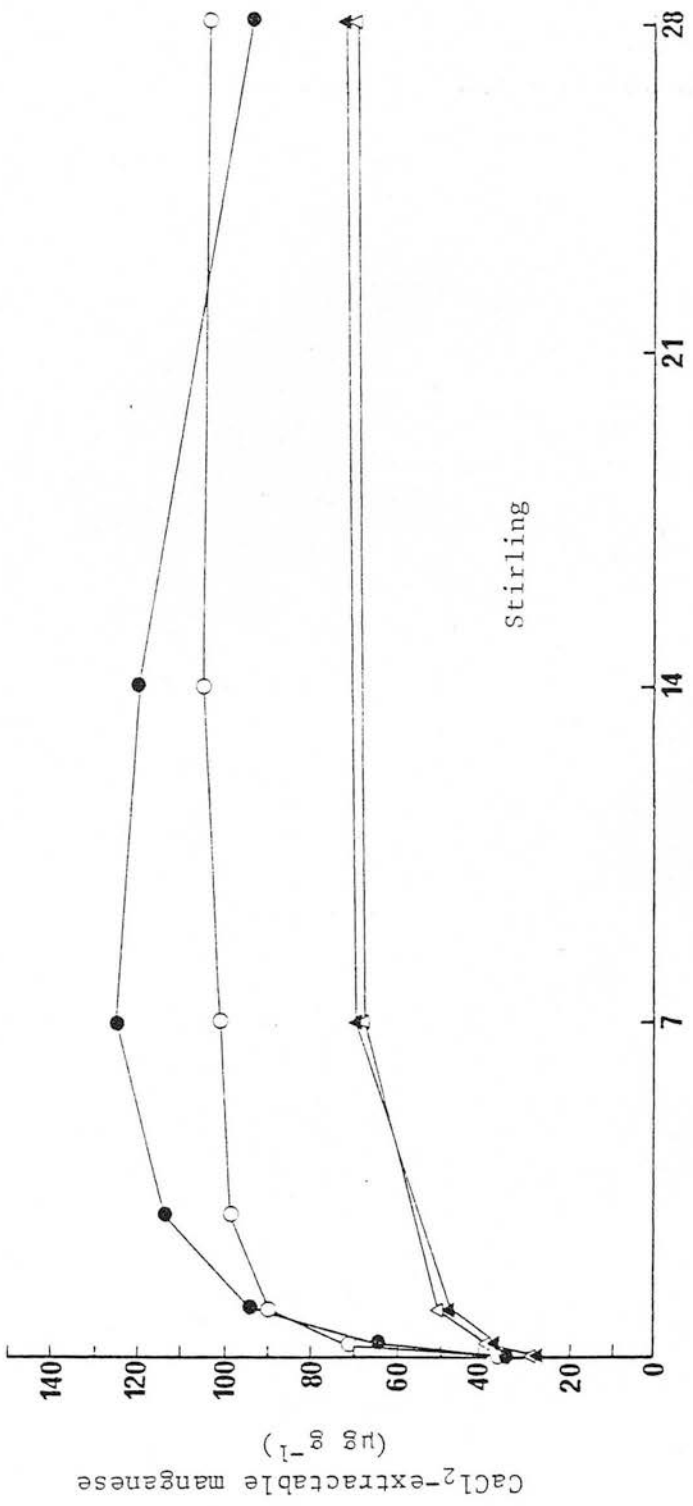

Figure 5.6 Changes in concentrations of $CaCl_2$-extractable manganese in sterile, flooded and non-sterile, flooded Stirling soil at 30^0C and 1^0C.

●-non-sterile, 30^0C; O-sterile, 30^0C; ▲-non-sterile, 1^0C; Δ-sterile, 1^0C

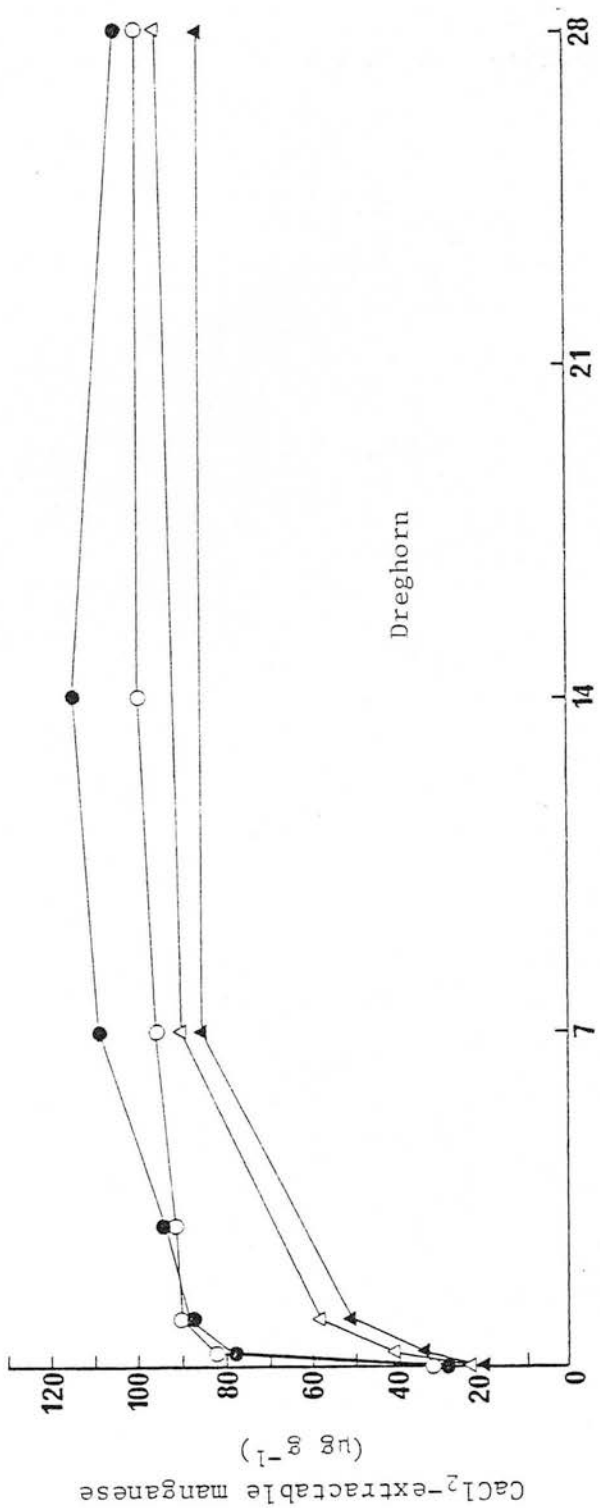

Figure 5.7 Changes in concentrations of $CaCl_2$-extractable manganese in sterile, flooded and non-sterile, flooded Dreghorn soil at 30^0C and 1^0C.

●-non-sterile, 30^0C; O-sterile, 30^0C; ▲-non-sterile, 1^0C; Δ-sterile, 1^0C

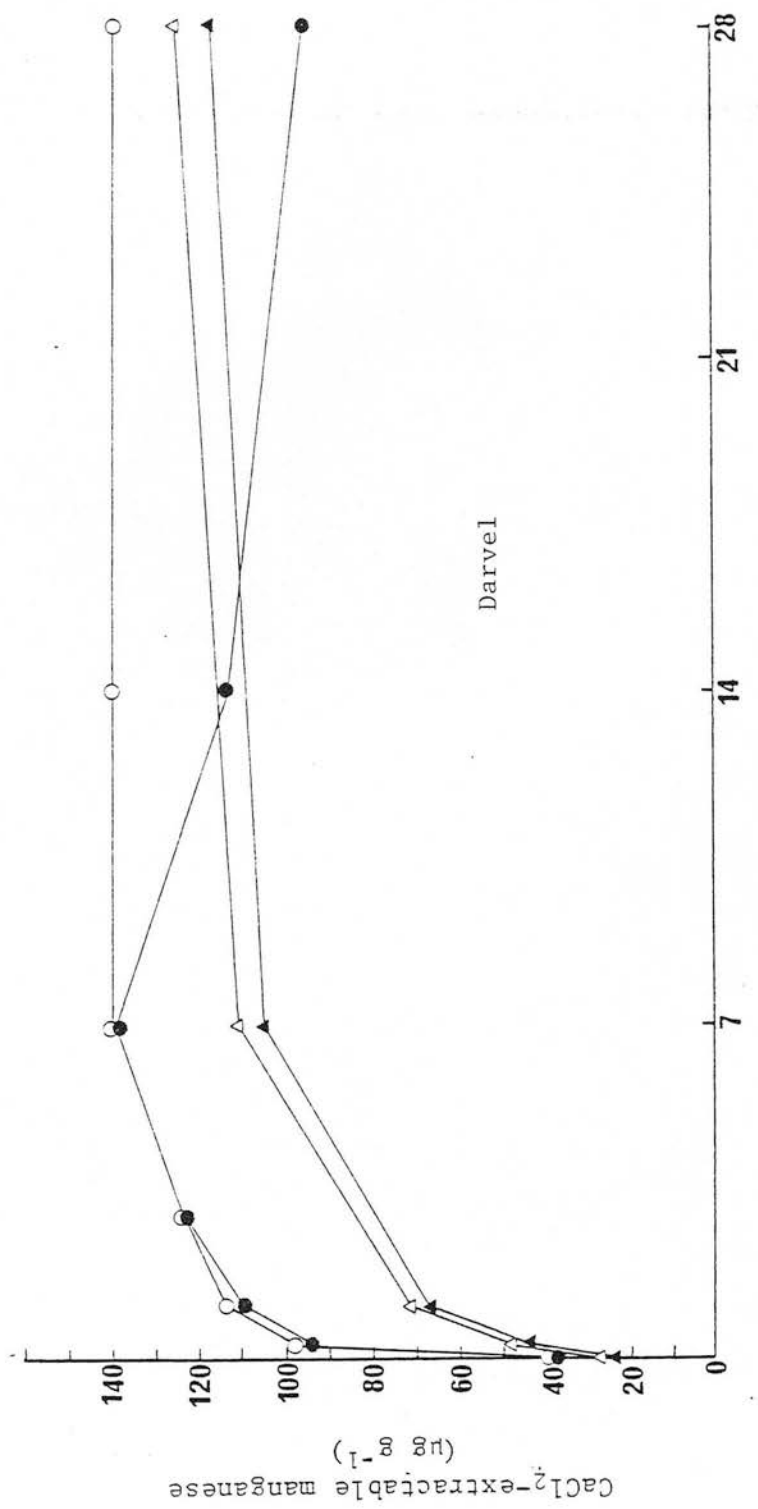

Figure 5.8 Changes in concentrations of $CaCl_2$-extractable manganese in sterile, flooded and non-sterile, flooded Darvel soil at 30^0C and 1^0C.

●-non-sterile, 30^0C; O-sterile, 30^0C; ▲-non-sterile, 1^0C; Δ-sterile, 1^0C

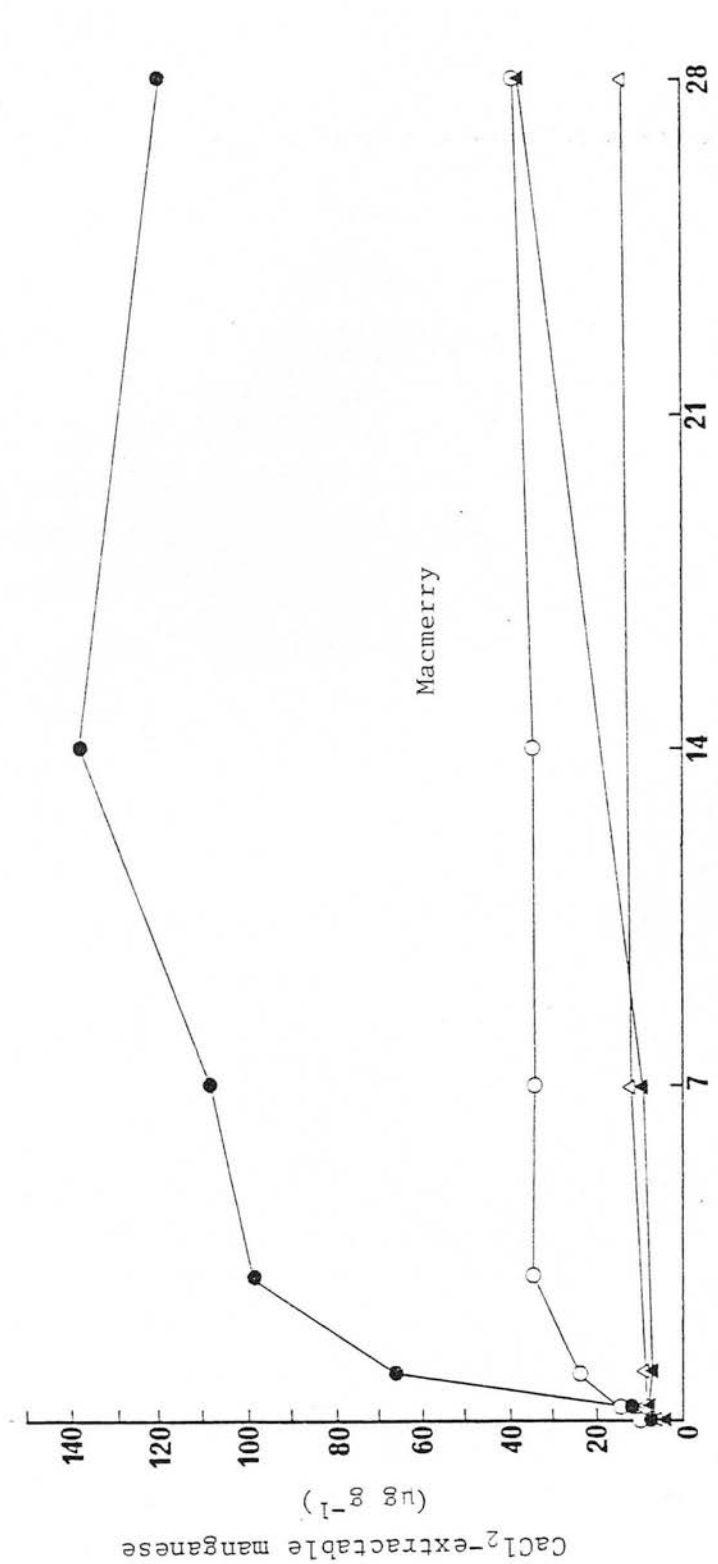

Figure 5.9 Changes in concentrations of $CaCl_2$-extractable manganese in sterile, flooded and non-sterile, flooded Macmerry soil at 30^0C and 1^0C.

●-non-sterile, 30^0C; O-sterile, 30^0C; ▲-non-sterile, 1^0C; Δ-sterile, 1^0C

to manganese release amounted to approximately 25% at peak values. The substantial rise in extractable manganese observed in the non-sterile soil incubated at 30°C (after 8 hours of submergence) was also observed at 1°C, albeit at a later period. Nearly a 4-fold increase in extractable manganese concentrations were observed between 7 and 28 days at the lower temperature; the sterile counterpart remained relatively constant during these times.

The reasons for the relatively sharp increases in extractable manganese concentrations in the non-sterile Macmerry soil are obscure in view of the fact that these trends were not apparent in the other soils studied. However, the observation that these increases occurred in the non-sterile soil only, indicates that microbial processes are responsible and in fact are operative at low temperatures (1°C) despite their apparent quiescence in nutrient culture (Plate 5.1).

5.3.6 Release of Manganese at 12°C

Generally, in the four soils studied the rates of manganese release at 12°C were intermediate between those at the two temperature extremes (Figs. 5.6-5.9) and followed similar trends in liberated manganese concentrations.

5.3.7 Mechanisms Responsible for the Chemical Reduction of Manganese

A prerequisite for the microbial reduction of higher-valency manganese in flooded soils is the disappearance of molecular oxygen, after which anaerobic organisms utilise the chemically bound oxygen of the oxide material (Russell, 1973). The similarity in $CaCl_2$-extractable manganese concentrations between the sterile and non-sterile samples in three of the four soils studied (Stirling, Dreghorn and Darvel) suggested that, initially, oxygen supply played no role in manganese reduction. In order to investigate this further, the four soils (sterile and non-sterile) were continuously shaken for 24 hours at 30°C in 0.05M $CaCl_2$ solution. During the extraction, the vessels remained open to the atmosphere to ensure complete aeration of the soil suspension. Results of this treatment were compared with those from flooded non-shaken soil (Table 5.3).

Of the four soils studied, as in the previous experiment, only the Macmerry showed any significant differences between the treat-

ments. An approximately 2-fold increase in extractable manganese was observed in the flooded non-sterile soil relative to the flooded sterile and both the aerobic samples.

Since no direct measurement of dissolved oxygen tensions were made it is not possible to say with certainty that the non-sterile Macmerry soil had become anaerobic. What is clear, however, is that for the other three soils, both after sterilisation and after taking precautions to maintain aerobic conditions, as much manganese passed into solution as in the non-sterile flooded soils. This strongly indicates that chemical rather than microbial (anaerobic) processes were responsible.

Table 5.3 Concentrations of $CaCl_2$-extractable manganese in flooded, non-shaken soils and soils shaken in aerobic suspensions for 24 hours.

Soil	Manganese ($\mu g\ g^{-1}$)			
	Flooded Non-shaken		Shaken Aerobically	
	Sterile	Non-Sterile	Sterile	Non-Sterile
Macmerry	24	67	37	32
Stirling	90	94	100	97
Dreghorn	90	88	89	85
Darvel	114	110	109	105

These results are contradictory to those of Yoshida and Kamura (1972) who indicated that manganese reduction in waterlogged soils was carried out mostly by soil microorganisms. Ingols and Enginum (1968), on the other hand, concluded that the reduction of MnO_2 to the Mn(II) ion in lake sediments was mediated via a chemical process

by reactions with organic matter present in the mud. The observation of Heintze (1957) that higher manganese oxides do not commonly occur in organic aerobic soils may further attest to the reducing properties of organic matter. Reactive H^+ ions in aromatic and aliphatic carboxyl and in phenolic and alcoholic hydroxyl groups or soil humic and fulvic acids are probably responsible.

The potential for manganese oxide reduction by fulvic acid compounds is quite substantial. Hayes *et al* (1975), using various solvents on a H^+ saturated organic soil, found 0.8% to 14% of the organic matter content to be composed of fulvic acid material, depending on the extracting solution used; a water extract yielded 2.8% of fulvic acid. The strong reducing properties of the material can be readily demonstrated. The quantity of H^+ ions present in carboxyl (COOH) groups ranges from 610 to 910 milliequivalents per 100 g of fulvic acid material (Stevenson and Butler, 1969). Therefore, from the figure of Hayes *et al* (1975), in a water extract of a 3 g sample of the Stirling soil (O.M. 6.4%), for example, enough fulvic acid would be present to solubilise at least 884 µg of manganese (given proper conditions) - far in excess of the reactive manganese content of the soil (Table 5.2).

Zagicek and Pojasek (1976) found that dilute leachates of soil and other terrestial material reduced manganese oxides. Fulvic acid extracted from bog water, as well as model compounds containing functional groups similar to those of fulvic acid, also reduced manganese oxide material. In fact, fulvic acids have been found effectively to solubilise natural chlorite minerals, thus bringing substantial amounts of Al, Fe and Mg into solution (Kodama and Schnitzer 1973).

The fact that the activity of many soil enzymes shows little decline after sterilising doses of radiation (McLaren *et al*, 1962) cannot rule out their role in the reduction of oxide material. In radiation-sterilised soil, the presence of enzymes has been detected in dead cells or on soil particles (McLaren, 1962) and it is considered probable that they have a role in manganese oxide reduction (Garey and Barber, 1952; Vavra and Frederick, 1952). Alexander

(1977) thought that manganese dioxide could serve as an electron acceptor for respiratory enzymes.

5.3.8 Immobilisation of Manganese

Figures 5.6-5.9 show some decrease in extractable manganese in the non-sterile soil at 30°C after concentrations reached a maximum. According to Ponnamperuma (1972), the concentration of manganese in anaerobic soil solutions represents a balance between Mn(II) release via reduction processes and its removal from solution by the formation of insoluble complexes, cation exchange reactions and precipitation of $MnCO_3$. However in the present work, the immobilisation of extractable manganese occurred in the non-sterile soils only, thus indicating that microbial rather than chemical processes were responsible, as the latter could be expected to be similar in both sterile and non-sterile conditions.

In order to investigate this matter further, samples of the sterile, flooded Darvel soil (after 28 days' submergence) were inoculated with 0.1 ml of a water extract from its non-sterile counterpart (prepared by shaking 10 g of the non-sterile Darvel soil with 30 ml of water for 30 minutes). After inoculation, the tubes were immediately resealed and replaced in the 30°C incubator. Figure 5.10 shows the concentration of $CaCl_2$-extractable manganese in the sterile and inoculated Darvel soil. The results showed that inoculation brought about a decrease in extractable manganese at some time after a further 8 days' submergence. Therefore, the reintroduction of microorganisms appears to have influenced immobilisation. At least two possibilities could account for these findings: firstly, manganese incorporation into microbial tissue could decrease solution concentrations; secondly, microbial transformations of another soil constituent such as iron which indirectly affects manganese solubility (e.g. by coprecipitation with iron hydroxides or carbonates). These processes are discussed in the following sections:-

5.3.8.1 Microbial immobilisation

Estimates of the magnitude of biomass in soils range from 100

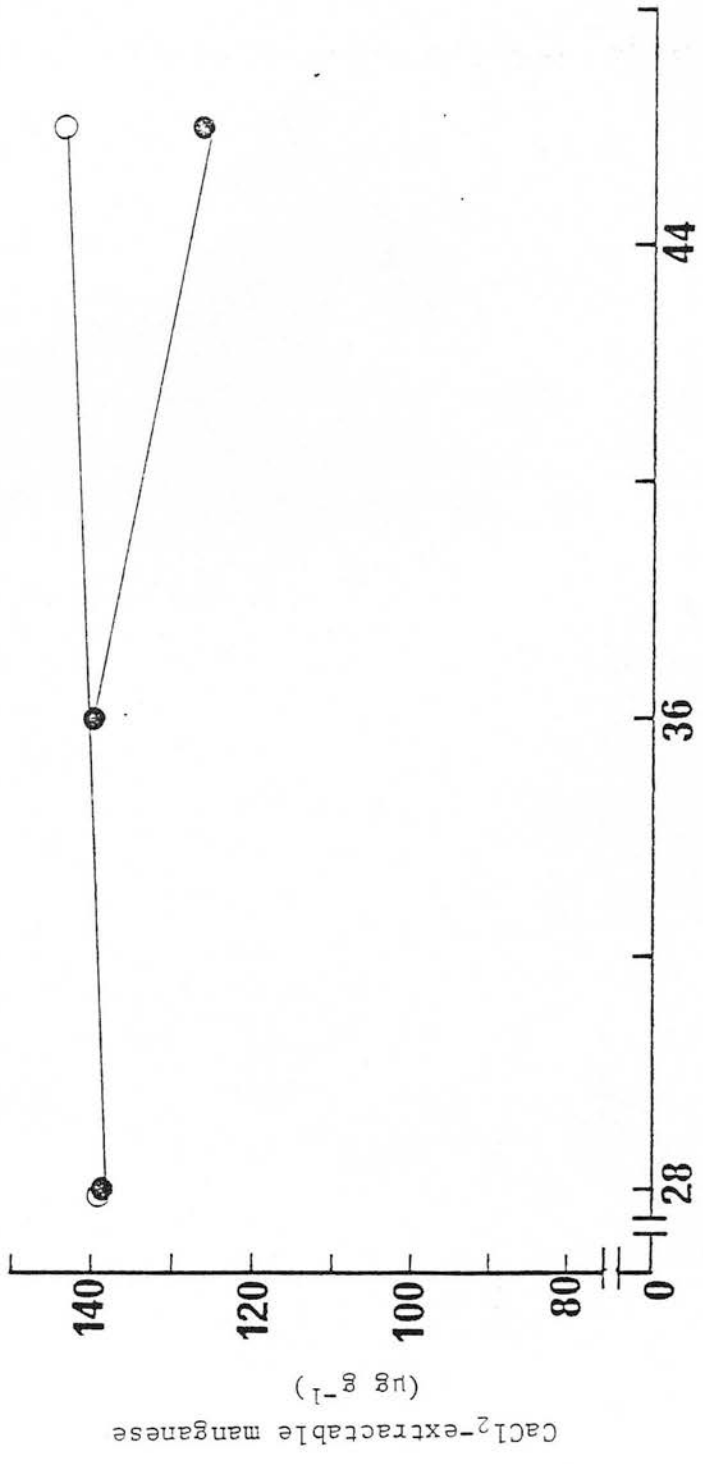

Figure 5.10 Concentrations of $CaCl_2$-extractable manganese in sterile Darvel soil following inoculation after 28 days' submergence.

O-sterile; ●-inoculated

to 4000 kg/ha of bacteria on a live weight basis. This is equivalent to 0.01 to 0.4% of the total soil mass. Despite the fact that the manganese concentration in microbial tissue rarely exceeds 0.05% (Alexander, 1977) and that the numbers and viability of bacteria would be depressed in air-dry soils, the onset of favourable conditions through flooding for facultative and strict anaerobes (less oxygen, less competition, liberation of organic and inorganic nutrients, stabilisation of pH to near-neutral values) could markedly enhance proliferation. In a closed system such as a sealed centrifuge tube, the exponential growth of many microbial populations could immobilise previously liberated manganese (and other nutrients) in microbial tissue. Once the nutrient supply became limited however, microbial proliferation would cease and a steady state would be attained; concentrations of extractable manganese should then reach an equilibrium.

5.3.8.2 Reaction with iron compounds

The transformations of iron in submerged soils are similar to those of manganese in many respects (Ponnamperuma, 1972). Since the transformations of iron occur, for the most part at a lower redox potential than manganese, the oxidation of ferrous iron, Fe(II), would commence prior to that of manganese. Fresh formation of ferric (Fe(III)) compounds could occlude or form co-precipitates with manganese (Turner and Patrick, 1968; Collins and Buol, 1970). This may be supported by the fact that a red-brown precipitate became visible in the non-sterile tubes only, approximately coinciding with decreases in extractable manganese concentrations. However, no attempts were made to analyse the precipitate.

Results of $CaCl_2$-extractable iron for the four non-sterile soils incubated at $30^{o}C$ are represented in Figure 5.11. The determination of iron in $CaCl_2$ extracts was not totally satisfactory and there was often a large variation in values between replicate samples. Generally, the data show (Figure 5.11) that, with the exception of the Macmerry soil, concentrations of extractable iron reached peak values within a day of submergence before declining. Decreases in extractable iron can probably be explained by an increase in soil

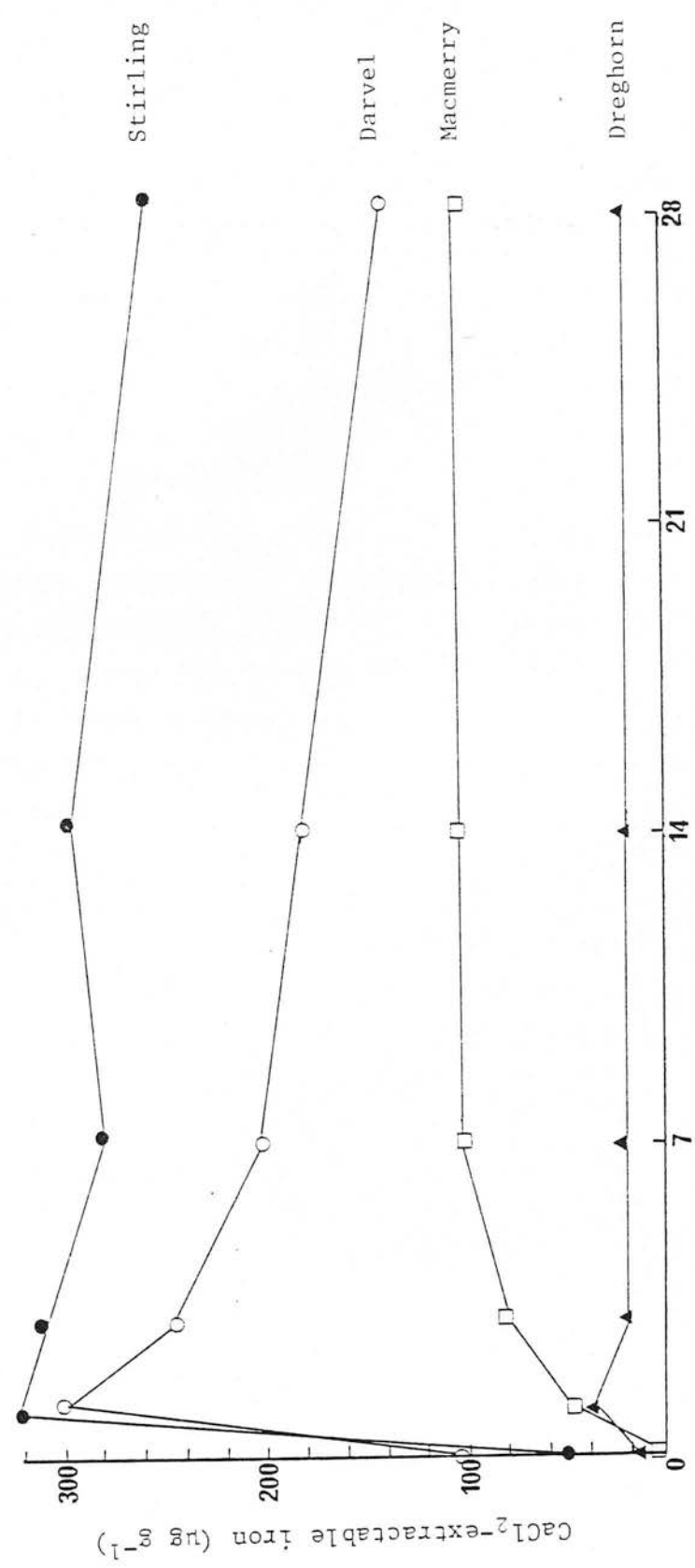

Figure 5.11 Concentrations of $CaCl_2$-extractable iron in non-sterile, flooded soils at 30^0C

●-Stirling; O-Darvel; □-Macmerry; ▲-Dreghorn

pH with the subsequent precipitation of $Fe_3(OH)_8$ or $Fe_3O_4.nH_2O$ (Ponnamperuma, 1972; Choudhury, 1979). Ponnamperuma (1972) stated that the increase in pH of acid soils can be attributable to the reduction of iron:-

$$Fe(OH)_3 + e^- \rightleftharpoons Fe(OH)_2 + OH^-.$$

Observed decreases in extractable iron during the first 24 hours of submergence were accompanied by pH shifts from 6.2 to 6.7, 5.5 to 6.2, 6.2 to 7.3 and 6.0 to 7.0 for the Macmerry, Stirling, Dreghorn and Darvel soils, respectively. The fact that decreases in extractable manganese occurred at later periods indicates the initial predominance of solubilisation processes over immobilisation.

Extractable iron concentrations in the sterile flooded soils, although substantially lower, in most cases decreased in a similar manner to those observed in the non-sterile flooded soil. The precipitation of lower quantities of iron in the sterile soil may not influence manganese concentrations to any marked degree. Other factors in the non-sterile system however, must play a role. Measurements of other soil parameters, especially redox potential, would be useful.

5.4 SUMMARY AND CONCLUSIONS

Experiments using four submerged soils under sterile and non-sterile conditions showed that a substantial amount of manganese was solubilised or reduced by chemical mechanisms. Chemical reduction of manganese could arise from (1) the reaction with higher manganese oxides by microbially synthesised organic compounds (e.g. fulvic acids) present in the soil prior to waterlogging, or (2) enzymatic systems that remain operative despite eradication of the microbial population. However, a direct microbial contribution to the reduction of manganese in three out of four submerged soils studied appeared to be small.

The observed decreases in $CaCl_2$-extractable manganese values appear to be attributable to a biologically mediated mechanism. Direct microbial immobilisation or indirect effects on manganese solubility via biological action on other soil constituents are

possibilities.

As expected, the act of grinding the soil accentuated the rate of change in concentration of $CaCl_2$-extractable manganese in flooded soils. The effect was much more pronounced on the Dreghorn soil than on the Macmerry. A greater proportion of more coarse particle sizes in the former was probably partly responsible. Other parameters however, e.g. relative aggregate size, or the occurrence of concretionary materials, could also have been responsible.

Although the frequent drying and wetting of soils is commonplace in natural field conditions, the effects of using air-dried soils (especially those stored for prolonged periods) on subsequent manganese release should be kept in mind. Yoshizawa (1966) found water extracts from air-dried (or heated) soils had greater reducing power than those from moist soil. Apparently the act of air-drying increased the amount of water-soluble organic matter which had a reducing capacity. Manganese in soils is especially sensitive to drying processes, as discussed in Section 2.6.5. It is not surprising, therefore, to observe variations in manganese release with the nature and length of the air-drying process prior to soil submergence. A more desirable approach would be to study chemical and microbiological factors affecting manganese release in soils which had been maintained in field moist condition prior to submergence. Unfortunately, gamma sterilisation of moist samples raises new problems. For example, Salonius *et al* (1967) noted a much greater solubilisation of organic matter in irradiated moist soil relative to dry material. They attributed this to the formation of peroxides which are formed when water is irradiated. Freeze-drying a soil prior to sterilisation may minimise the reducing effects of slow air-drying processes, without introducing the problems associated with irradiation of moist soils. A comparison of manganese release from submerged soils that had been previously air-dried for varying periods of time would also be a useful investigation.

SECTION 6

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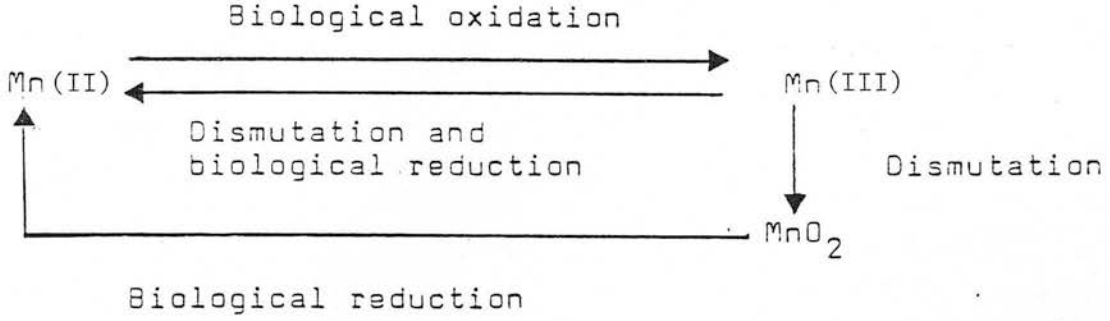
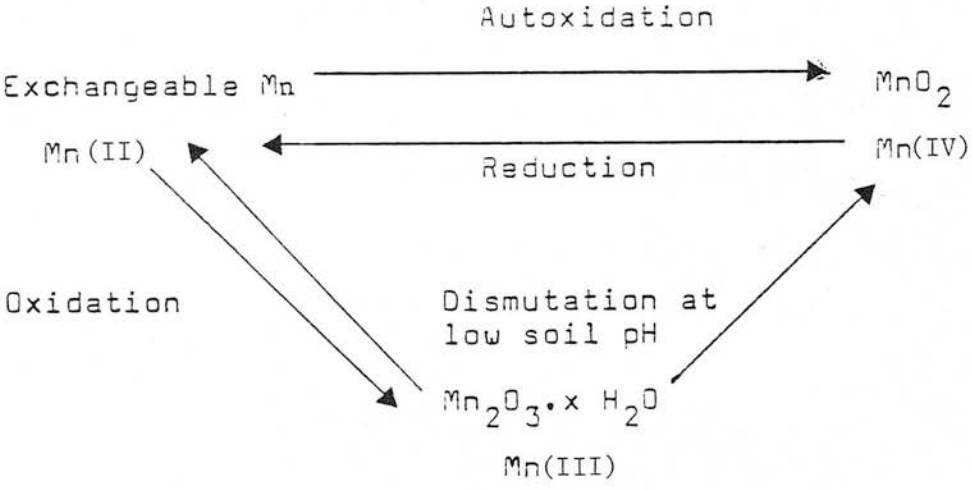
APPENDICES

A. Proposed Manganese Cycles in Soil

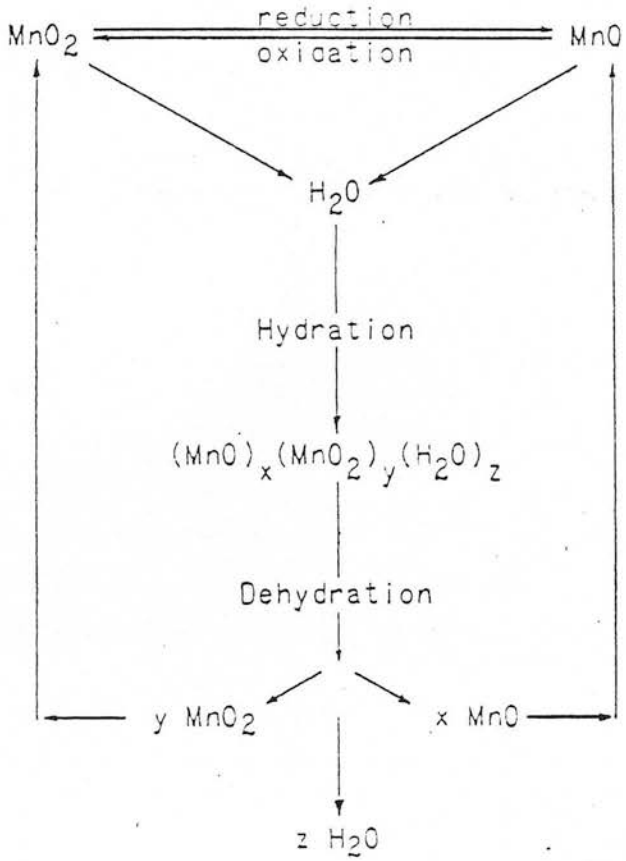


Mann and Quastel (1946)

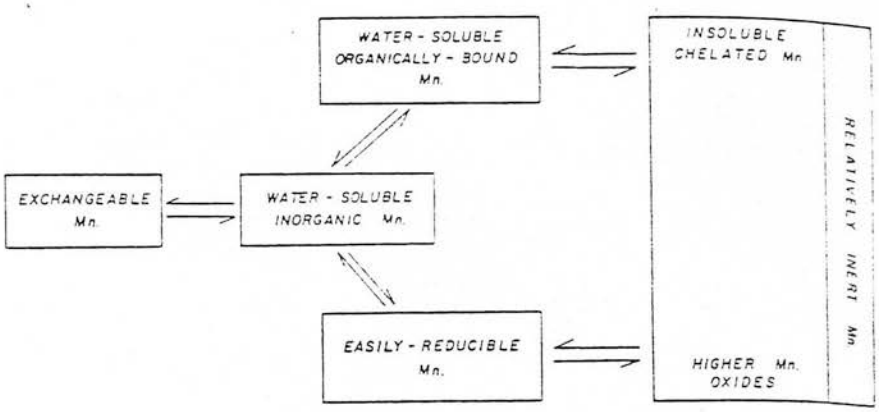


Dion and Mann (1946)

A. Contd



Fujimoto and Sherman (1948)



Ghanem *et al* (1971)

B.1 Investigation of the Changes in $CaCl_2$-Extractable Manganese with Air-Drying

An experiment was undertaken with the Giffordtown soil (from the 1978 field trial) in a preliminary investigation of the effects of air-drying on extractable soil manganese. A year later, an identical study was carried out using a Hexpath soil from the 1979 field trial site.

A large composite sample of the soil was passed through a 2 mm sieve and thoroughly mixed. The soil was then spread evenly to a depth of 1 cm on a polythene sheet in the glasshouse and left to dry at ambient temperature. At daily intervals during the drying period, the soil was mixed thoroughly and subsampled. A portion of the soil was dried overnight at 105°C for moisture content determination while another portion was extracted with 0.05M $CaCl_2$.

The results of the drying experiments on the Giffordtown and Hexpath soils are shown in Figure B.1.1. Gradual air-drying of the former soil from the field-moist (29% moisture) to the air-dried (2% moisture) state resulted in nearly a three-fold increase in the concentration of extractable manganese. After a period of 7 weeks of storage of the air-dried soil, a further five-fold increase in extractable manganese was observed.

Similarly, an approximately three-fold increase in extractable manganese occurred with the Hexpath soil from the moist to the air-dried state. Storage of the soil in the air-dried state resulted in a further substantial increase but not such a drastic one as that observed with the Giffordtown soil. Nevertheless, levels increased approximately three-fold during the 142 day storage period following air-drying. Bartlett and James (1980) noted similar increases in ammonium acetate-extractable manganese in an air-dried soil stored over a 9 week period.

Such increases in extractable manganese values occurred regardless of the soil pH. Samples of the Giffordtown soil (slurry mixture of 50 g air-dried soil and 125 ml of H_2O or 0.01M $CaCl_2$) were adjusted to different pH values with $Ca(OH)_2$ or HCl and freeze-dried. The pH and $CaCl_2$ extractable manganese values were determined after

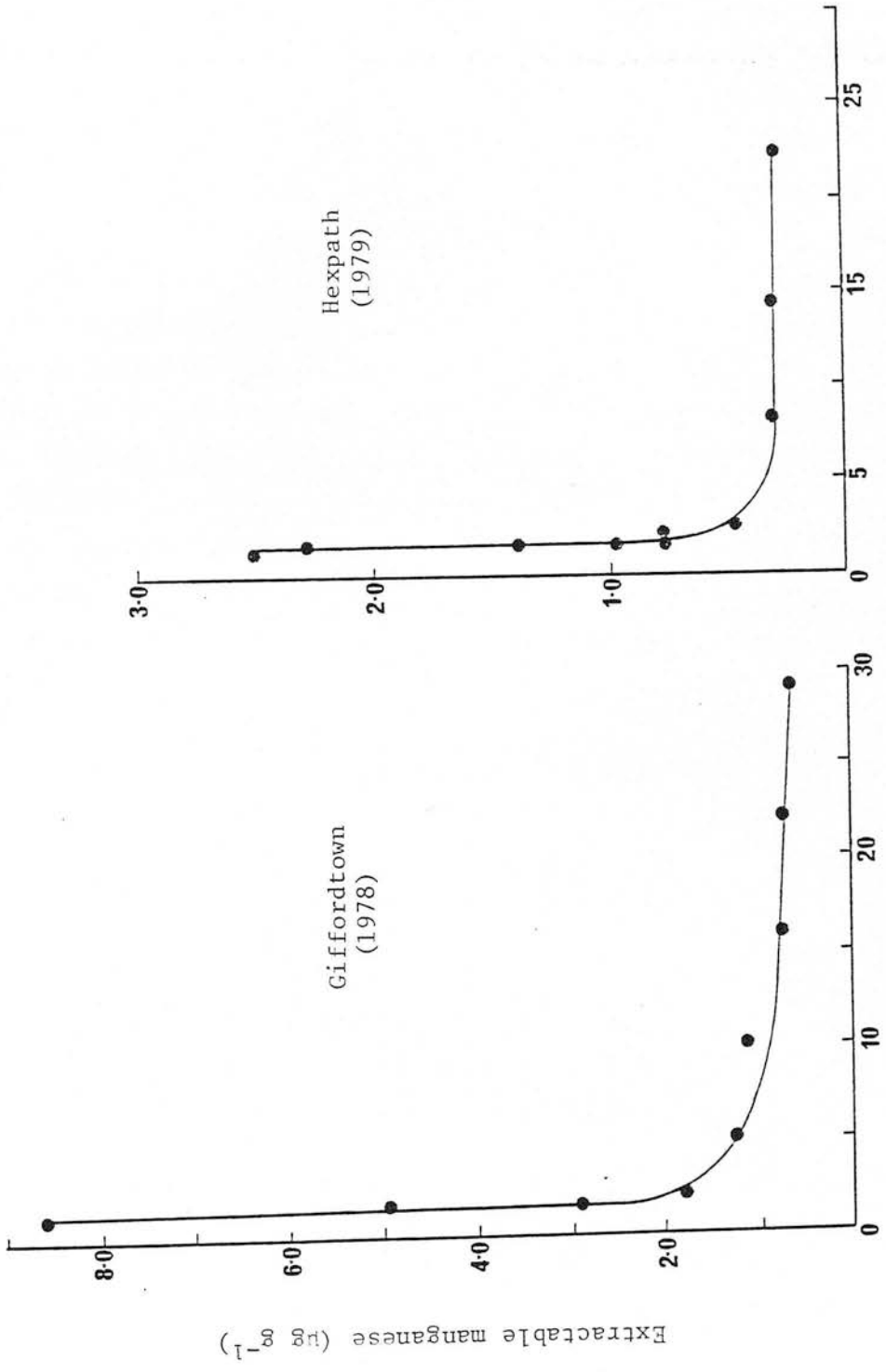


Figure B.1.1 Relationship between per cent moisture and extractable manganese for the Giffordtown and Hexpath soils.

0 and 26 days of storage. An increase in manganese levels with time was observed for all treatments (Table B.1.1). Soil pH, however, remained constant.

Given relatively constant drying conditions, the rate at which a soil reaches the air-dried state will depend on its initial moisture content at the time of sampling. As is evident from Figure B.1.1 the continued increase in manganese solubility after a soil is dried would create difficulties in interpretation of results if soil samples from any particular area varied greatly in moisture content. This would be especially critical in heavy-textured soils where the water-holding capacity is greatest and may be affected by compaction. However, with the light-textured soils encountered in this study, moisture contents of samples from differently consolidated areas within a particular field did not vary greatly. This is evident from moisture content values of soils selected at random from several of the experimental sites (Tables B.1.2 and B.1.3). Thus desiccation rates to the air-dried state would be similar for soil samples originating from the same field.

Table B.1.1 The effect of storage on the pH and extractable manganese of pH-adjusted Giffordtown soil

pH (H_2O)		Manganese ($\mu g\ g^{-1}$)	
1 day	26 days	1 day	26 days
5.4	5.4	2.2	3.0
5.8	5.8	2.0	2.8
5.9	5.9	1.2	1.8
6.1	6.1	1.9	2.8
6.8	-	0.9	1.5

Table B.1.2 Moisture contents of soils at 3 levels of consolidation, sampled at random from 2 experimental sites

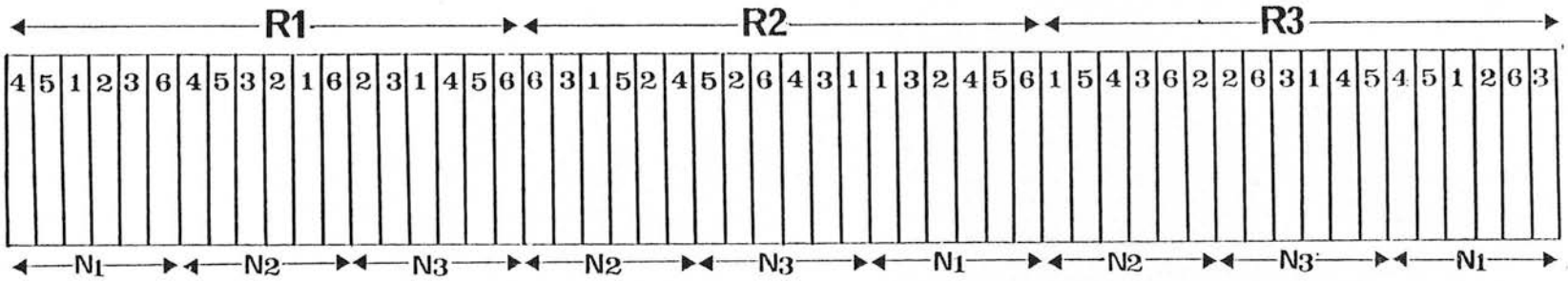
Soil	Moisture content g/100 g oven-dry soil		
	Loose*	Normal*	Compact*
Giffordtown	31.6	32.6	33.2
	34.8	30.0	34.8
	35.5	33.9	37.0
	34.0	32.5	34.2
	35.5	31.9	33.7
Mean	33.9	32.2	34.6
Standard deviation	1.5	1.4	1.5
Macmerry	30.3	36.7	28.7
	30.5	30.2	27.6
	27.2	26.5	33.5
	30.9	36.6	30.3
	29.7	28.5	34.2
Mean	29.7	31.7	30.8
Standard deviation	1.5	4.7	2.9

* Seedbed consolidation treatments (see 3.3.1.1)

Table B.1.3 Moisture contents of soils in and between tractor wheel tracks at 2 sites

Soil	Moisture content g/100 g oven-dry soil	
	In wheel tracks	Between wheel tracks
Hexpath	23.9	24.4
	22.1	22.0
	25.3	24.1
	24.8	24.4
	24.2	24.5
Mean	24.1	23.8
Standard deviation	1.2	1.1
Darvel	18.2	17.1
	15.1	14.0
	14.4	12.9
	18.3	17.2
	16.8	15.3
Mean	16.6	15.3
Standard deviation	1.8	1.9

B.2 - B.4 Experimental Designs of Field Trials 1978-1980



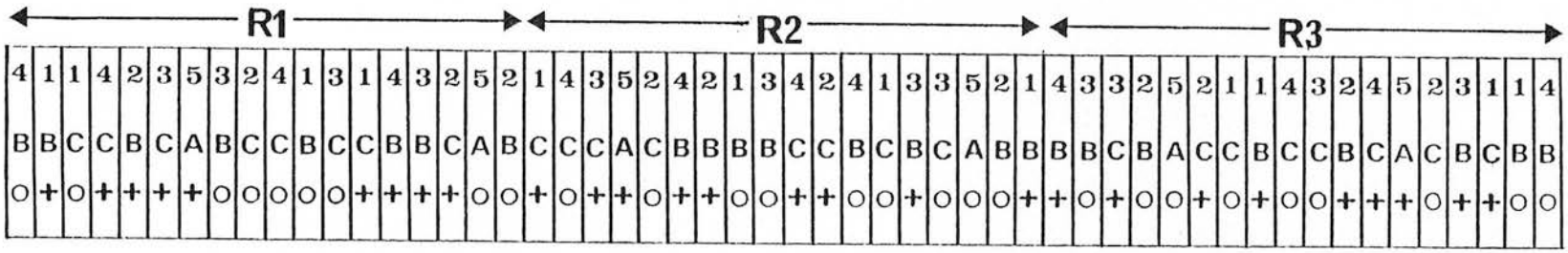
B.2 Experimental design of the field trial on the Giffordtown soil, 1978

Legend:

R1,R2,R3-replicates

1-6-seedbed consolidation treatments (1-L; 4-N; 6-CP; 2,3 and 5 were treatments not monitored)

N1,N2,N3,-fertiliser application rates



B.3 Experimental design of the field trial on the Hexpath soil, 1979

Legend:

R1,R2,R3-replicates

1-5 - seedbed consolidation treatments - (1-L; 2-N; 3-CP1; 4-CP3; 5-NBB)

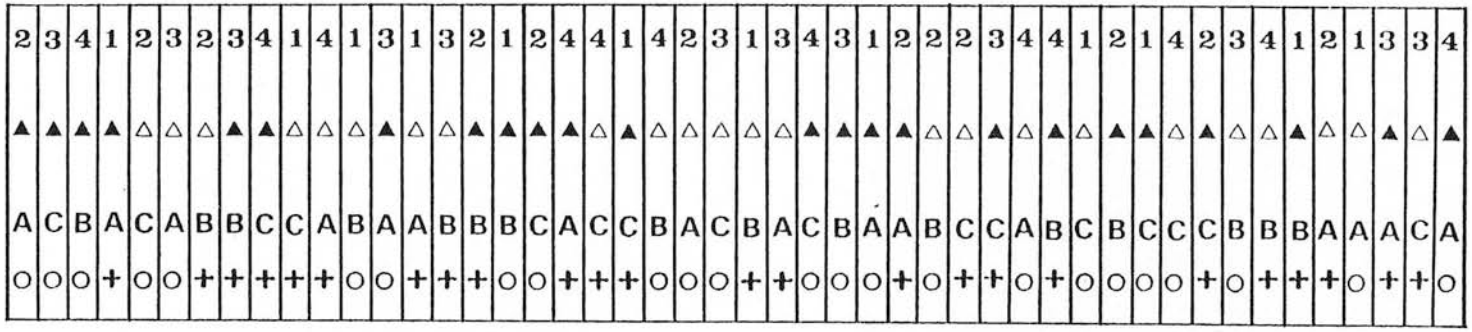
A-fertiliser broadcast before drilling (BB)

B-fertiliser broadcast at drilling (BA)

C-fertiliser combine-drilled (C)

+-with manganese sulphate spray

O-without manganese sulphate spray



B.4 Experimental design of the field trial on the Carpow soil, 1980

Legend:

1-4 seedbed consolidation treatments - (1-L; 2-N; 3-CP1; 4-CP3)

Δ-N1 fertiliser application rate

▲-N2 " " "

A-fertiliser broadcast before drilling (BB)

B-fertiliser broadcast at drilling (BA)

C-fertiliser combine-drilled (C)

+-with manganese sulphate spray

O-without manganese sulphate spray

B.5 The Translocation of ^{54}Mn within the Plant

Experimental Materials and Methods

Barley seeds were sown in 1225 cm^3 plastic pots at a density of 5 per pot on sieved (<2 mm) Darvel soil. After several weeks of growth, plants were sprayed with ^{54}Mn-labelled $MnSO_4$ solution, prepared by mixing equal quantities of carrier-free ^{54}Mn (4.0 $\mu Ci\ ml^{-1}$) and 0.3% $MnSO_4$. Each pot received two 2 ml doses, with a 24 hour drying period between each dose. The soil surface was previously covered with absorbent filter paper, thus preventing any incorporation of spray into the soil and hence to the root system. At later growth stages, plant material was counted for activity and qualitative and quantitative assessments of translocation were made.

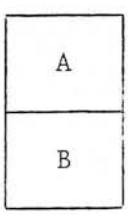
B.6 Bulk Density Determinations of Soil Clods from Uniformly Compacted Pots

1. Random areas of two pots uniformly compacted in the same manner.

Pot 1 Bulk Density	Pot 2 Bulk Density
1.32	1.39
1.39	1.31
1.40	1.35
1.37	1.37
1.38	1.43
1.36	1.28
1.30	1.41
1.44	1.42
1.40	1.39
1.43	1.44

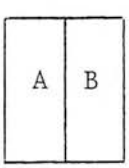
2. Specific areas of two compacted pots.

Pot 3



Bulk Density	
A	B
1.47	1.33
1.30	1.40
1.39	1.35

Pot 4



1.60	1.52
1.51	1.62
1.57	1.47

B.7 Example of a Calculation for the Determination of the Percentage of Plant Nitrogen Derived from a ^{15}N-labelled Fertiliser

A = Fertiliser source - e.g. $Ca(NO_3)_2$ of which 5.5 atom per cent of the nitrogen is ^{15}N.

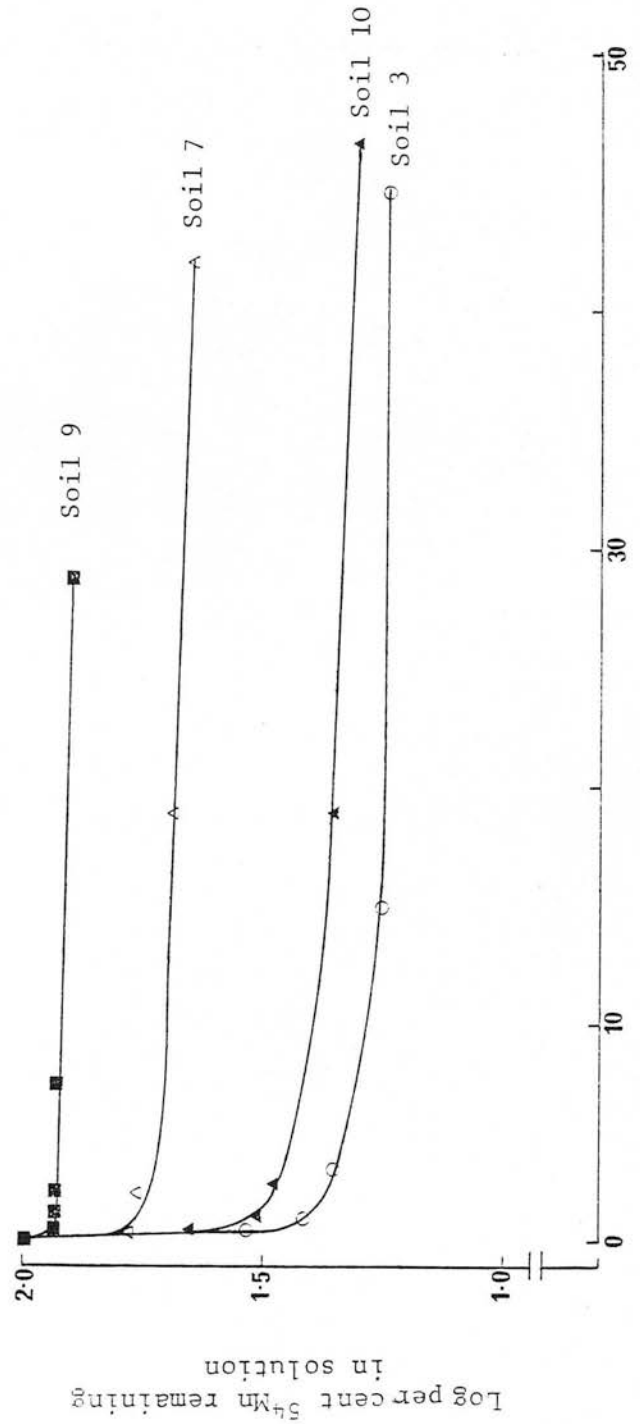
B = Natural abundance of ^{15}N in the environment = 0.37 atom per cent (Hauck and Bremner, 1976).

C = Atom per cent of ^{15}N in plant tissue determined by mass spectrometry = 2.42.

Therefore the per cent derived from the fertiliser source =

$$\frac{C-B}{A-B} \times 100$$

$$= 39.84\%$$



C.1 Log per cent ^{54}Mn remaining in solution with time.

C.2 Mn_L Value Determinations from Individual Pots of the Three Experiments

		Consolidation		
	Experiment	Unconsolidated	Intermediate compaction throughout	Heavy compaction throughout
1.	Giffordtown	15.0	15.8	15.9
		17.2	14.1	18.3
		16.2	21.2	17.0
		Unconsolidated	Surface compacted	Compacted throughout
2.	Hexpath	50.5	92.5	75.4
		82.6	113.4	92.0
		44.4	92.1	77.6
		52.8	110.0	70.2
		51.6	114.8	91.9
		51.0	97.0	72.2
3.	Darvel	81.2	76.1	91.0
		70.2	76.7	121.8
		60.6	86.3	112.8
		73.8	78.2	106.2

C.2.a Analysis of variance for Mn_L value determinations

Hexpath soil

Source of variation	df	SS	MS	VR
Among groups (among consolidation treatments)	2	6860.27	3430.13	26.31***
Within groups (error, replicates)	15	1955.62	130.37	
Total	17	8815.88		

Standard error of difference between means = 6.59
*** - significant at 0.1% level

Darvel soil

Source of variation	df	SS	MS	VR
Among groups (among consolidation treatments)	2	2951.54	1475.77	16.75***
Within groups (error, replicates)	9	792.79	88.09	
Total	11	3744.33		

Standard error of difference between means = 6.64
*** - significant at 0.1% level

D. The Release of Manganese from the Sterile, Flooded and Non-sterile, Flooded Soils at $1^{\circ}C$, $12^{\circ}C$ and $30^{\circ}C$

The release of manganese in sterile, flooded and non-sterile, flooded Stirling soil at 1°C, 12°C and 30°C

Time (hours)	Determination	Manganese (µg g^{-1})					
		1°C		12°C		30°C	
		Sterile	Non-sterile	Sterile	Non-sterile	Sterile	Non-sterile
0	A	30	30	34	31	37	35
	B	28	27	33	28	37	35
0.5	A	31	28	-	-	41	39
	B	31	32	-	-	39	42
2	A	34	33	40	39	55	46
	B	35	34	37	36	51	46
8	A	42	37	-	-	73	65
	B	39	38	-	-	71	64
24	A	51	48	67	60	93	93
	B	51	49	66	60	88	95
72	A	-	-	81	87	98	115
	B	-	-	79	83	100	117
168	A	80	70	96	104	102	127
	B	57	71	93	104	101	123
336	A	-	-	99	107	105	123
	B	-	-	97	106	105	117
672	A	73	73	99	102	105	93
	B	69	74	95	102	103	93

The release of manganese in sterile, flooded and non-sterile, flooded Dreghorn soil at 1°C, 12°C and 30°C

Time (hours)	Determination	Manganese (μg g^{-1})					
		1°C		12°C		30°C	
		Sterile	Non-sterile	Sterile	Non-sterile	Sterile	Non-sterile
0	A	22	21	25	22	31	29
	B	24	21	25	24	32	29
0.5	A	28	23	-	-	34	33
	B	25	23	-	-	34	33
2	A	29	27	36	34	58	48
	B	30	27	36	34	57	49
8	A	42	33	-	-	82	77
	B	40	34	-	-	82	79
24	A	59	52	81	77	90	87
	B	58	52	82	75	89	89
72	A	-	-	92	91	93	94
	B	-	-	93	88	92	95
168	A	90	87	97	98	94	111
	B	90	84	96	95	97	107
336	A	-	-	108	111	102	115
	B	-	-	102	107	101	115
672	A	96	86	98	102	98	105
	B	95	87	100	98	103	105

The release of manganese in sterile, flooded and non-sterile, flooded Darvel soil at 1°C, 12°C and 30°C

Time (hours)	Determination	Manganese (μg g^{-1})					
		1°C		12°C		30°C	
		Sterile	Non-sterile	Sterile	Non-sterile	Sterile	Non-sterile
0	A	29	23	32	32	40	39
	B	25	25	32	29	39	39
0.5	A	32	28	-	-	43	43
	B	32	29	-	-	46	42
2	A	33	33	43	42	70	66
	B	39	30	46	42	73	64
8	A	49	44	-	-	100	94
	B	47	44	-	-	98	93
24	A	72	68	98	92	115	112
	B	72	66	95	92	114	109
72	A	-	-	111	112	124	124
	B	-	-	115	107	124	124
168	A	111	106	130	128	141	139
	B	112	107	130	124	140	140
336	A	-	-	135	129	141	111
	B	-	-	135	125	138	114
672	A	127	119	134	104	136	106
	B	125	116	130	103	138	86

The release of manganese in sterile, flooded and non-sterile, flooded Macmerry soil at 1°C, 12°C and 30°C

Time (hours)	Determination	Manganese (μg g^{-1})					
		1°C		12°C		30°C	
		Sterile	Non-sterile	Sterile	Non-sterile	Sterile	Non-sterile
0	A	8.0	7.5	8.1	7.6	9.4	8.7
	B	8.1	7.4	8.1	7.5	9.8	8.5
0.5	A	7.9	7.7	-	-	10.5	8.4
	B	8.0	7.5	-	-	9.9	8.0
2	A	8.6	8.0	10.4	8.6	12.8	9.7
	B	8.6	8.0	9.4	7.8	11.2	9.3
8	A	9.6	8.5	10.5	9.3	14.8	11.9
	B	9.6	8.3	11.3	9.5	14.5	12.4
24	A	9.5	8.5	12.4	9.6	23	68
	B	9.1	7.2	11.9	10.0	25	66
72	A	-	-	18	58	34	100
	B	-	-	18	50	35	98
168	A	12.0	9.5	20	84	33	108
	B	11.7	9.8	20	84	33	108
336	A	-	-	21	103	34	136
	B	-	-	21	98	34	139
672	A	14.3	30	21	103	44	119
	B	14.2	47	22	97	35	121